MO (genetically modified organism) has been a subject of heated debate in the public since the first commercial production of GM crops in 1996. The governments around the world regulate GM crops vigorously before they are allowed to grow commercially in their countries. Improvement of traits through GM has been largely concentrated on resistance to herbicides and pest insects. Despite the limited number of GM traits, a record 18 million farmers planted 175 million hectares (432 million acres) of GM crops (mainly canola, cotton, maize, soybean and sugar beet) in 27 countries in 2013 according to the latest data from International Service for the Acquisition of Agri-Biotech Applications (ISAAA).

Supporters of GMO view that GM crops are effective solutions for feeding the poor and hunger in the world, and believe that biotechnology is an essential tool for our society to meet with the increasing challenges in climate change, cropland decline, population growth, and increase in demand of food and feed. However, opponents of GMO argue that the impacts of GM crops on human health and the environment need to be studied more carefully before they are permitted to grow in large scale, and insist that prematurely authorizing GM crops to enter the food and feed supply systems is dangerous. These arguments primarily stem from some characteristics of GMOs, e.g. a transgene and a selectable marker gene are frequently arranged together and are co-inserted into the genome in a random fashion. One of the frequently used marker genes is nptII (neomycin phosphotransferase type II), which confers resistance to Kanamycin and Neomycin although the antibiotics have limited use in medicine nowadays and gene nptII exists in a wide range of soil microorganisms.

The First GMO Apple

Arctic Apples, developed by Okanagan Specialty Fruits (OSF, a British Columbia biotech firm), are one of the typical GM crops. The major improvement of Arctic Apples (Arctic Golden Delicious and Arctic Granny Smith) over their non-transgenic counterparts (e.g. conventional Golden Delicious and Granny Smith) is that the GM fruit do not turn brown after being sliced (Figure 1). This non-browning trait is accomplished by inserting a genetic construct into the apple genome, which can suppress the key enzymes called polyphenol oxidases (PPOs) responsible for flesh browning when cut (Xu 2013b). Based on OSF, the non-browning apples have more eye appeal (no yucky browning) and more nutritional and health benefits (the health-supporting antioxidants are not burned up by the browning reactions). These positive appeals and benefits would stimulate more fresh consumption, thereby more sales of apple fruits. Arctic Apples could also help reduce waste related to browning.

Currently, OSF is still awaiting regulatory approval of Arctic Apples from both the Canadian and US governments to enter the markets. The OSF petition for the deregulated status of Arctic Apples was filed to USDA-Animal and Plant Health Inspection Service (APHIS) in May 2010. The review process administrated by APHIS has included two opportunities for the public to comment. The first public comment opportunity was ended on 11 September 2012. Based on its environmental and plant pest risk assessments, APHIS concluded in August 2013 that Arctic Golden Delicious and Arctic Granny Smith varieties are unlikely to pose a plant pest risk, and recommended that the two Arctic Apple varieties be granted non-regulated status. The second public comment opportunity was ended on 30 January 2014. To date, final decision on deregulation of Arctic Apples has not been made by APHIS although it can be expected anytime now.

Objections to GMO Apples

While Arctic Apples remain unavailable on the market due to the regulatory process, anti-GMO groups have directed their enormous negative efforts towards the fruit. For example, Friends of the Earth similarly published an article of headline “Frankenapple: Bad news no matter how you slice it.” “Does the GMO Arctic Apple threaten bees and possibly human health?” reads of the headline of an article available at gneducation.org. In the wake of the positive assessments on Arctic Apples from APHIS, Friends of the Earth has taken their action against the fruit to market. The most resilient one was the news release on 7 November 2013...
interpreting the position of McDonald’s and that of Gerber/Nestle on Arctic Apples in responding to the inquiries of Friends of the Earth. The title of the news release reads “McDonald’s, Gerber say no to GMO apple.” However, OSF countered this on November 2013 with an article “McDonald’s and Gerber Have Not ‘Rejected’ Arctic Apples” in Food Safety News by referring to the same letters from McDonald’s and Gerber.

If the anti-GMO groups’ action already imposes challenges to OSF, the apple industry’s objection creates a real problem for Arctic Apples. The U.S. Apple Association (Vienna, VA), the Northwest Horticultural Council (Yakima, WA), and the BC Fruit Growers’ Association (Kelowna, BC, Canada), have all urged the U.S. and/or Canadian governments not to approve the deregulated status for Arctic Apples. Their major concern is the possible negative impact of GM apples on the industry image in both domestic and international markets due to the public uneasy perception on GMO. Different from the anti-GMO groups, however, the industry is supportive of GMO research and thinks that the safety of Arctic Apples should not be an issue if the fruit are deregulated by the federal authorities after extensive reviews and risk assessments.

All these controversies around Arctic Apples could be attributed to the technical limitations of the existing biotechnology although the same or similar GM procedures had been used in other crops. In the case of Arctic Apples, kanamycin resistance gene nptII was used as a selectable marker and inserted together with the PPO suppression transgene PGAS (Xu 2013b). The transgene is derived from apple genome and is reconstructed to target multiple PPO genes through a technique called sense cosuppression or sense-RNA interference (RNAi). Since RNAi is less commonly used in other GM crops, it is also become a target of critics for the possible unintended effects on human health.

What if a non-browning apple variety is free of any foreign DNA (e.g. the kanamycin resistance gene nptII), but is developed by knocking-out the same PPO genes in their native genomic locations using a novel means of genetic manipulation? Should such non-browning apples be regulated in the US? The answer would likely be “no.” Would such non-browning apple be considered non-GMO and equivalent to conventional bred? Would such non-browning apples be acceptable to anti-GMO groups, the apple industry as well as the public? The answers would likely be “yes.”

Precision Genome Editing as a Better Alternative to GMO

Thanks to the latest scientific breakthroughs, several novel means of genetic manipulation based on programmable nucleases (Kim and Kim 2014) have been developed to allow precision genome editing or engineering (PGE), i.e. precise DNA sequence editing in the genome. Programmable nucleases are enzymes that can be engineered to make a cut or double-stranded break (DSB) at predetermined specific sites in the genome. There are three major classes of programmable nucleases for precision genome editing (PGE): zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspersed short palindromic repeats (CRISPR/Cas9) (Figure 2).

ZFNs (Figure 2A) are a set of engineered zinc finger proteins fused with an endonuclease called FokI that can make a cut or double-stranded break (DSB) of DNA. ZFNs are designed to bind to specific sites in the genome, but two ZFNs are required in order to make a cut in the targeted site as FokI functions as a dimer (Puchta and Fauser 2014). TALENs (Figure 2B) are also designer DNA-binding proteins fused with FokI, but the DNA binding domain of TALENs comprises an array of repeats that are highly modular, allowing effective designing of TALENs to recognize virtually any DNA sequences. Due to this modular feature in their DNA binding domain, TALENs are easier to be engineered than ZFNs. Consequently, TALENs are more specific in DNA binding than ZFNs, thereby minimizing off-target events (Xu 2012 Puchta and Fauser 2014). CRISPR/Cas9 (Figure 2C) finds its target DNA through a guide RNA, i.e. the guide RNA sequences define the specific genomic site, which is accomplished by base paring between RNA and DNA. This makes CRISPR/Cas9 based genome targeting relatively simpler and more straightforward than ZFNs and TALENs. Such simplicity has quickly allowed CRISPR/Cas9 the choice of method for precision genome editing (Puchta and Fauser 2014; Xu 2013a).

The consequence of a cut or double-stranded-break (DSB) of DNA created by the programmable nucleases is to activate DNA repair pathways in cell (Sander and Joung 2014), i.e. non-homologous end-joining (NHEJ) and homology-directed repair (HDR) (Figure 3). In most cases, DNA repairing through pathway NHEJ is precise. However, errors such as mismatches, short deletions or insertions can occur in NHEJ, leading to targeted mutations. HDR is an alternative pathway of DNA repair when a DNA donor template is available. In HDR, the sequence information from the donor template is copied to restore the broken ends. DNA repairing through pathway HDR provides many opportunities for desired site-specific changes in DNA sequences. For
Figure 3. A diagram for DNA repair pathway-non-homologous-end-joining (NHEJ) or homology-directed repair (HDR) triggered by double-stranded breaks (DSBs). The upper-panel shows a DSB induced by programmable nucleases ZFNs, TALENs or CRISPR/Cas9 at a specific genomic site. The lower-left panel shows the NHEJ-mediated DNA repair, which can sometimes produce deletion (red dotted lines) and/or insertion (green lines) mutations of variable length at the DSB site. The lower-right panel shows the HDR-mediated repair pathway, which can introduce precise point mutations or insertions from a single- or double-stranded DNA donor template (blue lines). Note that a desirable gene of any source can be included in the template DNA, allowing gene replacement or gene transfer. The black lines stand for DNA strands. Adapted from Sander and Joung (2014).

Precision genome editing (PGE) using ZFNs, TALENs or CRISPR/Cas9 has been successfully conducted in many model plant (Arabidopsis and tobacco) and field crops (rice, wheat and sorghum) species (Xu 2013a) as well as specialty crops, such as Citrus (Jia and Wang 2014) and Tomato (Brooks et al. 2014). Depending upon how the programmable nucleases and the DNA repair pathways are used/triggered, the precision genome editing (PGE) improved crops may be categorized into three groups: PGE1, PGE2 and PGE3 (Table 1). PGE1 stands for those that are generated using a programmable nuclease followed by NHEJ DNA repair pathway, and are free of any foreign DNA in the genome. PGE2 represents those that are also produced by a programmable nuclease, but a native DNA derived donor template is used to trigger the HDR DNA repair pathway. Therefore, PGE2 plants are also free of any foreign DNA. PGE3 plants are obtained similarly as PGE2 plants. However, a foreign gene is included in the DNA donor template and integrated into the genome at a predetermined site. Since the PGE1 and PGE2 plants are indistinguishable from cultivars developed from conventional mutation breeding, these two classes of plants should not be regulated as GMO. However, regulatory reviews are required for the PGE3 plants due to the presence of foreign genes in the genome (Table 1).

Regulatory Decisions with Precision Genome Editing

In the US, the Federal government, which is represented by three powerful agencies USDA-APHIS, FDA and EPA, has been reinforcing example, if the target is a disease susceptibility gene, introducing a template carrying a disease resistance gene to repair the cut can lead to the former being replaced, converting a disease susceptible cultivar into a resistance one. Certainly, through the HDR pathway, a foreign gene could be also introduced to a predefined genomic site (Figure 3).
its GMO policy based on the Coordinated Framework for Regulation of Biotechnology, a policy document that was published by the Office of Science and Technology Policy (OSTP) in 1986. Being adaptive and responsive to advances in biotechnology and to stakeholders’ feedback, the US regulatory system has been much improved over the past 30 years. When reviewing the ZFN-12 maize plants that belong to the PGE1 group (Table 1) from Dow AgroScience, in which a gene (IPK1) is site specifically disrupted by ZFNs, USDA-APHIS determined that these plants fall outside of the agency’s authority. Gene IPK1 encodes an enzyme called inositol-1,3,4,5,6-pentakisphosphate 2-kinase, which catalyzes the final step in phytate biosynthesis in corn seeds. Knocking out of IPK1 would lead to lower contents of grain phytate, which is desirable. This is because “phytate accounts for about 75% of total seed phosphorus, is an anti-nutritional component of feed grains and contributes to environmental pollution through the waste stream” (Shukla et al. 2009). The decision on the ZFN-12 maize plants from APHIS effectively permits Dow AgroScience to commercialize the corn with reduced phytate if the company chooses doing so. This suggests that precision genome editing technology can help developers develop crop cultivars without the need to go through the expensive and time-consuming regulatory process.

In tree fruit, long juvenility is a severe constrain for genetic improvement. The GM ‘FasTrack’ breeding system of plum, which was developed by scientists at the Appalachian Fruit Research Laboratory of USDA-ARS, Kearneysville, West Virginia, can induce fruit from plum trees less than one year old from seed (Figure 4). An important application of this system is to allow backcross to be conducted in plum trees like in annual crops, which has been impossible in the past. Although the plum ‘FasTrack’ system is a GMO-based system, progeny free of transgene, called ‘null segregant (NS) lines’ can be selected after multiple rounds of backcross using the ‘FasTrack’ system. When reviewing the ‘NS’ lines of plum free of any foreign DNA, USDA-APHIS again ruled that these new plums are fall outside the agency’s regulatory authority. Although the new plums have nothing to do with precision genome editing, the APHIS’ decision on this case further suggests that improved new crops without foreign DNA can be considered as conventionally bred cultivars.

**Future Apple Varieties Developed with Precision Genome Editing**

Given the rapid advancing pace in precision genome editing, it is certain that more and more crops, including tree fruits such as apple, will be developed using this technology. I believe that the future precision genome editing improved apples, including those of non-browning trait, will not be regulated by APHIS, nor objected by the anti-GMO groups, the apple industry and the consumers.

**Table 1. Comparison of traditional GM crops and the precision genomic editing improved crops.**

<table>
<thead>
<tr>
<th>Genetic manipulation method</th>
<th>Category</th>
<th>DNA repair pathway</th>
<th>Foreign gene</th>
<th>Gene placement</th>
<th>Disruption of other genes</th>
<th>Other foreign DNA</th>
<th>Need for regulation</th>
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<tr>
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<td>PGE3</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>GMO (cisgenic)</td>
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</tr>
</tbody>
</table>

PGE: precision genome editing; NHEJ: non-homologues-end-joining; HDR: homology-directed repair.

**Figure 4.** A GM FasTrack plum line produces fruit in less than a year after being planted from seed. Plum seedlings usually take 3-10 years to set fruit. Photo by Chinnathambi Srinivasan at URL: http://www.aphis.usda.gov/biotechnology/downloads/reg_loi/Drs%20Scorza%20and%20Callahan%20Final.pdf.

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