

REVIEWS

Genome editing of crops: A renewed opportunity for food security

Fawzy Georges and Heather Ray 

Jene Quests Corporation, Saskatoon, SK, Canada

ABSTRACT. Genome editing of crop plants is a rapidly advancing technology whereby targeted mutations can be introduced into a plant genome in a highly specific manner and with great precision. For the most part, the technology does not incorporate transgenic modifications and is far superior to conventional chemical mutagenesis. In this study we bring into focus some of the underlying differences between the 3 existing technologies: classical plant breeding, genetic modification and genome editing. We discuss some of the main achievements from each area and highlight their common characteristics and individual limitations, while emphasizing the unique capabilities of genome editing. We subsequently examine the possible regulatory mechanisms which governments may be inclined to use in assessing the status of genome edited products. If assessed on the basis of their phenotype rather than the process by which they are obtained, these products will be categorized as equivalent to those produced by classical mutagenesis. This would mean that genome edited products will not be subject to the restrictions imposed on genetically modified products, except in some cases where the mutation involves a large sequence insertion into the genome. We conclude by examining the potential of societal acceptance of genome editing technology, reinforced by a scientific perspective on promoting such acceptance.

KEYWORDS. CRISPR/Cas9, food security, genome editing, mutagenesis, plant breeding, regulatory issues, societal acceptance

Correspondence to: Fawzy Georges; Jene Quests Corporation, 134 Egnatoff Way, Saskatoon, SK, Canada, S7K 7R9; Email: fawzy.georges@jenequests.com

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INTRODUCTION

An important milestone in the history of humanity is predicted to be achieved by the middle of the 21st century when Earth's population will have reached a record high of over nine billion people. A population burst of this enormity will necessarily impose an unprecedented huge demand on the planet's resources and capabilities. Additionally, the current cycle of climatic changes that the planet is undergoing is bound to have a negative impact on natural ecosystems and inflict damaging environmental stresses on agricultural productivity.

Of immediate concern is the disproportionate imbalance between the agricultural lands needed for food production, and the steady rise in urban development needed to accommodate this population increase at the expense of farm lands. Although the increase in human population is expected to be centered mostly in underdeveloped countries, its effects will be felt throughout the globe in terms of demands for sustainable food resources. Therefore, the production of efficient and much improved crops is crucial for meeting and maintaining the potential demands of the expected increase in population.

The substantial scientific revolution made in the fields of plant genetics and molecular biology during the last quarter of the 20th century made possible genetic manipulation of metabolic pathways in crops in a highly targeted manner, which led to the achievement of improved new varieties, some of which could not have been obtained by conventional breeding. Transgenic or genetically modified (GM) crop varieties with new traits and enhanced characteristics were produced and in some cases commercialized throughout the world. Despite the promise these crops held for global food security, GM crops later become associated with generally unsubstantiated concerns over health and environmental safety, which were then elevated into political issues. In turn, this led to the imposition of restrictions, particularly in most European countries, on the production or importation of food items of GM origin. In other areas, such as North America, Asia and many countries of the third world, the

use of GM crops and products thereof is considered in some cases safe and unobjectionable.

Over the past 20 years, through advances in the field of bacterial and plant research, novel techniques have emerged which now allow sequence-specific mutations to be made efficiently and with high precision through the CRISPR/Cas9 technology (Bortesi & Fischer, 2015; Puchta & Fauser, 2014). Several previous methods for sequence-specific mutations, such as zinc finger nucleases and the transcription activator-like effector nuclease system (TALENs) and the use of oligonucleotides (Abdallah et al., 2015), seem to have been swept aside in favor of this technique (CRISPR/Cas9), which promises to be simpler, more flexible and more accurate. This momentous concept of genome editing (GenEd) technology is expected to reduce many of the opinion gaps that have contributed to the opposition to the GMO technology and severely hampered its progress. In this article, we briefly examine the progress of the current, relatively short but dynamic, era of crop genomics research, looking at important accomplishments and potential challenges. We comparatively examine the fundamental differences between GM, GenEd and classical plant breeding with a view to evaluating their capacities to contribute to the future of global food security through agriculture. We conclude by putting into perspective the challenges arising from mixed societal views and the resilience required by scientists to move forward.

Traditional Plant Breeding

Crop improvement through conventional breeding practices has been an ongoing quest for quality and productivity enhancements. In its simplest form, crop improvement by breeding has been accomplished by selection of pre-existing, natural mutants such as those yielding more, providing better flavor or more easily harvested. Many generations of selection among mutants went into the production of nearly every "traditional" crop. Many traditional crops are far removed from their progenitor species in phenotype, and some are nearly

incapable of independent reproduction; maize is an example.

Plant breeders gradually developed the science of crossing different plants and selecting the best genetically recombined progeny. Conventional breeding of crops is achieved primarily through crossing different lines and looking for recombination of existing genes, sometimes using lines which are not useful agriculturally but have one trait of high interest, followed by selection of the most useful progeny lines. It also may use wide crosses, with wild species which normally would cross very rarely or not at all with the crop. This has been done to introduce individual genes or suites of genes, such as those conferring complex traits such as salt tolerance from a particularly tolerant wild relative. This is supplemented through the use of high-dose irradiation or chemical mutagenesis, modifying or cleaving chromosomes in random locations. Plants recovered from these treatments rely on their natural mechanisms to repair their damaged DNA and regenerate. Such plants may incorporate an enormous number of random mutations throughout their genome, along with rare chromosome rearrangements. Typically, the mutant is backcrossed several times over a number of generations to segregate the desired mutation away from most of the other changes to the genome, sometimes taking several years.

Mutation has frequently been used to generate new traits not identified in the original crop. For example, the gene encoding acetolactate synthase (ALS) has been mutated in many crop species, to provide tolerance of Group 2 herbicides (Tan et al., 2005). It can also be used to remove undesired traits, such as the formerly high erucic acid content of rapeseed (Roscoe et al., 2001).

Traditional plant breeding and agronomic advances have done a great deal to bring average yields closer to what used to be record yields. This, however, is a trend which is weakening as most of what can be easily done has been done, and average yield increases in major crops are slowing. As well, these high yields come at a high cost to what lies downstream and downwind of the farm, with soil, rivers and lakes and even oceans grossly contaminated by

conventional application of fertilizer, pesticides and herbicides.

Genetically Modified Crops

GM crops are produced by the transfer of genes (transgenes) or gene elements of known function and their integration into random locations along the chromosome of the recipient plant (host plant). The donor species of the transgene may or may not be able to cross with the host plant; it could be a non-related plant, animal or microorganism. In some cases, transgenes may be native to the species, being re-introduced with a different context of expression to increase or decrease the gene's temporal or spatial activity.

A transgene is introduced into the host using a variety of DNA delivery (transformation) systems. Most common is that involving the mediation of organisms that are naturally capable of escorting genetic elements, such as *Agrobacterium tumefaciens*, or by plant viral vehicles. Other methods use delivery by physical means, such as DNA-coated particles or electroporation of protoplasts that can easily regenerate. When expressed, the transgenic product(s) will enable the host plant to exhibit the effect or phenotype corresponding with its intended function.

For traits which require the expression of a complex battery of genes, it is possible to transform the plant with multiple genes in a single event (gene stacking) (Que et al., 2010). Sometimes the same effect can be achieved through the use of single genes that have regulatory functions such as signaling enzymes (Georges et al., 2009) or transcription factors (Gao et al., 2011). Gene stacking can also be used to introduce multiple unrelated traits more quickly than by classical breeding (Que et al., 2010).

The 4 major crops that are in the forefront of approved GM crops for production worldwide are soybean, maize, cotton and canola. Several other approved GM crops such as eggplant, papaya, potato, rice, sugarbeet, sunflower, sugarcane, and tomato are also in production around the world, but with lesser prominence.

Thus far, the predominant GM trait conferred on most transgenic crops has been herbicide (largely glyphosate) or insect resistance (HR and IR, respectively) genes. These traits are of high agronomic value, since minimizing yield losses to weeds and pests increases the yield potential of limited areas of farmlands, particularly in the absence of possibilities for land expansion or in the face of decreasing agricultural areas. Thus, the technology offers increased returns per unit of cultivated land.

The introduction of glyphosate HR varieties of several crops (soybean, canola, cotton, corn), followed shortly by the release of glufosinate HR crops in the mid-1990s, has been accredited with drastically improving weed management and permitting the widespread adoption of no-till agriculture. Both herbicides are considered safe and environmentally friendly relative to most herbicides, and were in use in agriculture long before their use in transgenic crops, with very few adverse effects on the environment. In addition, the fact that glyphosate has a short half-life in the soil reinforces its suitability for controlled usage. Thus the use of HR crops has greatly contributed to better weed management using less harmful chemicals than those used in conventional agriculture, and less frequently. The technology also led to better soil conservation and environmental protection. However, some weed species later developed herbicide resistance, particularly to glyphosate, as discussed below.

Insect resistant varieties of maize were also introduced in the mid-1990s, using single genes of bacterial origin, *Bacillus thuringiensis* (Bt), that encode a class of crystalline (cry) proteins which are toxic to a wide spectrum of crop pest insects, with no measurable effects on beneficial species (Gewin, 2003; Gatehouse et al., 2002). In addition to arresting insect infestation, the trait also proved useful in reducing the incidence of infection by secondary pathogens (Diaz-Gomez et al., 2016). Furthermore, non-GM maize planted near the GM-Bt varieties were not as heavily infested by Bt-target pests as they would normally be, which was attributed to eggs being deposited on Bt leaves (Tabashnik, 2010). The strategy of cultivating non-GM lines near the Bt lines served to

impede the selective pressure which would result in insects developing resistance to the toxins (Tabashnik, 2010). Nevertheless, challenges similar to those in the HR situation have also emerged as discussed below.

Genome-Edited Crops

Mutations at defined sites in the genome, rather than the random non-specific changes produced by radiation or chemical mutagenesis, have long been desired by crop developers. In comparable applications, GenEd technology provides a much more controlled and faster approach to introducing specific alterations of target-gene functions at precise locations in the genome for the purpose of gene silencing or enhancement of gene expression. Variants such as the insertion or deletion of full-length genes or expression cassettes at predetermined positions in the chromosome are also possible through GenEd methodologies (Schiml et al., 2014).

One of the main instruments now being used in GenEd procedures is the CRISPR/Cas9 system (Jinek et al., 2012). The CRISPR/Cas9 and related systems have recently moved to the forefront of the GenEd technology for plants as well as other organisms. Several previous methods continue to be used, but the greater simplicity, accuracy and development speed promised by CRISPR/Cas9 have brought it into quick prominence.

Details of the functioning mechanism of the CRISPR/Cas9 system have now been described many times (Hsu et al., 2014; Doudna & Charpentier, 2014). Essentially, it comprises a DNA-specific nuclease, guided by an RNA:DNA match, acting at a particular sequence of about 20 base pairs (bp) and creating a double-strand break. Such breaks are typically repaired by the non-homologous end-joining repair mechanism, an error-prone repair system, which places the cut ends together, repairs the lesion, and frequently adds or loses a base pair or a few base pairs in the process, leading to a mutation. The most frequent result is a small deletion, which often creates a frame-shift mutation (Jiang et al., 2013).

If a nearby sequence has high homology to the ends, the lesion may be repaired using homologous repair, which is much more accurate. High copy numbers of the nearby sequence might be added on a plasmid or by other means, and can introduce a new defined sequence into the double-strand break. Also, at the same site, larger sequences can be deleted. The change of a few bp at such a site would constitute GenEd. If a large sequence derived from another species is inserted, it would be considered GM.

To date, gene editing has been successfully undertaken, at least as far as the initial mutated plant, for example in *Arabidopsis* (Schiml et al., 2014; Li et al., 2013; Feng et al., 2013), poplar (Fan et al., 2015), *Brassica oleracea* and barley (Lawrenson et al., 2015), maize (Svitashev et al., 2015), soybean (Li et al., 2015), sorghum and rice (Jiang et al., 2013). The number of examples is increasing rapidly, and transmission to the next generation has been shown in several cases (Schiml et al., 2014; Feng et al., 2013; Lawrenson et al., 2015; Svitashev et al., 2015; Li et al., 2015).

Multiple variations have been developed to adjust the properties of the CRISPR system. The precision of the match can be varied somewhat by using a slightly longer or shorter RNA: DNA guide (Lawrenson et al., 2015). In some cases, the nuclease activity for one strand has been removed, leaving a nickase activity (Cong et al., 2013). Two complementary nickases each recognizing a 20 bp sequence can generate long single-strand stretches of DNA, which will have a strong tendency to search for homologous binding (Cong et al., 2013). This method should be more effective for bringing about larger insertions or deletions (Schiml et al., 2014). Other recent developments include the use of a disarmed nuclease, lacking nuclease activity, which will competitively bind a defined site to block the access of other molecules such as transcription factors and down-regulate or turn off the expression of a gene (Gilbert et al., 2013). Where promoters are highly similar for a number of genes in a pathway, it may be possible to block expression of every gene in the pathway, or in a polyploid plant, to block expression of the homeologues

genes of all the subgenomes if they contain short stretches of very high homology (Endo et al., 2015). Similarly, a CRISPR/Cas9 complex can retain transcriptional activators close to a site (Konermann et al., 2015), or hold a specific repair enzyme at the site, causing site-specific mutation without cleaving the DNA backbone (Nishida et al., 2016). Further bacterial genera have comparable genes to Cas9, some of which are now being analyzed and developed (Zetsche et al., 2015; Steinert et al., 2014), and further advances are expected to arise from all the variations on this theme (Schaeffer & Nakata, 2015).

Studying gene expression, function and regulation in plants through bioinformatics and microarray analysis often necessitated the use of transgenic approaches (GM) for gene function verification (Abdeeva et al., 2013). However, recent advances in the CRISPR/Cas9 technology has shown that functional genomics studies involving gene function interrogation can be carried out in a far superior and easier manner, including the large number of genes which are transcribed but not translated (Konermann et al., 2015).

The applied versatility of GenEd technology also extends to an area of high significance in agriculture, disease control. We have monocultures, or next to monocultures, for many crops. Even where many varieties are grown they may descend from a rather small pool of founder lines. The large areas of monoculture bring about large opportunities for the disease organism that mutates to a form that can infect it.

Plants, however, carry many specific disease-resistance genes which can mutate or recombine into new forms which are resistant to newly-mutated diseases. In many instances resistant and susceptible alleles of a gene are only different by a few bp (Bryan et al., 2000). If such genes have been sequenced and resistance alleles are known, even in different species, GenEd can be used to mutate the susceptible forms to resistance directly rather than by a series of crosses and back crosses. GenEd can also be used to develop mutants in polyploid plants where mutants are often difficult to isolate, particularly in recent polyploids where all homeologues of a gene may be

expressed. Powdery mildew of wheat (*Blumeria graminis* f. sp. *tritici*), for instance, is difficult to find resistance to. Natural recessive mutations in the *MLO* gene, conferring a high level of persistent disease resistance, had previously been obtained in the diploid barley (Piffanelli et al., 2004), but not in wheat. Using the TALENS GenEd system, this was accomplished by mutating all 3 homeologous alleles of the wheat *MLO* locus which encodes a suppressor of a defensive response; the triply-mutated lines appeared to be resistant to the fungus (Wang et al., 2014). CRISPR/Cas9 was also used to mutate one of the alleles (Wang et al., 2014), and presumably the triple mutant could be obtained using this method. This demonstrates the potential of gene editing methods, provided there is adequate knowledge of the gene under investigation.

Similarly, CRISPR/Cas9 methods have shown excellent promise for suppressing viral infections, for many of which there is no good method of control. However, because pathogenic viruses replicate their genomes using the machinery within the plant cell, they are susceptible to an introduced Cas9 nuclease which recognizes their sequences and specifically destroys them, in a manner parallel to its original function in bacteria. For example, Gemini viruses have some highly conserved sequences which have been targeted using CRISPR/Cas9 to good effect (Ali et al., 2016). As well, some Cas-like nucleases (e.g. from *Francisella novicida*) act on RNA, rather than on DNA, offering the possibility of deleting key sequences of RNA viruses when they infect and begin to replicate in a plant cell (Fondong et al., 2016). In these cases, however, it would be necessary for the plant to carry the nuclease and guide sequences in its genome, that is, to be a GM plant prepared to commit GenEd.

For the purpose of creating a mutation in a plant using CRISPR or a comparable system, it is essential to have a reasonable knowledge of the target gene. For getting the mutation apparatus into the cell, the components of the system whether carried on a vector or assembled *in vitro* can be introduced by methods similar to the established biolistic and *Agrobacterium tumefaciens* protocols. In some cases, the large

Cas9 protein and synthetic RNA can be complexed *in vitro* and used to transform protoplasts by a polyethylene glycol or other carrier (Woo et al., 2015). Generally, continued activity of the nuclease in the plant genome is not wanted. However, as the cells divide the gene editing activity will be diluted out. In the case of *Agrobacterium*-inserted genes, they will segregate away from the site of the nuclease activity, to which they are usually not linked.

GM and GenEd Crops – Biological and Political Challenges

The first-generation GM crops which have been released have been widely successful. Yields increased and fields became cleaner, and losses to the target insect pests decreased greatly. However, several weeds have now mutated to be resistant to glyphosate or glufosinate (Beckie, 2013), while some insects have mutated to be Bt resistant, although efforts were made to limit these effects, which were foreseen and planned against (Tabashnik et al., 2013). Such efforts had limited success because the GM crops were too well embraced by growers, who did not all maintain the reserve areas and other strategies recommended to slow the appearance of weed and insect resistance (Beckie, 2013; Tabashnik et al., 2013). Such problems, however, are not limited to GM crops. For example, the use of conventional mutant ALS crops has led to widespread development of weeds resistant to Group 2 resistant herbicides without any transgenes being involved (Beckie, 2013). Therefore, where variables such as pest or weed resistance are concerned, this should not be considered as a technological flaw as no technology can offer guarantees against such challenges. GM or GenEd crops do not provide perfect nor permanent solutions to the weed and pest pressure, at least in the near-monoculture form in which GM crops have been used to date. Rotations and periodic returns to tillage may need to be widely adopted.

While GenEd is much more precise than GM, sometimes the GM approach is needed to facilitate dealing with a particular crop

problem. An example is the capacity to destroy viruses discussed above (Wang et al., 2014; Ali et al., 2016). Therefore, scientists need to continue to make a rational, cogent case for GM.

In addition to weed and pest adaptation challenges, other unexpected side effects may also arise. However, these are not confined to crops produced by transgenic methodologies alone. Because each of the techniques discussed above – conventional breeding, mutation breeding and GM and GenEd technologies – is based on genetic alterations of the original genotype, the chances of unintended and possibly harmful side effects arising from any of these techniques are comparable. Of note is the example of potato lines, obtained by conventional breeding, which were found to produce hazardous levels of the harmful steroidal alkaloid demissidine, a toxin not found in either parent, *Solanum tuberosum* and *Solanum brevidens* (Laurila et al., 1996). In wheat and related cereals, gluten constitutes a family of protein allergens which affect individuals with an inability to digest these proteins, leading to celiac disease, involving serious intestinal inflammation and, perhaps, non-celiac wheat intolerance (Czaja-Bulsa, 2015). Some evidence supports a shift to more frequently toxic gluten epitopes in modern European wheat cultivars relative to landraces, and a clear reduction of gluten epitope diversity (van den Broeck et al., 2010). Therefore, it is rather irrational to consider that genetic changes produced by GM and, perhaps, GenEd technologies as more hazardous than those produced by conventional breeding or mutation breeding, which may also lead to unintended gene combinations or mutations. In all cases, multiple cycles of field testing and analysis eliminate most harmful or less than efficacious effects; however, the products of classical breeding undergo less extreme scrutiny than GM crops. If the standards applied to GM crops were applied with equal rigour to the results of classical plant breeding, few new cultivars would survive the process.

The difficulties which have been put in the way of transgenic crop development have, of course, slowed its transition from lab to farmers' fields. Universities and public bodies have

generated extensive knowledge of plant biology using GM methods and have developed many useful approaches to biotic and abiotic stress resistance, among others. Often their practical application has been limited not by the biology, but by the timidity of funders, IP costs, the cost of carrying out GM trials, the difficulty of getting public/private partnerships in place and the difficulty of getting public acceptance of very reasonable GM possibilities over the noise of alarmists. In Canada, for example, some federal research bodies and funding agencies stopped GM research for those reasons. Presumably other similar institutions and many small companies with good knowledge of seed production have likewise been stopped. As a result, the field is left to those few who can afford it – a very small number of very large corporations. This means that research for the public good is very disadvantaged in comparison to the profit motive, and that most released GM crop varieties have been tied to herbicides or Bt, with restrictions on use of saved seed. Numerous other improvements were also accomplished by GM approaches but never released or allowed to be pursued due to the continuing political opposition to the production of food from GM sources (Schmidt, 2005).

This is so even in North America, which has developed largely science-based categorizations which consider how the plant differs from the former plant. In Europe a new line is assessed scientifically, but its release is then conditional on political factors, at which point many lines have been blocked for several years (Smyth & Phillips, 2014). Even for the largest companies, the difficulties put in the way of GM crops have been formidable. As a consequence, BASF closed its European GM crop development in 2012 (Cookson & Bryant, 2012). Other companies have stopped developing transgenic crops for this market, at a high cost to the EU (Raybould & Poppy, 2012), because conventional methods, with more cultivation and larger amounts of pesticides and herbicides, continue to be used in Europe. These are regarded as triumphs by anti-GMO activists.

Other countries, those which export crops to Europe in particular, are necessarily affected by European regulations on the one hand and

attitudes on the other. Anti-GM sentiment in Europe necessarily influences regulatory decisions in other nations if they would lose their European markets as a consequence. This attitude makes decisions more complex for growers in exporting countries.

Regulatory Decisions on GenEd Crops

Regulatory decisions to date regarding GenEd crops have generally followed the standards set for mutation breeding, i.e. product-based categorization. For example, GenEd mushrooms (a fungus, not a plant) developed with reduced tendency to brown (through CRISPR-based mutation of a polyphenol oxidase gene) were judged not to require GM regulation (Waltz, 2016b), and the USDA has also judged that plants produced by this technology will not be considered as GM (Waltz, 2016a). In Canada, GenEd organisms will fall under the present, plants expressing novel traits (PNT), regulations, i.e., phenotype or product-based (Wolt et al., 2016).

The EU directives as presently written cover those organisms which could not be developed by natural means, which should mean that GenEd organisms which do not add any new sequence from other species should be non-regulated (Hartung & Schiemann, 2014), since the same mutation could occur naturally although at low frequency. However, the EU regulations also are process-based, and anti-GMO forces are presently attempting to have all GenEd lines classed with GM lines, to be considered as a result of an unnatural process. This position if carried to its logical extreme would also exclude the use of all varieties with any history of radiation or chemical mutagenesis, meaning that it would reduce the increase in crop yields of the last century by about half. The regulatory status of GenEd plants in Europe remains uncertain, following many delays in committee decisions. France has recently requested a decision on the matter from the European Court of Justice (Byrne, 2016).

With a view to maintaining and increasing crop yields and reducing losses, it must be hoped that GenEd crop varieties, when they appear, will be treated as mutation events unless they actually do incorporate foreign

DNA. There appears to be no scientific reason for them to be treated otherwise.

GenEd being treated as equivalent to conventional mutagenesis will have several advantages, one of them being that lower development and approval costs will permit decentralization of the present situation in which a small number of companies control nearly all the GM crop landscape. Such decentralization is only viable provided these companies do not gain sole license to all the patents being pursued in the GenEd crop area. Such a renewal of opportunity will encourage development of improved varieties of a greater number of species, permitting increased yield and reduced losses to common plant diseases and stress conditions. It will allow useful variations to be developed which are not profitable to a company past the first seed sale, and will encourage the development of locally adapted varieties, or precise and useful changes in locally preferred varieties.

Genome Editing of Crops – A Science Perspective

While much of the purpose and philosophy behind the development of the GM-crops technology over the past 40 years is indistinguishable from those that instigated the evolution of the GenEd technology today, which is to improve agriculture and serve humanity, the latter differs in that it is performed with higher precision and increased efficiency. For the most part it does not constitute incorporation of foreign transgenes into edible crops. Rather, the technology offers a superior alternative to conventional breeding by random mutagenesis, which is often afflicted by non-specific mutations that can go undetected for generations. However, despite the fact that the GenEd approach aims to eliminate many of the concerns and uncertainties that victimized the GM technology in the past, it should be emphasized that both GM and GenEd technologies should remain somewhat complementary. By avoiding the objectionable shortcomings believed in some cases to have been associated with the GM methodologies, such as transgene integration at random sites in the plant chromosome or the inability to remove undesired excess genetic material such as

remnants of plasmids, it is anticipated that with time and effective dissemination of information, the new technology will find public support and earn the trust of consumers worldwide, as well as revive the crippled momentum and focus on developing acceptable ways for better agriculture. However, such optimism should be conveyed with caution, especially in view of the negative mindset currently in place in the public mind toward GM crops, created by anti-GM activist groups. It is possible that, with this mindset, the public may not hail GenEd crops as a readily acceptable alternative, at least initially. This represents a sizable challenge for scientists while trying to contradict the negative campaigns and recapture public trust. If it is expected of science to find solutions to the ever growing problems of food scarcity and malnutrition in the world, while keeping up with the increasing demands for safe and more efficient agriculture, particularly in the face of the rapid changes in climate and Earth's population, it is imperative to allow science the necessary latitude to develop such solutions.

Also, it is the responsibility of scientists to keep a constant flow of information in a manner that would promote positive societal understanding of the goals and means of science, while providing a clear account of risks vs. benefits as well as emphasizing the risks of opportunities lost. Such interaction with society contributes to guard against the spread of misinformation.

Genome Editing of Crops – Societal Perspective

Plant breeders and seed companies have forfeited a degree of public trust by the development of some plants, particularly market vegetables and fruits, which are selected much more for shipping quality than for flavor; the “cardboard” strawberry, the flavorless plum, the bouncing tomato, have all been the subject of exasperation and mockery by consumers who are acquainted with better tasting varieties. The drop in flavor is obvious but the savings incurred in better shipping and longer keeping varieties are largely invisible. Although these varieties have been produced via conventional

mutagenesis and selection, unfortunately some parts of the public have misconceived that they were produced through transgenic approaches. This points to the potential danger of GenEd crops being erroneously judged if this misconception is left uncorrected, and further reinforces the need for keeping the public well informed.

While GM lines have been opposed because of a false confusion with shipping-oriented plant breeding, there is also genuine resentment of the licensing controls imposed by large companies to maximize profits. Perhaps respective governments should exercise more control, through their legislations and regulation policies, over the establishment of reasonable licensing rules (Leyser, 2014).

Thus, learning from the GM experience, the GenEd approach, although superior and much more precise, is likely to face similar challenges depending on how governments perceive the technology and whether they afford it fair regulatory consideration. This will ultimately contribute to shaping the public perception of the technology. To date public discussion concerning societal acceptance of the new technology has barely begun. An increase in informed opinion should be of benefit to such acceptance. This can only result if extensive public information is made available.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ORCID

Heather Ray  <http://orcid.org/0000-0002-2911-5886>

REFERENCES

Abdallah NA, Prakash CS, McHughen AG. Genome editing for crop improvement: Challenges and opportunities. *GM Crops Food* 2015; 6(4):183-205; PMID:26930114; <http://dx.doi.org/10.1080/21645698.2015.1129937>

- Abdeeva I, Abdeev R, Bruskin S Piruzian E. Transgenic Plants as a Tool for Plant Functional Genomics. In: Çiftçi YO, ed. *Transgenic Plants - Advances and Limitations*. pp 259-284 INTECH 2013; 259-284.
- Ali Z, Ali S, Tashkandi M, Zaidi SS, Mahfouz MM. CRISPR/Cas9-mediated immunity to geminiviruses: differential interference and evasion. *Sci Rep* 2016; 6:2691; <http://dx.doi.org/10.1038/srep26912>
- Beckie HJ. Herbicide-Resistant (HR) Crops in Canada: HR Gene Effects on Yield Performance. *Prairie Soils Crops J* 2013; 6:33-39.
- Bortesi L, Fischer R. The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol Adv* 2015; 33(1):41-52; <http://dx.doi.org/10.1016/j.biotechadv.2014.12.006>
- Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP, McAdams SA, Faulk KN, Donaldson GK, Tarchini R, Valent B. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi-ta. *Plant Cell* 2000; 12(11):2033-2045; PMID:11090207; <http://dx.doi.org/10.1105/tpc.12.11.2033>
- Byrne J. France asks ECJ to decide if plants from new breeding techniques are GMO. 14-Oct-2016. <http://www.feednavigator.com/Regulation/France-asks-ECJ-to-decide-if-plants-from-new-breeding-techniques-are-GMOs>.
- Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. *Science* 2013; 339(6121):819-823; PMID:23287718; <http://dx.doi.org/10.1126/science.1231143>
- Cookson C, Bryant C, Chaffin J. An end to GM crop development for Europe. *Financial Times*. 16 January 2016.
- Czaja-Bulsa G. Non coeliac gluten sensitivity-A new disease with gluten intolerance. *Clin Nutri* 2015; 34(2):189-94; PMID:25245857; <http://dx.doi.org/10.1016/j.clnu.2014.08.012>
- Diaz-Gomez J, Marin S, Capell T, Sanchis V, Ramos AJ. The impact of *Bacillus thuringiensis* technology on the occurrence of fumonisins and other mycotoxins in maize. *World Mycotoxin J* 2016; 9(3):475-486; <http://dx.doi.org/10.3920/WMJ2015.1960>
- Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science* 2014; 346(6213):1258096; PMID:25430774; <http://dx.doi.org/10.1126/science.1258096>
- Endo M, Mikami M, Toki S. Multi-gene knockout utilizing off-target mutations of the CRISPR/Cas9 system in rice. *Plant Cell Physiol* 2015; 56(1):41-47; PMID:25392068; <http://dx.doi.org/10.1093/pcp/pcu154>
- Fan D, Liu T, Li C, Jiao B, Li S, Hou Y, Luo K. Efficient CRISPR/Cas9-mediated Targeted Mutagenesis in *Populus* in the First Generation. *Scientific Reports* 2015; 5:12217; <http://dx.doi.org/10.1038/srep12217>
- Feng Z, Zhang B, Ding W, Liu X, Yang DL, Wei P, Cao F, Zhu S, Zhang F, Mao Y, Zhu JK. Efficient genome editing in plants using a CRISPR/Cas system. *Cell Res* 2013; 23:1229-1232; PMID:23958582; <http://dx.doi.org/10.1038/cr.2013.114>
- Fondong VN, Nagalakshmi U, Dinesh-Kumar SP. Novel functional genomics approaches: a promising future in the combat against plant viruses. *Phytopathol* 2016; 106(10):1231-1239; PHYTO-03; <http://dx.doi.org/10.1094/PHYTO-03-16-0145-FI>
- Gao SQ, Chen M, Xu ZS, Zhao CP, Li L, Xu HJ, Tang YM, Zhao X, Ma YZ. The soybean GmbZIP1 transcription factor enhances multiple abiotic stress tolerances in transgenic plants. *Plant Mol Biol* 2011; 75(6):537-53; PMID:21331631; <http://dx.doi.org/10.1007/s11103-011-9738-4>
- Gatehouse AM, Ferry N, Raemaekers RJ. The case of the monarch butterfly: a verdict is returned. *Trends Genet* 2002; 18(5):249-51; PMID:12047949; [http://dx.doi.org/10.1016/S0168-9525\(02\)02664-1](http://dx.doi.org/10.1016/S0168-9525(02)02664-1)
- Georges F, Das S, Ray H, Bock C, Nokhrina K, Kolla VA, Keller W. Over expression of *Brassica napus* phosphatidylinositol-phospholipase C2 in canola induces significant changes in gene expression and phytohormone distribution patterns, enhances drought tolerance and promotes early flowering and maturation. *Plant Cell Environment* 2009; 32(12):1664-1681; <http://dx.doi.org/10.1111/j.1365-3040.2009.02027.x>
- Gewin V. Genetically Modified Corn— Environmental Benefits and Risks. *PLoS Biol* 2003; 1(1):e8; PMID:14551906; <http://dx.doi.org/10.1371/journal.pbio.0000008>
- Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, Lim WA. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* 2013; 154:442-451; PMID:23849981; <http://dx.doi.org/10.1016/j.cell.2013.06.044>
- Hartung F, Schiemann J. Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. *Plant J* 2014; 78:742-752; PMID:24330272; <http://dx.doi.org/10.1111/tbj.12413>
- Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 2014; 157(6):1262-1278; PMID:24906146; <http://dx.doi.org/10.1016/j.cell.2014.05.010>
- Jiang W, Zhou H, Honghao B, Fromm M, Yang B, Weeks DP. Demonstration of CRISPR/Cas9/gRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic Acids Res* 2013; 41(20):e188; PMID:23999092; <http://dx.doi.org/10.1093/nar/gkt780>
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012; 337(6096):816-821; PMID:22745249; <http://dx.doi.org/10.1126/science.1225829>
- Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O. Genome-scale transcriptional

- activation by an engineered CRISPR-Cas9 complex. *Nature* 2015; 517:583-600; PMID:25494202; <http://dx.doi.org/10.1038/nature14136>
- Laurila J, Laakso I, Valkonen JPT, Hiltunen R, Pehu E. Formation of parental type and novel alkaloids in somatic hybrids between *Solanum brevidens* and *S. tuberosum*. *Plant Sci* 1996; 118:145-155; [http://dx.doi.org/10.1016/0168-9452\(96\)04435-4](http://dx.doi.org/10.1016/0168-9452(96)04435-4)
- Lawrenson T, Shorinola O, Stacey N, Li C, Østergaard L, Patron N, Uauy C, Harwood W. Induction of targeted, heritable mutations in barley and *Brassica oleracea* using RNA-guided Cas9 nuclease. *Genome Biol* 2015; 16(1):1; PMID:25583448; <http://dx.doi.org/10.1186/s13059-015-0826-7>
- Leyser O. Moving beyond the GM Debate. *PLoS Biol* 2014; 12(6):e1001887; PMID:24914954; <http://dx.doi.org/10.1371/journal.pbio.1001887>
- Li JF, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J. Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nat Biotechnol* 2013; 31:688-691; <http://dx.doi.org/10.1038/nbt.2654>
- Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, Huang L, Ward RT, Clifton E, Falco SC, Cigan AM. Cas9-guide RNA directed genome editing in soybean. *Plant Physiol* 2015; 169(2):960-970; PMID:26294043; <http://dx.doi.org/10.1104/pp.15.00783>
- Nishida K, Arazoe T, Yachie N, Banno S, Kakimoto M, Tabata M, Mochizuki M, Miyabe A, Araki M, Hara KY, Shimatani Z, Kondo A. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science* 2016; 353(6305):aaf8729; PMID:27492474; <http://dx.doi.org/10.1126/science.aaf8729>
- Piffanelli P, Ramsay L, Waugh R, Benabdelmouna A, D'Hont A, Hollricher K, Jørgensen JH, Schulze-Lefert P, Panstruga R. A barley cultivation-associated polymorphism conveys resistance to powdery mildew. *Nature* 2004; 430(7002):887-891; PMID:15318221; <http://dx.doi.org/10.1038/nature02781>
- Puchta H, Fauser F. Synthetic nucleases for genome engineering in plants: prospects for a bright future. *Plant J* 2014; 78(5):727-741; PMID:24112784; <http://dx.doi.org/10.1111/tpj.12338>
- Que Q, Chilton MDM, de Fontes CM, He C, Nuccio M, Zhu T, Wu Y, Chen JS, Shi L. Trait stacking in transgenic crops: challenges and opportunities. *GM Crops* 2010; 1(4):220-229; PMID:21844677; <http://dx.doi.org/10.4161/gmcr.1.4.13439>
- Raybould A. Poppy GM. Commercializing genetically modified crops under EU regulations: objectives and barriers. *GM Crops Food* 2012; 3(1):9-20; PMID:22430852; <http://dx.doi.org/10.4161/gmcr.18961>
- Roscoe TJ, Lessire R, Puyaubert J, Renard M, Delseny M. Mutations in the fatty acid elongation 1 gene are associated with a loss of β -ketoacyl-CoA synthase activity in low erucic acid rapeseed. *FEBS Letters* 2001; 492(1):107-111; PMID:11248246; [http://dx.doi.org/10.1016/S0014-5793\(01\)02243-8](http://dx.doi.org/10.1016/S0014-5793(01)02243-8)
- Schaeffer SM, Nakata PA. CRISPR/Cas9-mediated genome editing and gene replacement in plants: transitioning from lab to field. *Plant Sci* 2015; 240:130-142; PMID:26475194; <http://dx.doi.org/10.1016/j.plantsci.2015.09.011>
- Schiml S, Fauser F, Puchta H. The CRISPR/Cas system can be used as nuclease for in planta gene targeting and as paired nickases for directed mutagenesis in *Arabidopsis* resulting in heritable progeny. *Plant J* 2014; 80(6):1139-1150; PMID:25327456; <http://dx.doi.org/10.1111/tpj.12704>
- Schmidt CW. Genetically modified foods: breeding uncertainty. *Environ Health Perspect* 2005; 113:A526-A533; PMID:16079054; <http://dx.doi.org/10.1289/ehp.113-a526>
- Smyth SJ, Phillips PW. Risk, regulation and biotechnology: the case of GM crops. *GM Crops Food* 2014; 5(3):170-177; PMID:25437235; <http://dx.doi.org/10.4161/21645698.2014.945880>
- Steinert J, Schiml S, Fauser F, Puchta H. Highly efficient heritable plant genome engineering using Cas9 orthologues from *Streptococcus thermophilus* and *Staphylococcus aureus*. *Plant J* 2014; 84:1295-1305; <http://dx.doi.org/10.1111/tpj.13078>
- Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM. Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. *Plant Physiol* 2015; 169(2):931-945; PMID:26269544; <http://dx.doi.org/10.1104/pp.15.00793>
- Tabashnik BE. Communal Benefits of Transgenic Corn. *Science* 2010; 330(6001):189-190; PMID:20929767; <http://dx.doi.org/10.1126/science.1196864>
- Tabashnik BE, Brévault T, Carrière Y. Insect resistance to Bt crops: lessons from the first billion acres. *Nat Biotechnol* 2013; 31(6):510-521; <http://dx.doi.org/10.1038/nbt.2597>
- Tan S, Evans RR, Dahmer ML, Singh BK, Shaner DL. Imidazolinone-tolerant crops: history, current status and future. *Pest Management Sci* 2005; 61(3):246-257; <http://dx.doi.org/10.1002/ps.993>
- van den Broeck HC, de Jong HC, Salentijn EMJ, Dekking L, Bosch D, Hamer RJ, Gilissen LJWJ, van der Meer IM, Smulders MJM. Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: wheat breeding may have contributed to increased prevalence of celiac disease. *Theor Appl Genet* 2010; 121:1527-1539; PMID:20664999; <http://dx.doi.org/10.1007/s00122-010-1408-4>
- Waltz E. CRISPR-edited crops free to enter market, skip regulation. *Nat Biotechnol* 2016a; 34:582; <http://dx.doi.org/10.1038/nbt0616-582>

- Waltz E. Gene-edited CRISPR mushroom escapes US regulation. *Nature* 2016b; 532:293; PMID:27111611; <http://dx.doi.org/10.1038/nature.2016.19754>
- Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL. Simultaneous editing of 3 homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat Biotechnol* 2014; 32(9):947-951; <http://dx.doi.org/10.1038/nbt.2969>
- Wolt JD, Wang K, Yang B. The Regulatory Status of Genome-edited Crops. *Plant Biotechnol J* 2016; 14:510-518; PMID:26251102; <http://dx.doi.org/10.1111/pbi.12444>
- Woo JW, Kim J, Kwon SI, Corvalán C, Cho SW, Kim H, Kim SG, Kim ST, Choe S, Kim JS. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nat Biotechnol* 2015; 33:1162-1164; <http://dx.doi.org/10.1038/nbt.3389>
- Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* 2015; 163(3):759-771; PMID:26422227; <http://dx.doi.org/10.1016/j.cell.2015.09.038>