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Consumer acceptance of food crops developed by genome editing

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Abstract:

One of the major problems regarding consumer acceptance of genetically modified organisms (GMOs) is the possibility that their transgenes could have adverse effects on the environment and/or human health. Genome editing, represented by the CRISPR/Cas9 system, can efficiently achieve transgene-free gene modifications and is anticipated to generate a wide spectrum of plants. However, the public attitude against GMOs suggests that people will initially be unlikely to accept these plants. We herein explored the bottlenecks of consumer acceptance of transgene-free food crops developed by genome editing and made some recommendations. People should not pursue a zero-risk bias regarding such crops. Developers are encouraged to produce cultivars with a trait that would satisfy consumer needs. Moreover, they should carefully investigate off-target mutations in resultant plants and initially refrain from agricultural use of multiplex genome editing for better risk-benefit communication. The government must consider their regulatory status and establish appropriate regulations if necessary. The government also should foster communication between the public and developers. If people are informed of the benefits of genome editing-mediated plant breeding and trust in the relevant regulations, and if careful risk-benefit communication and sincere considerations for the right to know approach are guaranteed, then such transgene-free crops could gradually be integrated into society.

(205 words)

Keywords:

Genome editing, crop, food, GMO, consumer, CRISPR/Cas9

Public attitude to GMOs and plant breeding by genome editing

Since the 1990s, commercial cultivation of genetically modified (GM) food crops, such as soybeans, corn and cotton, has expanded in some countries, particularly the USA, Brazil, Argentina, India, Canada and China (Brookes and Barfoot 2012). However, even in such permissive countries, not all people accept food products containing GM crops (Li, et al. 2015; Lucht 2015; Wunderlich and Gatto 2015). Additionally, no GM food crop has been commercially cultivated in many countries, including most of the EU countries (except in Spain, Portugal, Czech Republic, Slovakia and Romania) (Lucht 2015), New Zealand (The_US_Library_of_Congress 2014), and Japan (The_US_Library_of_Congress 2014). The negative attitude toward genetically modified organisms (GMOs) is associated with insufficient knowledge of GMOs, the lack of trust in developers and/or relevant regulations, poor risk-benefit communication, and ethical values (Lucht 2015; Siegrist 1999; Siegrist, et al. 2012; Tanaka 2004; Wunderlich and Gatto 2015; Zilberman, et al. 2013). These factors should be carefully considered if one believes that genetic engineering can address food security issues by breeding crops with a new trait, such as improved nutrition value, higher yields, pest and disease resistance and increased tolerance to environmental changes including higher or lower temperatures and drought. Moreover, plant breeding by genetic engineering might also contribute to a richer dietary life.

Genome-editing technology, such as zinc-finger nucleases (ZFNs) (Klug 2010), transcription activator-like effector nucleases (TALENs) (Joung and Sander 2013) and the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas system (Hsu, et al. 2014), can induce DNA double-strand breaks (DSBs) at target sites, and subsequently attain various types of genetic modification, thus potentially providing a myriad of agricultural benefits as one of the new plant breeding techniques (NPBTs) (Araki and Ishii 2015; Belhaj, et al. 2015; Hartung and Schiemann 2014; Nagamangala Kanchiswamy, et al. 2015; Voytas and Gao 2014; Weeks, et al. 2015; Wolt, et al. 2015). Some reviews have indicated that non-homologous end-joining (NHEJ) will be preferred in plant genome editing because the resultant plants are considered to contain no transgenes, which is one of the major concerns surrounding GM crops (Araki and Ishii 2015; Hartung and Schiemann 2014; Nagamangala Kanchiswamy, et al. 2015; Nagamangala Kanchiswamy, et al. 2015; Voytas and Gao 2014). Nonetheless, consumer acceptance of genome edited crops is not optimistic at present (Center_for_Food_Safety 2015; GM_Freeze 2016; GMWATCH 2014; Green_Peace 2015; IFOAM_EU 2015). The transgene-free crops appear to be, without sufficient explanation, similar to conventional GM crops because both methods appear to merely use genetic modification technology to alter crops. Can the public understand differences among such breeding techniques? Moreover, can all concerned carefully weigh the risks and benefits of each crop variety? Furthermore, how should various public views be considered when introducing such crops in the market? In this article, the bottlenecks of consumer acceptance of transgene-free crops developed via genome editing were explored. Moreover, the likelihood of social integration of such food crops was discussed.

Informing people about different breeding techniques

Various mutagenesis techniques have been used for decades in plant breeding and some of them are well-accepted practices in plant breeding. Reviews on the public perception of GMOs suggest that people must be informed of the technical differences between older breeding techniques and genome editing (Lucht 2015; Siegrist 1999; Wunderlich and Gatto 2015).

A classical plant breeding technique, chemical or radiation mutagenesis, results in entirely random mutations. Screening a plant of interest among a tremendous number of randomly modified plants proceeds primarily based on the phenotype, using traditional breeding selection techniques; thus, one decade or more is required to provide a new crop variety (European_Seed_Association 2015). Through random mutagenesis, at least 2543 cultivars in 175 plant species, including *Oryza sativa* (rice), *Triticum aestivum* (wheat), *Hordeum vulgare* （barley）, *Gossypium arboreum* (cotton), *Brassica napus* (rapeseed), *Helianthus annuus* (sunflower), *Citrus paradise* (grapefruit), *Malus pumila* (apple) and *Musa acuminate* (banana), have been developed worldwide and cultivated in Europe, Asia, North America, South America, and Australia thus far (Ahloowalia, et al. 2004). Thus, plants bred by random mutagenesis have now been worldwide accepted.

Newer breeding techniques, such as transgenesis, can more efficiently produce a cultivar with a new trait. The generation of a GM crop begins with extracellular DNA manipulation to construct a vector harboring a gene derived from an unrelated species or its relatives, or a specific DNA sequence that is intended for transfer. The plasmid construct is transferred into plant cells, such as protoplasts and callus cells, using *Agrobacterium*-mediated transformation, particle bombardment or polyethylene glycol. Modified cells are subsequently used to generate a GM plant. This breeding technique has provided cultivars with a trait such as herbicide or pest resistance for the developer or farmer’s benefits (Barfoot and Brookes 2014; Li, et al. 2015). However, the efficiency and specificity of the insertion of exogenous DNA in a plant genome has largely proven to be low. Thus, this technique requires several years to obtain a plant variety with an intentional trait.

Rather than extracellular DNA manipulation, genome editing proceeds as an intracellular genetic modification by introducing plasmid DNA harboring the nuclease gene via methods such as *Agrobacterium*-mediated transformation or directly introducing site-specific nucleases into plant cells. Editing the plant genome results in far more efficient gene modifications at target sites (Araki and Ishii 2015). In addition, genome editing is a versatile genetic engineering tool. It can disrupt an endogenous gene via the NHEJ pathway or copy a variant or add a transgene via the homology-directed repair (HDR) pathway. Of note, the CRISPR/Cas9 system is advantageous over the other two techniques, ZFNs and TALENs, regarding the ease of simultaneous editing at multiple sites across the genome (multiplex genome editing) (Cong, et al. 2013). Such features are anticipated to drastically facilitate rapid breeding based on plant genome information (European_Academies'_Science_Advisory_Council 2015) (European_Plant_Science_Organisation 2015; European_Seed_Association 2015). However, the nucleases could simultaneously create off-target DSBs at non-target sites. Despite an extremely low frequency, off-target DSBs could lead to indels of various lengths, including point mutations, in crop genomes (Shan, et al. 2013; Zhang, et al. 2014). There is a possibility that multiplex genome editing would increase off-target effects if improperly designed nucleases or guide RNAs (gRNAs, in the case of CRISPR/Cas9) are simultaneously introduced into plant cells. Although unintentional genetic changes may result in a silent mutation or loss of function, some could lead to a gain of function through a frameshift mutation, potentially affecting food safety or the environment (Araki, et al. 2014).

Gene addition via HDR is, in part, similar to transgenesis because it can add exogenous DNA at a target site via HDR. Moreover, NHEJ-mediated gene disruption is somewhat similar to random mutagenesis because it leaves only indels in the genome. However, genome editing differs from these techniques because it enables developers to rapidly perform crop breeding (less than one year) and readily produce a cultivar with multiple new traits. In addition, this breeding technique is marked by the unprecedented introduction of the artificially designed nucleases into plant cells. Despite such complicated features, it is essential to inform the public of the advantages and disadvantages of plant breeding by this robust genome engineering.

Risk-benefit communication

The major problems of risk-benefit communication for GM crops include the emphasis of the developer's and farmer's benefits, not the consumer's benefit, in addition to the consistent pursuit of a zero risk, and the unbalanced view of the risks and benefits (Davidson 2010; Lucht 2015; Siegrist 1999; Tanaka 2004; Zilberman, et al. 2013). However, GM crop developers have recently aimed to emphasize the consumer's benefit. For instance, *Arctic Apple* is a non-browning apple cultivar which is genetically engineered with a transgene that produces specific RNAs to silence the expression of at least four polyphenol oxidase (PPO) genes. RNA interference (RNAi) significantly reduces the expression level of PPOs, thus inhibiting the enzyme-mediated browning in the GM apple (Waltz 2015). *Innate Potatoe* is a GM potato (*Solanum tuberosum*) with two RNAi systems that lower the transcript levels for

Asn1 and *Ppo5,* subsequently limiting the formation of acrylamide precursor asparagine and black spot bruise (Waltz 2015). However, the mechanism of RNAi is not simple and might be difficult to understand for consumers.

Similarly, genome editing-mediated plant breeding would more efficiently produce consumer-directed crops (Table 1). Similarly to *Innate Potatoe*, potatoes were treated with TALENs to minimize the accumulation of reducing sugars during cold storage. As a result, a full *VInv*-knockout potato displayed no detectable reducing sugars in tubers, and processed chips with reduced levels of acrylamide were lightly colored, which benefitted both the consumers and farmers (Clasen, et al. 2015). A lipoxygenase3-deficient rice produced using TALENs is also expected to enhance storage tolerance (Ma, et al. 2015). A fragrant rice was generated by TALEN-mediated *OsBADH2* disruption (Shan, et al. 2015). In this rice, the content of a major fragrance component, 1-acetyl-1-pyrroline, increased from 0 to 0.35-0.75 mg/kg. By disrupting the soybean (*Glycine max*) *FAD2* with TALENs, the oil quality was improved, oleic acid increased from 20-80% and linoleic acid decreased from 50% to <4% (Haun, et al. 2014). It was also demonstrated that tomato (*Solanum lycopersicum*) fruit ripening can be regulated by CRISPR/Cas9-mediated *RIN* mutagenesis, potentially developing tomato varieties with a designed shelf life (Ito, et al. 2015). Focusing on the consumer's benefit might increase the social integration of crops produced via genome editing.

Regarding the food safety of GM crops, some rodent feeding experiments suggested tumor formation, poor development and early death in the animals upon consuming GM crops. These results sensationally emerged in mass media, with warnings that GM crops are unsafe for food consumption (Marshall 2007; Romeis, et al. 2013). However, accumulating evidence from other animal feeding experiments suggest that current GM crops in the marketplace are predominantly safe (EFSA_GMO_Panel_Working_Group_on_Animal_Feeding_Trials 2008), although some criticisms about scientific justification and methodology consistency of animal toxicity studies performed so far were raised (Bartholomaeus, et al. 2013; Zdziarski, et al. 2014). In contrast, it should also be noted that some food products which are not genetically modified may affect human health. For instance, potatoes naturally contain glycoalkaloids, such as α-chaconine and α-solanine, and display toxicity unless it is served without peeling (Friedman and Rasooly 2013). Thus, it is crucial to avoid a zero-risk bias and show the level of risk when considering the food safety of GM crops. Regarding the environmental risk of GM crops, there is serious concern about transgene flow from GM crops to its relatives and wild species, which potentially affects biological diversity. The occurrence of transgene flow in rapeseed, maize (*Zea mays*), cotton and creeping bentgrass has been reported from Canada, Japan, Mexico, Switzerland and the USA (Ryffel 2014). Previous discussions surrounding the food safety and environmental risk of GM crops underscore the need of careful risk communication on crop breeding by genome editing (GM_Freeze 2016; GMWATCH 2014; Green_Peace 2015; IFOAM_EU 2015).

Crop varieties developed via NHEJ appear to be attractive from consumer perspectives. The plant varieties could increase consumer acceptance due to the lack of transgenes, which the public is primarily concerned about (Nagamangala Kanchiswamy, et al. 2015; Voytas and Gao 2014). Moreover, such transgene-free plants will not cause transgene flow. However, plant breeding by genome editing requires further scrutiny. The unprecedented introduction of artificial nucleases could induce off-target DSBs and result in unwanted, off-target mutations in resultant plants. Although the off-target effect would be reduced if the nucleases are introduced in the form of a ribonucleoprotein, rather than plasmid DNA (Woo, et al. 2015), the risk of off-target mutations can be substantial, depending on the selection of the target site and the design of the targeting molecules (Shan, et al. 2013; Zhang, et al. 2014). Higher-fidelity CRISPR/Cas9 variants have been developed and some of them displayed no detectable genome-wide off-target effects (Kleinstiver, et al. 2016; Ran, et al. 2013; Zetsche, et al. 2015). Moreover, methodologies on genome-wide profiling of off-target effects are available to validate each nuclease or gRNA of the CRISPR/Cas9 system before generating genome edited organisms (Kim, et al. 2015; Tsai, et al. 2015). However, a recent review on plant genome editing suggests that most of the relevant reports have not evaluated the occurrence of off-target mutations in resultant crops

(Araki and Ishii 2015) (Table 1).

With regard to on-target gene disruption via NHEJ, the potential environmental implications also require scrutiny, in particular if the gene disruption confers herbicide or disease resistance (Butler, et al. 2015; Li, et al. 2012; Wang, et al. 2014; Zhou, et al. 2015). Notably, the cultivation of Clearfield rice with an *ALS* variant, which is a herbicide-resistant crop developed by a non-GM technique (Tan, et al. 2005), has led to the emergence of herbicide-resistant weeds by hybridization with wild species in Italy and the USA (Burgos, et al. 2014; Busconi, et al. 2012). This example underscores the importance of considering the potential environmental risks of a trait induced in genome edited crops, in addition to the ecological characteristics of the field surrounding the cultivated land. Moreover, genome edited crops with disease resistance (Li, et al. 2012; Wang, et al. 2014) could have ecological implications between plants and microorganisms in the long-term and wide-scale cultivation of such crops. This possibility is supported by the ecological issue associated with the large-scale cultivation of GM cotton in China. Due to the adoption of *Bacillus thuringiensis*-toxin cotton and the reduction of pesticide use, a minor pest has progressively acquired pest status in cotton production (Lu, et al. 2010).

Weighing the benefits and potential risks is crucial for determining the use of a new technology in society. However, when it comes to GM crops, such a viewpoint has been challenging since it is difficult to predict the potential environmental risk following large-scale cultivation, food safety following widespread consumption and economic impact on trade and industry. Even if the public is well informed of plant breeding by genome editing, a social decision requires careful discussions on the risk-benefit balance for each crop variety.

Trust in developers

One of the obstacles in the public acceptance of GMOs is the fact that many consumers receive information solely from the media, internet, and other sources (Lucht 2015; Wunderlich and Gatto 2015). However, public trust in developers also impacts the perception of the benefits and risks of GMOs, which may affect their acceptance (Lucht 2015; Siegrist 1999; Siegrist, et al. 2012; Tanaka 2004). Therefore, developers are required to carefully address off-target mutations as well as on-target modification in plant genome editing because it entails the introduction of artificially designed nucleases (Fig. 1).

As discussed above, most of the relevant crop reports have not addressed off-target mutations in resultant plants (Araki and Ishii 2015)(Table 1). Pauwels et al. assert that the use of next-generation sequencing is currently not always significant in the risk assessment of GM plants (Pauwels, et al. 2015). However, Huang et al. propose whole-genome resequencing for interrogating off-target mutations in the plant genome editing (Huang, et al. 2016). Indeed, Zhang et al. recently reported detailed data on CRISPR/Cas9-mediated mutagenesis at 11 genes in rice (Zhang, et al. 2014). In the rice gene editing, an off-target mutation occurred at only one putative off-target site in one of 11 target genes. Moreover, whole genome sequencing showed that the number of indels and single-nucleotide polymorphisms (SNPs) in genome edited rice is comparable with that of wild-type rice. However, detecting all off-target mutations across a plant genome appears to be challenging due to the unclear distinction between small off-target mutations and reading errors, SNPs or spontaneous mutations during cell culture. Moreover, there is currently no consensus regarding the means of assessing off-target effects or mutations in genome edited crops (Joung 2015). Conversely, if available, a risk assessment from bench-to-table via a field trial would avoid significant adverse effects on the environment and ensure the safety of a food ingredient, thus fostering public acceptance of plants developed via genome editing (Fig. 1). Whole exome sequencing, which analyzes all of the protein-coding regions (approximately 10% in the rice genome), might be an efficient and practical method because an off-target mutation in an exome is more likely to exert a serious influence on a protein function than in the remaining region (Hashmi, et al. 2015).

Trust in regulation

GM crops have remained the subject of risk assessments regarding the potential impact of exogenous DNA and/or relevant genetic modification on the environment and food safety prior to field trials and food consumption in many countries (Chen and Lin 2013; Davidson 2010; Li, et al. 2015). The Cartagena Protocol on Biosafety is an international agreement to ensure the safe handling, transport and use of living modified organisms (LMOs; the technical legal term that is closely related to GMO) generated by modern biotechnology (The_Convention_on_Biological_Diversity 2016). The number of ratifying countries is 170 as of January 2016, including China, EU, New Zealand and Japan, however, some countries such as Argentina, Australia, Canada and the USA, where GM crops are cultivated for commercial purpose, have not ratified the protocol. Another important issue is that different GMO regulations have been enacted worldwide. Some countries, including Argentina, Canada, Japan, India and the USA, regulate GM crops under product-based GMO regulations, whereas others, such as Australia, China, EU and New Zealand, regulate GM crops under process-based regulations (Araki and Ishii 2015; Araki, et al. 2014; Davidson 2010; Lucht 2015; Ramessar, et al. 2008). Such regulatory mosaicism regarding GM crops in global society are partially to blame for public distrust in the regulation of GM crops (Lucht 2015; Zilberman, et al. 2013). Some people question the appropriateness of the management of foreign trade of GM crops, while others wonder which regulation system, process-based or product-based, is superior.

Transgene-free plants produced via genome editing might bypass product-based GMO regulation, which is based on the existence of exogenous DNA such as transgenes in the final product (Araki and Ishii 2015; Araki, et al. 2014; Nagamangala Kanchiswamy, et al. 2015; Voytas and Gao 2014). Some groups advocate that such transgene-free crops should not be regulated (Camacho, et al. 2014), even under process-based GMO regulation (European_Academies'_Science_Advisory_Council 2015; European_Plant_Science_Organisation 2015; European_Seed_Association 2015; Hartung and Schiemann 2014).

We surveyed the regulatory status of food crops generated by NPBTs (Table 2). The US Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) regards plants resulted from intragenesis or cisgenesis as non-regulated if the introduced gene or gene element is not derived from “plant pest”. Importantly, the US authority considers plants modified by ZFNs and TALENs to be non-regulated if such crops are null segregants. Likewise, the world’s first regulation for NPBTs, which was issued by the Argentine government, indicates the possible determination that some products without a transgene may not fall under product-based GMO regulation, Resolution no.173/15 of the Secretariat of Agriculture, Livestock and Fisheries (Whelan and Lema 2015). If researchers use CRISPR/Cas9 in the form of a ribonucleoprotein (Woo, et al. 2015), then the resultant crop variety may be deregulated in the US and Argentina where the product-based GMO regulation is adopted. Moreover, a sulfonylurea-tolerant canola variety developed by one of NPBTs, oligonucleotide-directed mutagenesis has recently been approved for food use, livestock feed and unconfined release in Canada with the product-based GMO regulation (Table 2). Importantly, this canola was judged as “Non-LMO” (Living Modified Organism that is defined by the Cartagena Protocol on Biosafety) (Table 2).

In contrast, New Zealand, which employs process-based GMO regulation, has a widely different regulatory direction than Argentina. Following a significant lawsuit concerning a regulatory decision on plant gene editing via NHEJ, New Zealand is currently amending relevant regulation under the Hazardous Substances and New Organisms (HSNO) Act 1996 (The_New_Zealand_Environmental_Protection_Authority 2015). In 2013, in response to an application by a local developer in New Zealand, a three-person committee appointed by the Environmental Protection Authority (EPA) board decided not to regulate transgene-free organisms because the organisms modified via NHEJ are, despite conformity with the legal GMO definition, similar to older breeding techniques, including random mutagenesis (EPA advice Application APP201381). The three-person EPA committee was allowed to make a decision about whether an organism is a new organism under the regulation. However, prior to this decision-making, the EPA officials recommended that such techniques should be considered similar to GMO technique under process-based regulation. An independent research council, the Sustainability Council of New Zealand, considered that the decision by the EPA was made according to misinterpretation of the HSNO Act and appealed the decision in the High Court (Araki, et al. 2014). Consequently, the High Court overruled the EPA decision on the deregulation of such transgene-free organisms in 2014 (Araki and Ishii 2015; Kershen 2015).

We can learn some lessons from this debate. First, the meaning of the GMO law, as well as the legal GMO definition, should be carefully considered in early discussions on the regulation of genome editing and other NPBTs. Second, scientific evidence is required prior to the final decision on the regulatory position. Third, the entire course of the regulatory discussion should be conveyed by an appropriate deliberation body. Both expert and public opinions should be reflected. Most importantly, although regulatory differences have emerged between Argentina and New Zealand, all countries are required to consider their regulatory status and some need to establish appropriate regulations for NPBTs including genome editing, while harmonizing individual regulations with those of global society.

Genome edited crops and ethical values

Some countries require involuntary or mandatory GMO labelling with or without a tolerance level (1~5%) (Lucht 2015; Ramessar, et al. 2008). In these countries, some people would, regardless of the existence of transgenes, demand the traceability of genome edited crops in field trials, and wish to know which food products contain genome edited crops in order to avoid purchasing or consuming them due to ethical values (Center_for_Food_Safety 2015; GM_Freeze 2016; GMWATCH 2014; Green_Peace 2015; IFOAM_EU 2015). The European

Plant Science Organisation requests the European Commission to uncouple the question of environmental risk and safety assessment from the

question of labeling (European_Plant_Science_Organisation 2015). Rather, labelling should be considered in order to develop genome edited crops ethically in society. Indeed, although the first GM grapevine grafting trial was approved by the competent ministry, the field trial was repeatedly disturbed by activists in France (Table 2) (Lemaire, et al. 2010). In so doing, careful thoughts are required for deciding whether to label all the transgene-free crops and the food products.

If risk assessment suggests that such a crop has an implication in environmental risks, the test growing should be conducted in an isolated field so as to carefully evaluate the impact on the environment (Fig. 1). However, it would be difficult to make a clear distinction between such a transgene-free crop and related species in the field and/or market. The introduction of a DNA tag into the crop genome would help us to readily confirm them (Tsukaya 2013). Some would oppose labelling based on DNA tagging because it requires additional gene modification via HDR, would likely place modified plants under GMO regulations, impose a burden on the system for food processing and distribution, and ultimately result in higher costs (Kling 2014). Nonetheless, DNA tagging should be considered as an option when cultivating such crops in the field and introducing them into the market (Araki and Ishii 2015). The primary agricultural use of genetic engineering is to address food security issues and contribute to a richer dietary life, not to affect the environment or confuse global society.

Conversely, if environmental risk assessment concludes that a transgene-free crop has no implication in environmental risks, the plant requires neither isolated field test nor GMO labelling (Figure 1). We recently proposed a regulatory model for genome edited plants (Araki and Ishii 2015). In this model, crops resulting from NHEJ-mediated mutagenesis were categorized as plants with gain-of-function (GOF) mutations or those with leaky or null mutations. If phenotypic differences of the two subcategories are considered from environmental risk point of view, plants with a GOF mutation and plants with a leaky or null mutation may be included in the plants with an implication in environmental risks and the plants with no implication in environmental risks, respectively.

Therefore, we propose that genome edited crops should be, while investigating off-target mutations, carefully assessed from the viewpoint of a novel trait prior to field test. Others also address the importance of a new trait regarding the regulation of genome edited crops (European_Academies'_Science_Advisory_Council 2015; European_Plant_Science_Organisation 2015; Hartung and Schiemann 2014). We could reconsider such a policy when it comes to more severe situations associated with global climate change and increased populations.

Recommendations

According to the aforementioned analyses, the pros and cons of food crop breeding by GMO and genome editing technologies were summarized from the consumer perspective (Table 3). Taking this summary into account, we make the following recommendations for the future integration of genome edited crops into society. First, developers should sincerely inform people of the advantages, disadvantages and limits of different plant breeding techniques, including genome editing. This technical explanation should be based on the viewpoint of the consumer, rather than the developer or administrative official. Second, the government must consider their regulatory status regarding genome editing. If necessary, relevant regulations, as a form of social norm, must be established or amended in a manner that people can trust in the regulatory decision on genome editing-mediated plant breeding, while harmonizing relevant regulations in global society. Advanced genetic engineering requires advanced and appropriate regulation. Third, risk-benefit communication must be carefully performed for each crop variety, in consideration of the off-target effects of genome editing. In doing so, people should not pursue a zero-risk bias, although the degree of health and environmental risks must be properly addressed by developers and regulators. Developers are encouraged to develop cultivars with a trait which would be favorably viewed by consumers. Moreover, they should initially refrain from the agricultural use of multiplex genome editing, since this approach would make it difficult to perform risk-benefit communication in the early days of genome editing. The government should aid and foster communication among the public and developers. Finally, the right to know approach should be considered for those who have difficulty accepting such engineered plants. If all the recommendations are sincerely considered, then consumers could gradually accept a genome edited crop as a new cultivar in the future.

Author Contribution Statement

TI conceived and designed the analysis. TI and MA surveyed and analyzed relevant reports. TI wrote the manuscript. All authors read and approved the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Ahloowalia B, Maluszynski M, Nichterlein K (2004) Global impact of mutation-derived varieties. . Euphytica 135:187–204.

Araki M, Ishii T (2015) Towards social acceptance of plant breeding by genome editing. Trends Plant Sci 20:145-149

Araki M, Nojima K, Ishii T (2014) Caution required for handling genome editing technology. Trends in biotechnology 32:234-237

Barfoot P, Brookes G (2014) Key global environmental impacts of genetically modified (GM) crop use 1996-2012. GM crops & food 5:149-160

Bartholomaeus A, Parrott W, Bondy G, Walker K (2013) The use of whole food animal studies in the safety assessment of genetically modified crops: limitations and recommendations. Critical reviews in toxicology 43 Suppl 2:1-24

Belhaj K, Chaparro-Garcia A, Kamoun S, Patron NJ, Nekrasov V (2015) Editing plant genomes with CRISPR/Cas9. Current opinion in biotechnology 32:76-84

Brookes G, Barfoot P (2012) GM Crops: Global Socio-Economic and Environmental Impacts 1996–2010. PG Economics Ltd., UK

Burgos NR, Singh V, Tseng TM, Black H, Young ND, Huang Z, Hyma KE, Gealy DR, Caicedo AL (2014) The impact of herbicide-resistant rice technology on phenotypic diversity and population structure of United States weedy rice. Plant physiology 166:1208-1220

Busconi M, Rossi D, Lorenzoni C, Baldi G, Fogher C (2012) Spread of herbicide-resistant weedy rice (red rice, Oryza sativa L.) after 5 years of Clearfield rice cultivation in Italy. Plant biology (Stuttgart, Germany) 14:751-759

Butler NM, Atkins PA, Voytas DF, Douches DS (2015) Generation and Inheritance of Targeted Mutations in Potato (Solanum tuberosum L.) Using the CRISPR/Cas System. PloS one 10:e0144591

Camacho A, Van Deynze A, Chi-Ham C, Bennett AB (2014) Genetically engineered crops that fly under the US regulatory radar. Nature biotechnology 32:1087-1091

Center_for_Food_Safety (2015) Environmental, Farmer, and Consumer Groups Demand Higher Standards for Genetically Engineered (GE) Crop Regulations. http://wwwcenterforfoodsafetyorg/press-releases/3967/environmental-farmer-and-consumer-groups-demand-higher-standards-for-genetically-engineered-ge-crop-regulations# Access: February 19

Chen H, Lin Y (2013) Promise and issues of genetically modified crops. Current opinion in plant biology 16:255-260

Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, Tibebu R, Davison S, Ray EE, Daulhac A, Coffman A, Yabandith A, Retterath A, Haun W, Baltes NJ, Mathis L, Voytas DF, Zhang F (2015) Improving cold storage and processing traits in potato through targeted gene knockout. Plant biotechnology journal 14:169-176

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science (New York, NY) 339:819-823

Davidson J (2010) GM plants: Science, politics and EC regulations. Plant Science 178:94-98

EFSA_GMO_Panel_Working_Group_on_Animal_Feeding_Trials (2008) Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Food Chem Toxicol 46:S2-S70

European_Academies'_Science_Advisory_Council (2015) Statement: New breeding techniques. http://wwweasaceu/fileadmin/PDF_s/reports_statements/Easac_14_NBTpdf Access: March 7, 2016

European_Plant_Science_Organisation (2015) Statement: Crop Genetic Improvement Technologies.

http://wwwepsoweborg/file/2147 Access: March 7, 2016

European_Seed_Association (2015) Regulatory approaches to modern plant breeding - the case of mutagenesis and new gene editing technologies. https://wwweuroseedseu/system/files/publications/files/esa_150543_0pdf Access: March 7, 2015

Friedman M, Rasooly R (2013) Review of the inhibition of biological activities of food-related selected toxins by natural compounds. Toxins 5:743-775

GM_Freeze (2016) The case for regulating Gene Edited crops. http://wwwgmfreezeorg/news-releases/266/ Access: March 7, 2016

GMWATCH (2014) "Genome editing": GM by another name. http://wwwgmwatchorg/news/archive/2014/15546-genome-editing-gm-by-another-name Access: February 19, 2016

Green_Peace (2015) Policy briefing Gene-editing of plants – GM through the back door? http://wwwgreenpeaceorg/eu-unit/Global/eu-unit/reports-briefings/2015/Greenpeace_Gene-editing_30112015%20-%202pdf Access: March 4, 2016

Hartung F, Schiemann J (2014) Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. The Plant journal : for cell and molecular biology 78:742-752

Hashmi U, Shafqat S, Khan F, Majid M, Hussain H, Kazi AG, John R, Ahmad P (2015) Plant exomics: concepts, applications and methodologies in crop improvement. Plant signaling & behavior 10:e976152

Haun W, Coffman A, Clasen BM, Demorest ZL, Lowy A, Ray E, Retterath A, Stoddard T, Juillerat A, Cedrone F, Mathis L, Voytas DF, Zhang F (2014) Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant biotechnology journal 12:934-940

Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. Cell 157:1262-1278

Huang S, Weigel D, Beachy RN, Li J (2016) A proposed regulatory framework for genome-edited crops. 48:109-111

IFOAM_EU (2015) New Plant Breeding Techniques Position paper. http://wwwifoam-euorg/sites/default/files/ifoameu_policy_npbts_position_final_20151210pdf Access: March 04, 2016

Ito Y, Nishizawa-Yokoi A, Endo M, Mikami M, Toki S (2015) CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. Biochemical and biophysical research communications 467:76-82

Joung JK (2015) Unwanted mutations: Standards needed for gene-editing errors. Nature 523:158

Joung JK, Sander JD (2013) TALENs: a widely applicable technology for targeted genome editing. Nature reviews Molecular cell biology 14:49-55

Kershen DL (2015) Sustainability Council of New Zealand Trust v The Environmental Protection Authority: Gene Editing Technologies and the Law. GM crops & food doi: 10.1080/21645698.2015.1122859:

Kim D, Bae S, Park J, Kim E, Kim S, Yu HR, Hwang J, Kim JI, Kim JS (2015) Digenome-seq: genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. Nature methods 12:237-243, 231 p following 243

Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, Keith Joung J (2016) High-fidelity

CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature 529:490-495

Kling J (2014) Labeling for better or worse. Nat Biotech 32:1180-1183

Klug A (2010) The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annual review of biochemistry 79:213-231

Lemaire O, Moneyron A, Masson JE (2010) "Interactive technology assessment" and beyond: the field trial of genetically modified grapevines at INRA-Colmar. PLoS biology 8:e1000551

Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nature biotechnology 30:390-392

Li Y, Hallerman EM, Liu Q, Wu K, Peng Y (2015) The development and status of Bt rice in China. Plant biotechnology journal doi: 10.1111/pbi.12464:

Lu Y, Wu K, Jiang Y, Xia B, Li P, Feng H, Wyckhuys KA, Guo Y (2010) Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. Science (New York, NY) 328:1151-1154

Lucht JM (2015) Public Acceptance of Plant Biotechnology and GM Crops. Viruses 7:4254-4281

Ma L, Zhu F, Li Z, Zhang J, Li X, Dong J, Wang T (2015) TALEN-Based Mutagenesis of Lipoxygenase LOX3 Enhances the Storage Tolerance of Rice (Oryza sativa) Seeds. PloS one 10:e0143877

Marshall A (2007) GM soybeans and health safety--a controversy reexamined. Nature biotechnology 25:981-987

Nagamangala Kanchiswamy C, Malnoy M, Velasco R, Kim JS, Viola R (2015) Non-GMO genetically edited crop plants. Trends in biotechnology 33:489-491

Nagamangala Kanchiswamy C, Sargent DJ, Velasco R, Maffei ME, Malnoy M (2015) Looking forward to genetically edited fruit crops. Trends in biotechnology 33:62-64

Pauwels K, De Keersmaecker SCJ, De Schrijver A, du Jardin P, Roosens NHC, Herman P (2015) Next-generation sequencing as a tool for the molecular characterisation and risk assessment of genetically modified plants: Added value or not? Trends in Food Science & Technology 45:319-326

Ramessar K, Capell T, Twyman RM, Quemada H, Christou P (2008) Trace and traceability--a call for regulatory harmony. Nature biotechnology 26:975-978

Ran FA, Hsu PD, Lin CY, Gootenberg JS, Konermann S, Trevino AE, Scott DA, Inoue A, Matoba S, Zhang Y, Zhang F (2013) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell 154:1380-1389

Romeis J, McLean MA, Shelton AM (2013) When bad science makes good headlines: Bt maize and regulatory bans. Nature biotechnology 31:386-387

Ryffel GU (2014) Transgene flow: facts, speculations and possible countermeasures. GM crops & food 5:249-258

Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu JL, Gao C (2013) Targeted genome modification of crop plants using a CRISPR-Cas system. Nature biotechnology 31:686-688

Shan Q, Zhang Y, Chen K, Zhang K, Gao C (2015) Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology. Plant biotechnology journal 13:791-800

Siegrist M (1999) A Causal Model Explaining the Perception and Acceptance of Gene Technology. J Applied

Social Psychology 29:2093-2106

Siegrist M, Connor M, Keller C (2012) Trust, confidence, procedural fairness, outcome fairness, moral conviction, and the acceptance of GM field experiments. Risk analysis : an official publication of the Society for Risk Analysis 32:1394-1403

Tan S, Evans RR, Dahmer ML, Singh BK, Shaner DL (2005) Imidazolinone-tolerant crops: history, current status and future. Pest management science 61:246-257

Tanaka Y (2004) Major Psycological Facotors Affecting Acceptance of Gene-Recombination Technology. Risk Analysis 24:1575-1583

The_Convention_on_Biological_Diversity (2016) The Cartagena Protocol on Biosafety https://bchcbdint/protocol Accessed 19 January 2016

The_New_Zealand_Environmental_Protection_Authority (2015) Consultation on wording of 'organisms not genetically modified' regulations in the Hazardous Substances and New Organisms Act. http://wwwepagovtnz/consultations/new-organisms/Pages/consultation-organisms-not-genetically-modified-regulationsaspx Accessed 19 January 2016

The_US_Library_of_Congress (2014) Restrictions on Genetically Modified Organisms: Japan. http://wwwlocgov/law/help/restrictions-on-gmos/japanphp Accessed 19 January 2016

The_US_Library_of_Congress (2014) Restrictions on Genetically Modified Organisms: New Zealand. http://wwwlocgov/law/help/restrictions-on-gmos/new-zealandphp Accessed 19 January 2016

Tsai SQ, Zheng Z, Nguyen NT, Liebers M, Topkar VV, Thapar V, Wyvekens N, Khayter C, Iafrate AJ, Le LP, Aryee MJ, Joung JK (2015) GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. Nat Biotechnol 33:187-197

Tsukaya H (2013) Design for controllability. EMBO reports 14:3

Voytas DF, Gao C (2014) Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS biology 12:e1001877

Waltz E (2015) Nonbrowning GM apple cleared for market. Nature biotechnology 33:326-327

Waltz E (2015) USDA approves next-generation GM potato. Nature biotechnology 33:12-13

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature biotechnology 32:947-951

Weeks DP, Spalding MH, Yang B (2015) Use of designer nucleases for targeted gene and genome editing in plants. Plant biotechnology journal doi: 10.1111/pbi.12448:

Whelan AI, Lema MA (2015) Regulatory Framework for Gene Editing and other New Breeding Techniques (NBTs) in Argentina. GM crops & food doi: 10.1080/21645698.21642015.21114698

Wolt JD, Wang K, Yang B (2015) The Regulatory Status of Genome-edited Crops. Plant biotechnology journal doi: 10.1111/pbi.12444:

Woo JW, Kim J, Kwon SI, Corvalan C, Cho SW, Kim H, Kim SG, Kim ST, Choe S, Kim JS (2015) DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nature biotechnology 33:1162-1164

Wunderlich S, Gatto KA (2015) Consumer Perception of Genetically Modified Organisms and Sources of

Information. Advances in nutrition (Bethesda, Md) 6:842-851

Zdziarski IM, Edwards JW, Carman JA, Haynes JI (2014) GM crops and the rat digestive tract: a critical review. Environment international 73:423-433

Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV, Zhang F (2015) Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. Cell 163:759-771

Zhang H, Zhang J, Wei P, Zhang B, Gou F, Feng Z, Mao Y, Yang L, Zhang H, Xu N, Zhu JK (2014) The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. Plant biotechnology journal 12:797-807

Zhou J, Peng Z, Long J, Sosso D, Liu B, Eom JS, Huang S, Liu S, Vera Cruz C, Frommer WB, White FF, Yang B (2015) Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. The Plant journal : for cell and molecular biology 82:632-643

Zilberman D, Kaplan S, Kim E, Hochman G, Graff G (2013) Continents divided: Understanding differences between Europe and North America in acceptance of GM crops. GM crops & food 4:202-208

Figure legend

Fig. 1 A proposed scheme for the development of a transgene-free food crop using genome editing

Non-homologous end-joining (NHEJ)-mediated plant breeding proceeds in the following manner. After designing the targeting domain of ZFNs and TALENs (guide RNAs of the CRISPR/Cas9 system), the specificity and off-target effect are validated in plant cell cultures. Next, plant cells modified by highly specific ZFNs, TALENs and CRISPR/Cas9 are subjected to an initial screen focused on on-target gene modifications. Subsequently, regenerated plants without significant off-target mutations are further selected. In addition to the acquired trait, the potential environmental impacts of the plants are evaluated in a laboratory. If the plants have an implication in environmental risks, such as the emergence of herbicide-resistant weeds by hybridization, test cultivation is carried out in an isolated field to evaluate their risks to the environment carefully. Moreover, the food product derived from such crops is subject to food safety assessment. If the plants have no implication in environmental risks, such plants are cultivated in a common field. Finally, the food product derived from the cultivar is subjected to a food safety assessment. In the case of plants with no implication in environmental risks, test cultivation may proceed without regulatory oversight. However, the food product would require food safety assessment since no implication in environmental risks does not necessarily imply food safety.

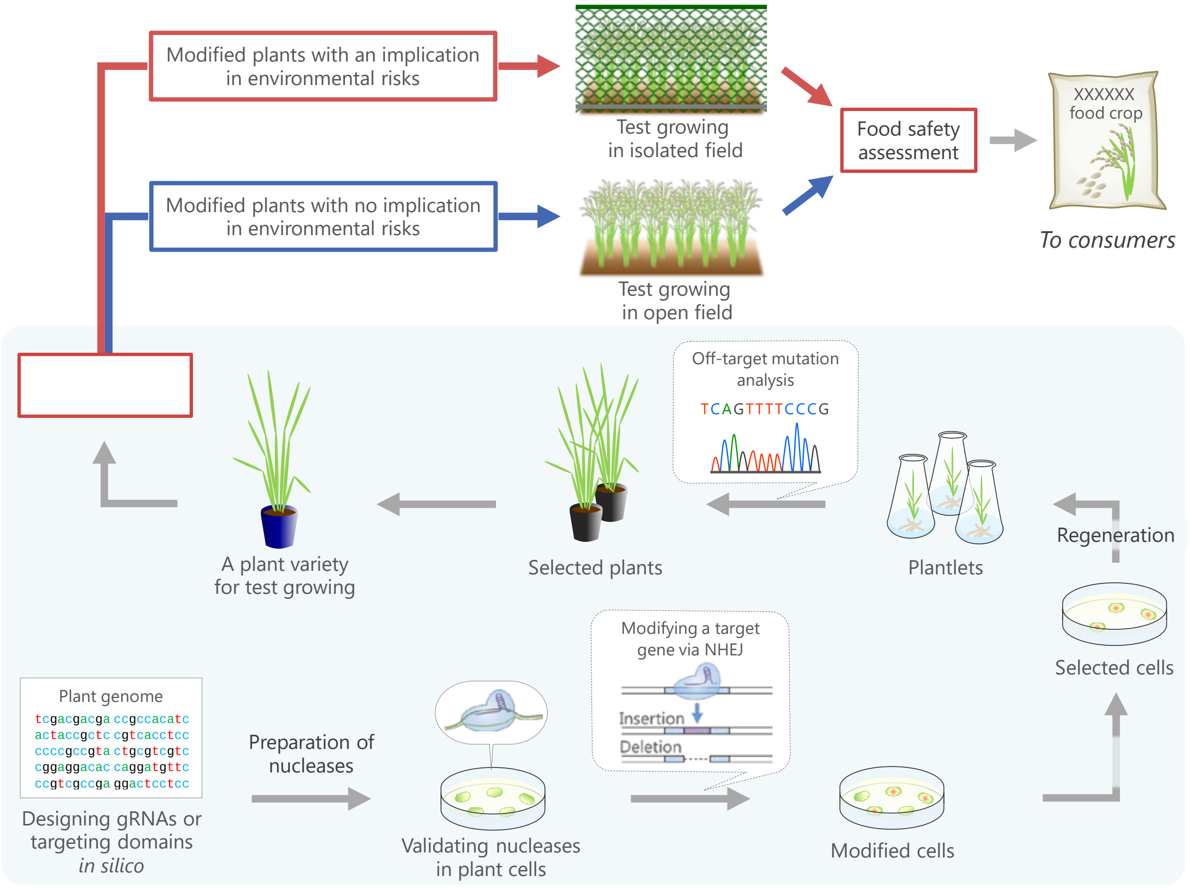

Table 1. Examples of food crops with potential consumer benefits that were attained via NHEJ-mediated mutagenesis.

Species	**Target gene**	**Genome editing**	**Efficiency of gene disruption (allele)**	**Genotyped subject**	**Off-target mutation analysis**	**Confirmed phenotype**	**Reference**
Potato	*VInv*	TALEN	28% (4) 11% (3) 28% (2) 33% (1)	T0	N.D.	No detectable reducing sugars	Clasen,et al.2015
Rice	*Lox3*	TALEN	29.0% (2) 45.0% (1)	T1	N.D.	Enhanced seed longevity	Ma,et al.2015
Rice	*OsBADH2*	TALEN	0, 12.5% (2) 28.6~78.1% (1)	T1	No (6 T0 plants)	Increased fragrance component	Shan,et al.2015
Soybean	*FAD2*	TALEN	33.3% (2) 66.6% (1)	T0	N.D.	Increased oleic acid and decreased linoleic acid	Haun,et al.2014
Tomato	*RIN*	Cas9	0~100% (2)	T1	N.D.	Changed fruit ripening	Ito,et al.2015

Table 3. The pros and cons of food crop breeding by GMO and genome editing technologies from the consumer perspective.

Technology	Pros	Cons
GMO	・Transgenesis is generally understood.	・Transgenic RNAi might be difficult to understand.
		・Transgene flow from GM crops has occurred in regions where a cross-compatible species exists.
	・Food safety is largely confirmed.	・There is public concern about the food consumption of GM crops with molecules such as BT toxin.
	・GMO regulations have been established based on the Cartagena Protocol on Biodiversity in 170 countries.	・Some countries which do not ratify the Cartagena Protocol on Biodiversity have uniquely developed GMO regulations.
	・The right to know is guaranteed by mandatory GMO labelling in some countries.	・People demand the right to know because GMO labelling is voluntary or no relevant policy exists in some countries.
Genome editing	・Gene addition via HDR would be easy to understand due to its similarity to transgenesis.	・Various types of mutagenesis via NHEJ and HDR are difficult to understand.
	・Transgene flow never occurs except for crops in which a transgene was added via HDR.	・There is no social consensus regarding the environmental risk assessment of genome edited crops in many countries.
	・Food safety can be improved over that of GM crops due to the lack of transgenes in crops modified via NHEJ, in addition to precise gene modification.	・There is no social consensus regarding the food safety assessment of genome edited crops in many countries.
	・The regulatory framework was established in Argentina and is being established in New Zealand.	・The regulatory response is delayed in many countries.
		・It is unclear whether the right to know is guaranteed or not.

BT: *Bacillus thuringiensis*

Table 2. The regulatory status of food crop varieties developed by new plant breeding techniques (NPBTs)

NPBT	Developer	Crop	Target gene modification (effect)	Novel trait	Application or inquiry content	Regulatory response	Response (year)	Regulator	Remarks	Ref
Grafting	French National Institute for Agricultural Research (INRA)	Grapevine	Delaying GFLV infection in grafts by GFLV coat protein expressed in GM rootstock	Resistance to grapevine fanleaf virus (GFLV)	Implementation of field trial	Approved the implementation	2010	French Ministry of Agriculture, Biomolecular Engineering Commission		Lemaire, et al. 2010
Intragenesis or Cisgenesis	University of Florida	Grapevine	Introduction of grapevine-derived anthocyanin regulatory gene (VvMybA1)	Red color in seed and berries	Regulated Article Letters of Inquiry	Non-regulated (non-plant pest)	2012	USDA APHIS		https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/Grapevine_Inquiry_BR_response_042412.pdf
Cisgenesis	Wageningen University	Apple	Introduction of apple-derived scab resistance gene (Vf)	Scab resistance	Regulated Article Letters of Inquiry	May be regulated	2012	USDA APHIS	Plant pest vector used	https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/aphis_response_schouten.pdf
ODM	Cibus Canda	Canola	Amino acid substitution (W574L) in BnAHAS1C and BnAHAS3A	Imidazolinone and sulfonylurea herbicide tolerance	Assessment of food safety and livestock feed and environmental safety	Approved the food use, livestock feed and unconfined release	2013	Health Canada, Canadian Food Inspection Agency		http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/canola-5715-eng.php http://www.inspection.gc.ca/active/scripts/database/pntvcn_submitdb.asp?lang=e&crops=1&company=26&trait=herbicide&events=all
ZFN	Dow AgroSciences	Maize	IPK1 knockout via NHEJ	Reduced phytate production	Regulated Article Letters of Inquiry	Non-regulated (non-plant pest)	2012	USDA APHIS	Null segregant	https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/DOW_ZFN_IPK1_052610.pdf
TALEN	Cellectis Plant Sciences	Soybean	Knockout of FAD2-1A and FAD2-1B via NHEJ	High oleic acid	Regulated Article Letters of Inquiry	Non-regulated (non-plant pest, non-noxious weed)	2015	USDA APHIS	Null segregant	https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/brs_response_cellectis_air_fad2k0_soy_cbidel.pdf
TALEN	Cellectis Plant Sciences	Soybean	FAD3 knockout via NHEJ	High oleic acid	Regulated Article Letters of Inquiry	Non-regulated (non-plant pest, non-noxious weed)	2015	USDA APHIS	Null segregant	https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-071-01air_resp.pdf
TALEN	Iowa State University	Rice	Knockout of OsSWEET11 and OsSWEET14 via NHEJ	Disease resisitance	Regulated Article Letters of Inquiry	Non-regulated (non-plant pest, non-noxious weed)	2015	USDA APHIS	Null segregant	https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/aphis_resp_isu_ting_rice.pdf
TALEN	Calyxt	Wheat	MLO knockout via NHEJ	Powdery mildew resistance	Regulated Article Letters of Inquiry	Non-regulated (non-plant pest, non-noxious weed)	2016	USDA APHIS	Null segregant	https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-238-01_air_response_signed.pdf

*This table shows the examples of regulatory responses to the application or inquiry on food crops developed by NPBTs in which a gene of interest was clearly indicated.

*USDA APHIS: United States Department of Agriculture Animal and Plant Health Inspection Service

*ODM: Oligonucleotide-directed Mutagenesis