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Review article

The Regulatory Status of Genome-edited Crops

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Summary

Genome editing with engineered nucleases (GEEN) represents a highly specific and efficient tool for crop improvement with the potential to rapidly generate useful novel phenotypes/traits. Genome editing techniques initiate specifically targeted double strand breaks facilitating DNA-repair pathways that lead to base additions or deletions by non-homologous end joining as well as targeted gene replacements or transgene insertions involving homology-directed repair mechanisms. Many of these techniques and the ancillary processes they employ generate phenotypic variation that is indistinguishable from that obtained through natural means or conventional mutagenesis; and therefore, they do not readily fit current definitions of genetically engineered or genetically modified used within most regulatory regimes. Addressing ambiguities regarding the regulatory status of genome editing techniques is critical to their application for development of economically useful crop traits. Continued regulatory focus on the process used, rather than the nature of the novel phenotype developed, results in confusion on the part of regulators, product developers, and the public alike and creates uncertainty as of the use of genome engineering tools for crop improvement.

Introduction

Genome editing with engineered nucleases (GEEN) has rapidly emerged as a leading tool for investigating gene function and for creating genetic variation using site-directed genomic alterations. When applied to economically important plant species, GEEN additionally provides a highly specific and efficient means to generate useful novel phenotypes. With the advent of genome editing as a readily accessible technology to the research community, the question arises as to how plants expressing unique traits derived by genome editing will be received by the public at large and treated within various regulatory domains. This question cannot be easily answered from a process viewpoint, as site-directed genome editing may range from mutations involving a single base change to transgene insertions. Addressing ambiguities regarding the regulatory status of genome-edited crops is critical to the application of genome editing for developing economically useful crop traits. In this review, progress in GEEN and related techniques in higher plants is considered with respect to the current regulatory status for genome-edited crops.

Genome editing techniques

The earliest example of genome editing (Table 1) in higher plants involved oligonucleotide-mediated mutagenesis (OMM) to cause site-specific gene targeting using chemically synthesized oligonucleotides with base replacement or addition caused by endogenous DNA-repair enzymes (Beetham *et al.*, 1999). The method differs from GEEN approaches in that OMM does not deliver a nuclease to the site of action. Optimized OMM trait development systems are resulting in the first genome-edited crops for commercial release (Pratt, 2012).

Recent discoveries and advances in genome editing use site-directed nucleases (SDNs) where engineering of the nuclease allows for highly specific targeting to any given gene of interest. The array of SDNs which have been used for genome editing in higher plants encompasses engineered meganucleases (EMNs also referred to as LAGLIDADG endonucleases or homing nucleases), zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats in conjunction with the associated Cas9 endonuclease (CRISPR/Cas9) (Gaj *et al.*, 2013; Osakabe and Osakabe, 2014). These various GEEN methods operate in a similar fashion to generate a double strand break (DSB) at a specific location in the genome and are comprised of engineered proteins consisting of a DNA binding domain to confer site specificity and an endonuclease domain to cause the DSB (Curtin *et al.*, 2012). A variety of natural DNA repair mechanisms involving nonhomologous end joining (NHEJ) or homologous recombination (HR) can be exploited to allow for targeted genome modifications *in vivo*. When NHEJ rejoins DSBs to repair broken chromosomes, the result is often imprecise, introducing mutations at the cut site that can alter gene function and serve as a source of induced genetic variation. Alternatively, DSB-induced HR can be used for highly specific gene targeting (homology-directed repair, HDR) involving either gene replacement or gene insertion enabling precise genome modification

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Table 1 Genome editing acronyms, terms and definitions (Breyer *et al.*, 2009; Kim and Kim, 2014; Osakabe and Osakabe, 2014; de Souza, 2012)

CRSPR	Clustered Regularly-Interspaced Short Paloindromic Repeats	Programable nucleases comprised of bacterially derived endonuclease (Cas9) and a single-guide RNA (sgRNA)
DSB	Double Strand Break	Cleavage in both strands of double-stranded DNA where the two strands have not separated
EMN	Engineered Mega Nuclease	Microbially derived meganucleases that are modified, fused, or rationally designed to cause site-directed DSB. Also referred to as LAGLIDADG endonucleases or homing nucleases.
GEEN	Genome Editing with Engineered Nucleases	Genetic engineering where DNA is inserted, replaced, or removed from a genome using SDN.
HDR	Homology-Directed Repair	A mechanism for DSB repair using a DNA sequence homologous to the break site that serves as a template for homologous recombination.
HR	Homologous Recombination	A genetic recombination process where two similar DNA strands exchange nucleotide sequences.
NHEJ	Non Homologous End Joining	A means for repair of DSB without the use of a homologous repair sequence. An error-prone process that often causes small insertions or deletions at the DSB site resulting in mutations.
OMM	Oligonucleotide Mediated Mutagenesis	Site-specific mutation with chemically-synthesized oligonucleotide with homology to the target site (other than for the intended nucleotide modification).
SDN	Site Directed Nuclease	Engineered DNA nucleases that are programmed to specific sites within the genome where they cleave a DNA chain by separating nucleotides.
TALEN	Transcriptional Activator-Like Effector Nuclease	Programmable nucleases comprised of the DNA binding domain of Xanthomonas-derived TAL effectors fused with FokI restriction endonuclease.
ZFN	Zinc Finger Nuclease	Programable nucleases comprised of the DNA binding domain of a zinc-finger protein and the DNA-cleaving nuclease domain of the FokI restriction endonuclease.

based on the exogenously introduced homologous template (Osakabe and Osakabe, 2014; Zhang *et al.*, 2013).

Generating a DSB at a specific chromosomal location is the critical step in site-directed genome engineering. In the case of EMNs, ZFNs and TALENs, gene targeting is accomplished through protein-based DNA recognition domains; whereas CRSPRs utilize a single guide RNA (sgRNA) for directing the accompanying Cas9 nuclease (Curtin *et al.*, 2012; Jones, 2015). The nature and specificity of GEEN methods vary, which influences both the way they are used and the ease of use.

Genome editing approaches

Approaches facilitated with recombinant DNA (rDNA)

To modify a plant gene through genome engineering, it is typically necessary to design and develop the SDN that is delivered to the plant cells by genetic transformation. In the case of HDR, a donor molecule must also be introduced. Following induction of nuclease expression and regeneration of plants, the plant events must be screened for the desired change. The process involves two independent loci of interest, the site of insertion for a transgene encoding the SDN and the target locus to be acted on by the SDN. These loci are not commonly linked and so the transgenic elements are readily removed by selection for null segregant lines in the T1 generation to produce subsequent plant generations that have been edited but are devoid of the tools used for the process (Curtin *et al.*, 2012).

The genome editing possibilities facilitated through GEEN may involve mutagenesis, gene replacement, gene editing, gene insertion, and site-directed deletions or inversions (Curtin *et al.*, 2012). The attributes of each in conjunction with the overall process for insertion, targeting, recovery and removal of transgenic elements may represent differing outcomes in terms of the way they will be assessed by regulators (see, Genome editing regulatory status and opinion). For instance, site-directed mutations are analogous to natural processes or mutation breeding with the exception that off-target effects can be greatly limited through design and selection of highly binding-specific SDNs or OMMs (Hartung and Schiemann, 2014) as well as by downstream selection to minimize undesired phenotypes. Additionally, the use of GEEN to deliver transgenes to a common site not only improves the efficiency and quality of transformations, but it can simplify the molecular characterization of events for product development and regulatory assessments.

Approaches involving other than rDNA

A further novel approach to genome editing is the possibility for SDN introduction through a means other than insertion of SDN-encoding genes into nuclear DNA (Kathiria and Eudes, 2014; Pauwels *et al.*, 2014). Such approaches include direct insertion into cells of the SDN (Martin-Ortigosa *et al.*, 2014) or of messenger RNA (mRNA) encoding the SDN (Yamamoto *et al.*, 2009), or the use of plasmid (Belhaj *et al.*, 2013) or viral vectors (Baltes *et al.*, 2014; Marton *et al.*, 2010) that do not integrate into the host genome. Delivering the genome editing machinery in these ways may circumvent transformation in difficult to transform plant systems therefore increasing the utility and accessibility of genome editing. These transient approaches also simplify the process of developing promising phenotypes, as they avoid regulatory triggers for rDNA techniques by eliminating the introduction of transgenic elements (see, Genome editing regulatory status and opinion). To date, approaches such as use of mRNA have been restricted largely to gene therapy considerations (Tavernier *et al.*, 2011), so their eventual utility for genome editing of crop plants is yet to be developed. On the other hand, direct introduction of protein for gene editing in plants currently shows good promise (Martin-Ortigosa *et al.*, 2014) as does the possibility for employing plasmid or viral vectors (Baltes *et al.*, 2014; Belhaj *et al.*, 2013).

Genome editing of economically important plants

Genome editing is being applied with increasing frequency to economically important plants to demonstrate proof of concept in terms of technical feasibility, regulatory acceptance and commercial viability (Table 2).

Early genome editing proof of concept in crop plants was demonstrated with OMM for expression of herbicide resistance in maize, rice, tobacco and wheat (Beetham *et al.*, 1999; Dong *et al.*, 2006; Iida and Terada, 2005; Kochevenko and Willmitzer, 2003; Okuzaki and Toriyama, 2004; Zhu *et al.*, 1999, 2000). Development is proceeding toward commercialization in 2016 of Cibus 5715 herbicide tolerant canola (Beetham *et al.*, 2005) which has been approved for use in Canada (Pratt, 2012), deemed not subject to regulation by USDA in the United States (Sparrow *et al.*, 2013) and is planned for release into European markets despite public questions as to the regulation of this technology (Harvey, 2014).

The use of an EMN for genome editing of an agronomic crop was first demonstrated using a native endonuclease modified through a structure-based protein design method to recognize and induce highly specific DSBs at a specific locus in maize, resulting in gene disruption through deletions or insertions of short base segments by NHEJ (Gao *et al.*, 2010). Recently, an EMN engineered from a yeast endonuclease, and also optimized for site recognition through a rational design approach, has been used to precisely target gene insertions into cotton plants for delivery of an herbicide tolerance gene at a specific, predefined site adjacent a previously inserted insect resistance gene (D'Halluin *et al.*, 2013).

A site-specific ZFN employing HDR has been used to disrupt the *IPK1* gene in maize by directed insertion of the *PAT* gene resulting in low-phytase maize with herbicide tolerance (Shukla *et al.*, 2009). Subsequently, a site-specific trait stacking approach using a ZFN was accomplished in maize by first producing a line containing an herbicide resistance gene and a linked synthetic ZFN target site, and then using ZFN-targeted site-specific integration of a second herbicide resistance gene flanked by the ZFN target site; thus allowing multiple trait stacking at a specific locus (Ainley *et al.*, 2013).

In the first instance of TALENs being applied toward crop improvement, the rice disease susceptibility gene *OsSWEET14* was modified by site-directed mutation to insert or delete nucleotide sequences ranging from 3 to 55 bp (Li *et al.*, 2012). Subsequent genetic segregation resulted in disease-resistant null segregant rice lacking the selection marker and TALEN genes. Further applications of TALEN technology in economically important plants have been shown for rice, barley and maize (Char *et al.*, 2015; Gurushidze *et al.*, 2014; Li *et al.*, 2012; Liang *et al.*, 2014; Shan *et al.*, 2013; Wendt *et al.*, 2013).

A Cas9-single guide RNA (sgRNA) system, representing a simplified form of the type II CRISPR/Cas9 system from *Streptococcus pyogenes*, has been successfully used for genome editing of both dicot and monocot crop species (Jiang *et al.*, 2013). Genes encoding Cas9/sgRNA and a nonfunctional mutant green fluorescence protein (GFP) were delivered by *Agrobacterium tumefaciens* to sorghum (as well as to Arabidopsis). In a second demonstration of the Cas9-sgRNA system, rice protoplast cells were transformed with constructs targeting the promoter region of the *OsSWEET14* and *OsSWEET11* bacterial blight susceptibility genes to contain mutated DNA sequences at the target sites.

Regulatory background

Worldwide, regulators dealing with the assessment of genetically modified (GM) crops generally support a scientific position that the plant phenotype arising from the application of a

Table 2 Examples of genome editing for value-added traits within economically important crops

Crop	Trait	Target	Technique	Approach	Citation
Barley	Phytase reduction	Promotor region of a gene in purple acid - phosphatase group (*HvPAPhy a*)	TALEN	*Agrobacterium*-mediated transformation, TALEN expression, NHEJ causing from 1 to 20 base deletions	Wendt *et al.*, 2013
Cotton	Herbicide tolerance and insect resistance stacking	Flanking genomic sequence of the *cry2Ae/bar* transgene locus of a GE cotton event	EMN	Biolistics introduction of meganuclease and repair template for homologous insertion of two herbicide tolerance genes (*EPSPS* and *HPPD*)	D'Halluin *et al.*, 2013
Maize	Herbicide tolerance	*AHAS108* and *AHAS109* encoding acetohydroxy acid synthase (AHAS)	OMM	Biolistic introduction of chimeric oligonucleotides with homologous repair resulting in single base replacement	Zhu *et al.*, 1999
Maize	Herbicide tolerance and phytase reduction	*IPK1* encoding inositol-1,3,4,5, 6-pentakisphosphate2-kinase	ZFN	Whiskers introduction with transient expression. (1) NHEJ with base deletion/insertion and (2) site-directed transgene insertion of a herbicide tolerance gene-expression cassette (*PAT*) with *IPK1* gene disruption	Shukla *et al.*, 2009
Maize	Male sterility	*MS26*, a maize fertility gene	EMN	*Agrobacterium*-mediated transformation to introduce genes encoding an EMN to cause base addition/deletion by NHEJ and resulting in male sterility	Djukanovic *et al.*, 2013
Rice	Herbicide tolerance	Specific codons of the endogenous rice acetolactate synthase (*ALS*) gene	OMM	Biolistic introduction of chimeric oligonucleotides with homologous repair resulting in single base replacement	Okuzaki and Toriyama, 2003
Rice	Bacterial blight resistance	Rice bacterial blight susceptibility gene *Os11N3* (*OsSWEET14*)	TALEN	*Agrobacterium*-mediated transformation, TALEN expression, NHEJ causing from nine base additions to 55 base deletions	Li *et al.*, 2012;
Rice	Bacterial blight resistance	Rice bacterial blight susceptibility genes *OsSWEET14* and *OsSWEET11*	sgRNA/Cas9 (variant of the CRISPR/Cas9 system)	Protoplast transfection with transient expression of Cas9/gRNA	Jiang *et al.*, 2013

given biotechnology process should be the focus of safety determinations. In practice, however, due to the governing statutes in various agencies, nations and regions of the world, there is a tendency for the process used in genetic engineering to determine the path to regulatory assessments and approval.

Canada is unique among nations currently evaluating GM crops for environmental release because of the strong adherence to the phenotype (i.e. a product basis) when determining the regulatory status of a plant expressing a novel trait (Smyth and McHughen, 2008). Thus, with this emphasis on the product versus the process, plant novel traits (PNTs) developed from conventional breeding, mutagenesis, transgenesis or genome editing will all be subject to a similar regulatory approval process. Crop phenotypes for herbicide resistance have been developed from each of the forgoing processes and have been subject to evaluation and approval by Canadian regulators as PNTs (Canadian Food Inspection Agency, 2015).

The regulatory paradigm followed in the United States under the coordinated framework distributes authority among the US Food and Drug Administration (FDA), Environmental Protection Agency (EPA) and Department of Agriculture (USDA). The coordinated framework relies on existing statutes for regulatory authority rather than on a national biosafety law as found elsewhere throughout the world. Even though the framework is ostensibly product based, process frequently comes into play for regulatory assessment of what are termed genetically engineered (GE) crops in the United States.

The FDA has a long-standing position of considering foods and feeds derived from rDNA technology as being as safe as their non-GE counterparts and maintains a strong focus on product-based considerations. The FDA evaluates safety of GE crops and their derived foods and feeds through informal consultation focused on compositional equivalence of the GE product and its non-GE comparator, especially as it relates to allergens, antinutrients and toxins (McHughen and Smyth, 2008). This process is evolving and a premarket notification procedure was suggested by FDA in 2001 in response to public pressure for a formal safety evaluation for GE-derived foods and feeds (http://www.fda.gov/Food/FoodScienceResearch/Biotechnology/).

The regulation of GE crops at EPA is more directed toward product through regulatory authority that is limited to traits developed to intentionally exhibit pesticide activity (McHughen and Smyth, 2008). Assessment within EPA is intended to regulate the pesticidal property rather than the crop. Thus, in the case of a crop expressing a Cry toxin derived from *Bacillus thuringiensis*, regulators focus on the plant incorporated pesticide—the expressed Cry protein—given that the transformed crop otherwise shows phenotypic similarity to the nontransformed phenotype (comparator).

The USDA serves as the lead US regulator under the coordinated framework and draws its authority to regulate GE crops to the extent that the derived plant may behave as a plant pest or noxious weed (McHughen and Smyth, 2008). Through this approach if elements of a plant pathogen are used in development of the GE crop, the crop falls within USDA regulatory purview but in other cases does not. For instance, plants transformed by *Agrobacterium tumefaciens* are typically subject to regulatory review by USDA whereas those transformed by biolistics are not when the gene donor, recipient and vector are otherwise not known plant pests (Camacho *et al.*, 2014).

Under European Union legislation, GM crops are specifically defined as "an organism ... in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination." Furthermore, techniques involving recombinant nucleic acid are explicitly cited as subject to regulation and mutagenesis is explicitly excluded (European Parliament, 2001). Thus, crops resulting from the process of genetic modification as defined are subject to special regulatory consideration, which sets them apart, for instance, from similar crops generated by mutagenic approaches which are explicitly excluded from EU regulatory consideration. This special consideration for GM crops adds many years to the development timelines for crops developed with rDNA techniques and intended for markets in Europe, and as of now the political process in Europe has restricted widespread entry of GM crops into commercial use (Smyth *et al.*, 2014). Recent and pending actions within the EU to allow for country by country GMO cultivation and food and feed approvals portend further restriction of commercialized GM crop use in Europe (European Commission, 2015; European Parliament, 2015).

The EU adheres to a precautionary principle, which enables, "where scientific data do not permit a complete evaluation of the risk,... to stop distribution or order withdrawal from the market of products likely to be hazardous" http://europa.eu/legislation_summaries/consumers/consumer_safety/l32042_en.htm. The EU precautionary approach and its unique application to the defined process of genetic modification is often reflected in emerging regulatory networks elsewhere in the world where new biosafety laws are being adopted and implemented (Bayer *et al.*, 2010; Gupta *et al.*, 2008; Okeno *et al.*, 2013). Elsewhere, countries such Australia, Argentina and Brazil have successfully adopted process-based regulatory approaches that may factor social and economic factors into a regulatory framework, but which achieve regulatory approvals in a manner relatively consistent with that seen in North America (Smyth and Phillips, 2014).

Genome editing regulatory status and opinion

The regulation of genome-edited crops has been widely discussed and reviewed by regulators and the scientific community (Breyer *et al.*, 2009; European Food Safety Authority Panel on Genetically Modified Organisms, 2012; Gruère and Rao, 2007; International Life Sciences Institute, 2013; Lusser and Davies, 2013; Lusser and Rodríguez-Cerezo, 2012; Lusser *et al.*, 2011, 2012; Pauwels *et al.*, 2014; Podevin *et al.*, 2012, 2013). The consensus arising from these deliberations shows that the degree of regulatory scrutiny on genome-edited crops will be determined by the nature of the DNA-repair process used, the characteristics and intended use of the phenotype that is developed and the existing regulatory strictures within the geopolitical region of release.

In the EU, failure to embrace transgenic technologies has hindered product development and caused industry retraction (Hope, 2013). Therefore, EU regulatory experts and scientists have been keen to explore GEEN and other new breeding technologies as a pathway for crop improvement that circumvents the near impossibility for crops defined as GM to obtain public and regulatory approval (Breyer *et al.*, 2009; Hartung and Schiemann, 2014; Pauwels *et al.*, 2014; Podevin *et al.*, 2012, 2013). While the ultimate hope is for a renewed recognition within the EU of the importance of product over process as a regulatory paradigm (Hartung and Schiemann, 2014), the more pragmatic goal at this time is that those aspects of GEEN technology that represent site-

directed mutation will not be regulated in the EU or throughout the world, with the exception of Canada and its PNT regulation (International Life Sciences Institute, 2013).

Regulatory discussion of a wide range of new breeding techniques applied to crop development was initiated in 2011 with an EU-convened international workshop that considered the techniques then available for site-directed genome editing (Lusser *et al.*, 2011). Based on the categorizations identified by this group, its elaboration by Podevin *et al.* (2012)—and accounting for the emergence of new techniques in the interim—a schema for regulatory characterization specific to genome editing techniques can be described (Figure 1). This schema considers the approach to DSB repairs that are achieved by NHEJ (SDN1), homologous recombination (SDN2) or transgene insertion (SDN3) and whether the technique for introduction of the GEEN is transient (Category 1), introduces rDNA within the plant genome with subsequent removal (Category 2) or entails stable plant genome integration of rDNA (Category 3). The OMM approach produces DSB repaired by NHEJ and therefore is analogous to SDN1 in terms of its regulatory characterization to the extent the changes are viewed as point mutations and not template insertions (Hartung and Schiemann, 2014; Lusser and Davies, 2013).

Category 1 techniques involve transient introduction of recombinant DNA using *in vitro* synthesized nucleic acids and DNA delivery methods that do not integrate into the host genome (Pauwels *et al.*, 2014). These techniques, therefore, resemble transgenic processes but produce phenotypes that are indistinguishable from plants obtained through conventional plant breeding. The techniques would include site-specific point mutations with oligonucleotides (OMM), site-specific random mutations by NHEJ (SDN1) and site-specific mutations with DNA repair via homologous recombination (SDN2). Novel techniques avoiding the use of rDNA through direct introduction of the nuclease or mRNA encoding the nuclease (Baltes *et al.*, 2014; Martin-Ortigosa *et al.*, 2014) to catalyse similar mutation events would also fall into this category.

Category 2 consists of stable introduction of rDNA into the host genome and an intermediate step involving expression of SDN1 or SDN2 to effect DSBs and repairs. Subsequent breeding selection for null segregants results in phenotypes that are indistinguishable from phenotypes obtained through conventional plant breeding. Therefore, evidence will generally be lacking in the product to indicate a transgenic process was involved in the intermediate step.

Plant phenotypes developed by SDN1 methods as described in either of the forgoing categories represent simple point mutations and with few exceptions (Canada) regulators do not consider crops developed by mutagenesis in the same context as GM crops. The regulatory opinions regarding plant phenotypes developed by SDN2 methods are not as clear, as the nature and extent of the edits used to effect the desired change in the phenotype obtained by the technique would influence opinions as to whether the phenotype represented a GM product. For instance, deletions are viewed as less consequential than are additions. And in the case of additions, the greater the number of bases added, the greater the level of regulatory concern. Important in this context is the determination as to whether the NHEJ accomplished by the technique is viewed as a template insertion into the genome (Lusser and Davies, 2013).

Finally, Category 3 involves techniques that result in stable integration of rDNA where GEEN is used to specifically target delivery of a transgene or multiple transgenes through insertion by homologous recombination (SDN3). Current examples of this technique involve the site-directed stacking of transgenes (D'Halluin *et al.*, 2013); thus, they simply represent a refined technique to accomplish transgenesis and would be considered no differently than GM products by regulators. The European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms—an expert panel providing independent scientific advice to EFSA on GMOs—has developed the regulatory opinion that existing EFSA guidance documents apply to the SDN3 technique (European Food Safety Authority Panel on Genetically Modified Organisms, 2012). But because the technique can specifically target transgene delivery into the genome, it has the potential to minimize potential hazards associated with gene disruption or regulatory elements in the recipient genome. Thus, plants developed using SDN3 methods may require less data for risk characterization than more conventional approaches to transgenesis.

Regulatory consideration of these various methodologies is an ongoing process as regulators throughout the world adhere to a case-by-case paradigm for decision-making. Therefore, until a significant body of regulatory decisions emerges, there will be uncertainty as to the regulatory classification of plants developed by GEEN and related techniques. The general scientific sentiment of regulators to date is that gene-edited crops are not uniformly subject to the same regulatory standards as transgenic crops (Lusser *et al.*, 2011), but some green and NGO groups as well as governance experts are beginning to argue otherwise over

Category / Method	Category 1 Transient expression resulting in site-specific DSB and repair	Category 2 Stable genomic introduction of rDNA with intermediate steps to generate transgene-free null segregants	Category 3 Stable genomic integration of recombinant DNA
SDN1* Site-directed random mutation involving NHEJ	Low	• Low for deletions • Case-by-case for addition • Higher as size of insertion increases	N/A
SDN2 site-directed homologous repair involving one or very few nucleotides	Case-by-case	Case-by-case	N/A
SDN3 site-directed transgene insertion	N/A	N/A	High, moderated for well characterized insertion sites

Figure 1 Relationship of site-directed genome approach to the anticipated degree of regulatory scrutiny of the plant phenotype obtained.
*Current uses of OMM are analogous to SDN1 in terms of regulatory scrutiny.

Table 3 Reponses of USDA to letters of inquiry regarding the regulatory status of plant phenotypes developed through genome editing

Date	Developer	Host	Trait	Editing technique	Transformation system	Nature of change	Regulatory determination*
(1) Transient expression resulting in site-specific DSB and repair							
Mar 2004	Cibus	Canola	Herbicide tolerance	OMM	NS**	Base insertion/deletion (NHEJ)	"the agency has no authority to regulate products created by mutagenesis techniques such as genoplasty"
May 2010	Dow AgroSciences	Maize	Reduced phytate production	ZFN (SDN1)	Whiskers delivered into embryonic cell cultures	Base deletion (NHEJ)	"induced deletions due to the use of zinc finger nuclease technology are not considered regulated articles"
Dec 2011	Cellectis	NS	NS	EMN (SDN1)	Biolistics, electroporation, mRNA	Gene deletion	"plants will not, in most cases, be regulated articles... because the meganuclease used is not from a plant pest and no plant pest sequences are inserted into the plant genome.... Also, there is no reason... to believe that changes to the plant genome generated by the deletion process would generate a plant pest, as long as no DNA is inserted into the plant genome during the deletion process."
Dec 2011	Cellectis	NS	NS	EMN (SDN2)	Biolistics, electroporation, mRNA	Targeted insertion (homologous repair)	"For... plants that use template DNA molecules, there are many potential changes to plant DNA.... For this reason, the Agency will consider case-by-case inquiries ..."
Mar 2012	Dow AgroSciences	Maize	Reduced phytate production	ZFN (SDN1)	Whiskers delivered into embryonic cell cultures	Base deletion (NHEJ)	"GE plants containing targeted deletions, caused by naturally-occurring DNA repair after the targeted break is made by zinc-finger nucleases, and in which no genetic material is inserted into the plant genome, are not regulated articles under 7 CFR part 340."
Mar 2012	Dow AgroSciences	Maize	Reduced phytate production	ZFN (SDN2)	Whiskers delivered into embryonic cell cultures	Targeted insertion (homologous repair)	"Zinc-finger nuclease techniques may also be used to create base-pair substitutions or insertion of genetic material into a plant genome. The Agency will consider case-by-case inquiries regarding the regulatory status of plants produced by these zinc-finger nuclease methods."
(2) Stable genomic introduction of recombinant DNA with intermediate steps to generate transgene free null segregants							
Aug 2014	Cellectis	Potato	Consumer safety and processing attributes	TALEN (SDN1)	Protoplasts treated with polyethylene glycol; PCR for selection of null segregants in regenerated plants	Base deletion (NHEJ)	"... the genetic material from plant pests was used to create the potato product; however, the potato plant regenerated from genetically engineered potato cells no longer contains the introduced genetic material. ... Therefore, APHIS does not consider this potato product... to be regulated under 7 CFR part 340."
May 2015	Iowa State University	Rice	Disease resistance	TALEN (SDN1)	Agrobacterium-mediated transformation; PCR for selection of null segregants in regenerated plants	Base deletion (NHEJ)	"... lines were developed using plant pests and genetic material from plant pests. However, the final rice plants do not contain any inserted genetic material and APHIS has no reason to believe that the plants... are plant pests... APHIS does not consider the five rice lines as to be regulated under 7 CFR part 340. Additionally,... APHIS has no reason to believe that the genetic engineering of your GE rice would increase the weediness of rice."

*Determination of USDA-APHIS-BRS relative its regulatory authority under the Plant Protection Act (7 CFR part 340). This does not preclude regulation by other regulatory authorities or statutes.

**Not specified.

concerns that these new technologies not escape regulatory scrutiny (Camacho *et al.*, 2014; Harvey, 2014). Ultimately, the regulatory approach taken toward plants developed using GEEN techniques will be constrained by the interpretation of language in existing national, regional and international statutes governing GM crops.

Within the United States, the USDA has provided guidance to product developers as to the regulatory status of products of modern biotechnology through responses to formal letters of inquiry submitted to the agency. These responses have broad relevance to FDA and EPA as well through the US coordinated framework. The guidance to date (Table 3) suggests that site-directed approaches that result in targeted deletions of endogenous nucleotides (SDN1) would not be regulated articles under USDA statutes, nor would approaches initiated with transgenesis that in intermediary steps selected for the absence of the transgenic elements (null segregants); however, for site-directed methods involving targeted oligonucleotide insertions or substitutions, further case-specific determinations would be required (Camacho *et al.*, 2014).

Public understanding of genome editing and regulatory implications

The plant research and development community is moving toward a number of new breeding technologies that represent options for increased innovation and which may find greater public and regulatory acceptance over the use of transgenic approaches. In addition to genome editing as discussed here, these new breeding technologies include, cisgenesis, intragenesis and grafting to GM rootstock, as well as some instances of RNA interference (Tait and Barker, 2011). The determination of the specific technology that should be employed in a given instance of crop improvement will depend not only on the best approach scientifically and technically, but the most viable approach in terms of public understanding and the regulatory pathway for approval (Chapotin and Wolt, 2007). The US regulatory framework is presently challenged in its ability to appropriately weigh and analyse novel breeding approaches, while over-regulating transgenic technologies with a clear record of safety (Camacho *et al.*, 2014). This limitation effects both the product developer's need for greater certainty in the regulatory process and the public's desire for appropriate governance of new technologies.

Early considerations of the regulation of GEEN and related technologies cite governance approaches and stakeholder involvement as seen in Europe as preferable to approaches in the US (Kuzma and Kokotovich, 2011); but this is of course an incomplete answer given the inability of the EU to make progress in the adoption of GM crops in general and the slowing pace in adopting regulatory positions on genome editing despite early leadership in the area. From 2007 through 2011, >35% of genome editing publications emanated from Europe but research progress is being rapidly outpaced by efforts in the United States (Kuzhabekova and Kuzma, 2014), perhaps because of a greater openness in the United States to technology innovation (Pew Research Center, 2015).

The general public's view of foods derived from products of modern biotechnology is perhaps the greatest hurdle faced for definition and implementation of regulatory processes that are consistent with scientific understanding of new plant breeding technologies including genome editing. A recent survey conducted comparing scientist and citizen views on a range of science, engineering and technology issues showed remarkable differences in understanding of issues from a scientific and public perspective (Pew Research Center, 2015). The most pronounced difference was on the question of safety of genetic engineering where 37% of the public at large responded that GM foods are generally safe to eat, whereas 88% of scientists interviewed recognized GM foods as generally safe. Given this large discrepancy and the difficulty in conveying concepts of modern biotechnology to the general public, there is considerable potential that the public may not immediately embrace genome editing. And in fact some experts argue that as an emerging technology genome editing should require greater scrutiny than well-established technologies such as transgenics (Araki and Ishii, 2015; Camacho *et al.*, 2014). Public questioning and precautionary mindsets feed the increasingly outsized influence of civil society campaigns in creating uncertainty as to the safety of GM foods. This influence ripples worldwide (Paarlberg, 2014) and, therefore, may outpace the ability of scientists to communicate the opportunities afforded by genome editing for crop improvement.

Needs within the regulated community

The need to rapidly innovate to introduce novel traits in crops is heightened by increased world food demand and increasing use of crops as sources of renewable energy (Edgerton, 2009). The opportunity for transgenic crop innovation is limited by regulatory hurdles and continued public unease (Pew Research Center, 2015; Smyth *et al.*, 2014). Transgenic technologies continue to elicit considerable public misunderstanding and mistrust despite 19 years of commercial use and over 181.5 million hectares in production globally in 2014 (James, 2014). Largely in response to effective pressure on the part of a broad spectrum of NGO and activist groups (Paarlberg, 2014) and the continuing public pressure it has engendered, the regulatory processes for transgenic GE crops (the so-called GMOs) are largely broken in many parts of the world. Implementation of national biosafety laws is encumbered in the developing world (Bayer *et al.*, 2010; Okeno *et al.*, 2013) and long delays in cultivation approvals are reducing the value of innovation in many regulatory domains (Smyth *et al.*, 2014). New breeding technologies, especially site-directed genome editing, are viable alternatives to transgenic crop production that provide new opportunities for innovation and which in many cases clearly involve a reduced degree of regulatory oversight.

Success in advancing GEEN and related technologies for crop improvement will be limited if public views and regulatory response continues to be captured within the overriding theme of GMOs. The continued reliance on process-based definitions as a guide to regulatory oversight—and the adoption of process-focused language in public discourse—detracts from appropriately gauged approaches toward the regulation of genome-edited crops. Thus, the focus on the nature of the novel plant phenotype/trait is lost as the appropriate paradigm for the safety assessment, which encumbers regulatory approvals for crops derived from both established and emerging plant breeding techniques. Lacking a fuller emphasis on this point means that the public may largely misunderstand genome editing and regulators will be faced with pressure to evaluate these products within existing biosafety frameworks. Fortunately, progress is being made by regulators in shaping sensible and pragmatic approaches toward the application of genome editing for crop improvement but at some point new product-

based paradigms for regulation of new breeding technologies must emerge.

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