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INVITED REVIEW

Regulatory aspects of genome-edited crops

Jeffrey D. Wolt[1] · **Bing Yang**[1] · **Kan Wang**[1] · **Martin H. Spalding**[1]

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Abstract Genome editing with engineered nucleases (GEEN) is a highly efficient means of generating useful traits in crops. The application of GEEN for multiple traits and crops is resulting in products that are already entering the marketplace. Genome editing represents diverse and robust techniques targeting highly specific genome locations to cause double-strand breaks and subsequent repair by homologous and non-homologous mechanisms. Genome edits range from simple random insertions or deletions to template additions to stacking of multiple transgenes at a specific locus. Consequently, it is not easy to generalize the regulatory view of genome editing for crop improvement since a case-specific consideration is necessary which, in many regulatory regimes, encompasses the process used and the genome edit achieved in addition to the phenotype derived. Key process considerations for regulators of a GEEN-derived product are the nature of the edit, specificity, the source of the editing machinery, and post-editing trait segregation. These considerations along with the nature of the derived trait and performance of the crop phenotype inform the safety and benefit of a given GEEN product. The view of regulators toward a particular genome-edited crop is driven by the degree that regulatory statutes are product-versus process-focused and will be shaped by emerging scientific and public views of genome-editing technology. In order that the public will not confuse genome-edited crops with genetically modified organisms (GMOs), scientists and regulators alike need to carefully frame and communicate GEEN techniques in terms of the specific process used and the benefits derived through development of useful crop traits.

✉ Jeffrey D. Wolt
jdwolt@iastate.edu

[1] Iowa State University, Ames, IA 50011, USA

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Introduction

A variety of new breeding techniques (NBTs) are being advanced for crop development that reflect both rapidly emerging technology and developer frustration with insurmountable regulatory approval timelines for transgenic crops (*i.e.*, genetically modified organisms (GMOs)) (Smyth *et al.* 2014). Genome editing with engineered nucleases (GEEN) represents an NBT that has rapidly emerged where there are numerous proofs of concept with crop products in development for commercial applications.

There are several GEEN techniques currently in use (Table 1), and promising new reagents for genome editing are forthcoming (Zetsche *et al.* 2015; Gao *et al.* 2016). The use of the clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) system, with further guide RNA innovations, achieves highly selective targeting of Cas9 nucleases and has received substantial recent notice largely because of its relative ease of use, wide array of targeting flexibility, and low cost (Hsu *et al.* 2014). However, numerous other GEEN reagents continue to find use and further improvements, and products with development potential have used targeted genome edits with oligonucleotides (Beetham *et al.* 1999; Gocal *et al.* 2002), zinc finger nucleases (Shukla *et al.* 2009), and transcriptional activator-like effector nucleases (TALENs: Li *et al.* 2012; Clasen *et al.* 2016) in addition to CRISPR-Cas9 (Science 2016).

Regulators and scientists alike have expressed interest and enthusiasm for GEEN-derived crops, and many have viewed this as a workaround for plant improvement that avoids the

Table 1 Genome editing terminology and methodologies for genome editing with engineered nucleases (GEEN)

Abbreviation	Term	Definition
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
GEEN	Genome editing with engineered nucleases	Genetic engineering where DNA is inserted, replaced, or removed from a genome using SDN
OMM	Oligonucleotide-mediated mutagenesis	Site-specific mutation with a chemically synthesized oligonucleotide with homology to the target site (other than for the intended nucleotide modification)
EMN	Engineered meganuclease	Microbial meganucleases that are modified, fused, or rationally designed to cause site-directed double-strand breaks. Also referred to as LAGLIDADG endonucleases or homing nucleases
ZFN	Zinc finger nuclease	Programmable nucleases consisting of the DNA binding domain of a zinc finger protein and the DNA-cleaving nuclease domain of the *Fok*I restriction endonuclease
TALEN	Transcriptional activator-like effector nuclease	Programmable nucleases consisting of the DNA binding domain of *Xanthomonas*-derived TAL effectors fused with *Fok*I restriction endonuclease
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeats	Programmable nucleases consisting of bacterially derived endonuclease (Cas9) and a guide RNA (gRNA)

regulatory and public acceptance pitfalls associated with GMOs (Camacho *et al.* 2014; Wolt *et al.* 2016). Communication around this point has fed public misunderstanding and elicited challenges from civil society groups. In addressing this and trying to establish a path forward for GEEN-derived crops, regulators have tended to make policy statements and taken case-specific actions that show continuing conundrums regarding the techniques used for crop improvement and whether the regulatory paradigm for bioengineered crops involves the process for generation of variation, the product derived, or perhaps both process and product.

The technique conundrum Generating phenotypic variation in crops involves a range of techniques including selective plant breeding to acquire traits within a species or among its close relatives, mutational breeding using chemicals or radiation, recombinant DNA (rDNA) techniques to move genes among unrelated species, and genome editing. Throughout much of the world, new crop phenotypes derived through selective plant breeding and mutagenesis are subject to minimal, if any, regulation aside from varietal registration. Canada is an exception, since it evaluates all novel plant traits (NPTs) regardless of technique used in their development (Smyth and McHughen 2008).

Among countries evaluating GMOs, an apparent dichotomy exists between those countries and regions, such as the EU, that follow a process model and those countries, such as the USA, that state adherence to a product-focused model. In fact, because of the statutory language or regulatory approaches used, there is no such dichotomy since there is a *de facto* focus on the process used wherever GMOs are subject to regulation. Additionally, there is widespread regulatory agreement that crops developed using rDNA techniques are subject to regulation, whereas conventionally and mutagenically derived crops are not.

The current uncertainties surrounding regulation of GEEN-derived crops are complicated by regulatory interest in both the specific approach applied and the type of edit achieved within the genome. Key process considerations for regulators for a product of GEEN are the nature of the edit (simple insertion or deletion, template addition, transgenesis), specificity (off-target effects), the source of the editing machinery (transient nuclease or encoding DNA), and post-editing trait segregation. Thus, simple insertions or deletions involving non-homologous end joining (NHEJ) or familiar template insertions using homology-directed repair (HDR) tend not to be of regulatory concern as long as editing is achieved with transient nuclease introduction or encoding DNA insertion with subsequent plant breeding selection to remove transgenic elements (null-segregant (NS) selection). This view seems consistent with the present regulatory approaches or opinions in the USA, Canada, and Argentina but has not been clarified elsewhere (Whelan and Lema 2015; Wolt *et al.* 2016).

Internationally, early regulatory opinion tended to recognize genome-editing techniques as site-directed mutation and thus analogous to less-specific chemical or radiation mutagenesis (Gruère and Rao 2007; Breyer *et al.* 2009; Lusser *et al.* 2011, 2012; European Food Safety Authority Panel on Genetically Modified Organisms 2012; Lusser and Rodríguez-Cerezo 2012; Podevin *et al.* 2012, 2013; International Life Sciences Institute 2013; Lusser and Davies 2013; Pauwels *et al.* 2014). As such, GEEN-derived crops were not considered subject to

regulatory oversight; however, pushback from some scientists and civil society groups has led to reconsideration. The crux of the issue appears to be whether the insertion of rDNA that encodes the engineered nuclease represents a transgenic event even when the transgenic elements are subsequently selected out of the finished phenotype in the process of plant breeding. The EU has delayed a decision over regulation of genome-edited crops, probably reflecting diversity of opinion in member states. German and Swedish regulators may view GEEN-derived crops involving double-strand breaks with NHEJ and subsequent NS selection as not subject to regulation (BVL 2015; Swedish Board of Agriculture 2015). Regulators in New Zealand originally considered GEEN as not subject to regulation, but civil society groups pressed the issue before the High Court of New Zealand and the position was subsequently reversed (Kershen 2015).

The process versus product conundrum In the USA, case-specific instances have indicated a clear regulatory opinion that the use of GEEN to develop a unique phenotype is not subject to regulation when subsequent plant breeding results in a NS selection (Wolt *et al.* 2016), and similar opinion has emerged in Argentina (Whelan and Lema 2015). This is less a regulatory opinion regarding genome editing than it is a determination that phenotypes where there is no evidence of insertional rDNA are not subject to regulation. This appears to be true even when the transformation is accomplished using *Agrobacterium tumefaciens*, a traditional process trigger for regulatory consideration by the USDA (https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/aphis_resp_isu_ting_rice.pdf). This paradigm is subject to change, however, since the USDA Animal and Plant Health Inspection Service has undertaken regulatory rulemaking proposing to define products of biotechnology as subject to regulation while explicitly excluding conventionally and mutagenically derived organisms (https://www.aphis.usda.gov/brs/fedregister/BRS_20160307.pdf). The language of the proposed rule complicates the distinction of product versus process as the driver for regulation by defining *products* of biotechnology by the *process* involved in their development. Thus, processes that involve insertion or deletion of DNA segments and directed genome altering (*i.e.*, genome editing) may be subject to regulatory oversight.

Other combinations of GEEN approaches and resulting changes in the genome would appear to be of regulatory interest irrespective of political domain and would include approaches that do not involve removal of DNA elements that encode the nuclease and template insertions that are novel or transgenic (Fig. 1). In addition, multiplexing procedures involving several simultaneous gene edits will elicit regulatory interest because of uncertainties regarding rearrangements that may occur. Finally, genome editing when used to facilitate gene drives (systems designed to rapidly spread a specific allele throughout a population through biased inheritance) will be of considerable concern to regulators until issues surrounding the environmental release of gene drives have been clarified (NASEM 2016).

Communicating the science and its implications

Oftentimes, vagueness in scientific communication has confused the path forward for regulation and commercialization of crops developed through genome editing. Clearly, not all genome edits are the same in terms of the nature of modifications achieved, which range from simple insertions or deletions to stacking of multiple transgenes; therefore, genome editing as a process provides no one answer as to the regulatory issues that may be involved. Because of this, regulatory opinions differ, reflecting the nature of the edit, the trait considered, and the operative regulatory statute. Regulatory opinions are in flux and will be responsive to public opinion. Broader deliberation must take place surrounding the needs, opportunities, and potential concerns associated with crops developed through genome editing before regulatory opinion and public opinion align. Until that occurs, there will be a high degree of uncertainty surrounding the future of genome editing as an NBT.

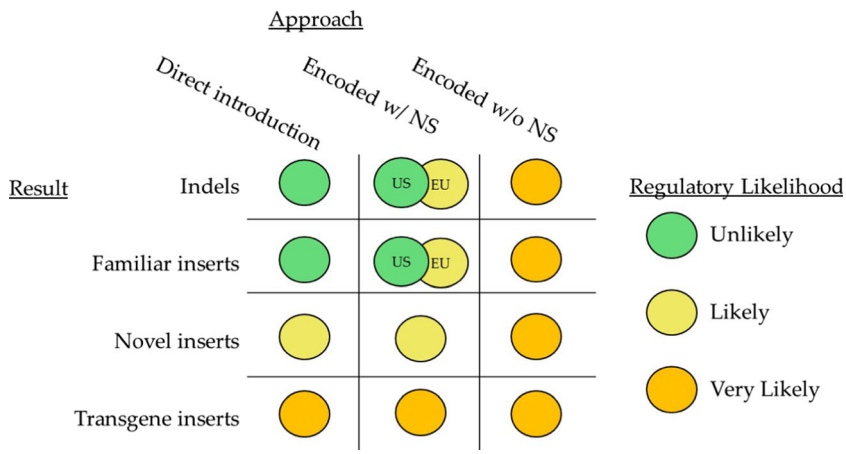

Figure 1. Current likelihood of regulatory oversight for crop phenotypes generated through genome editing as a function of the approach used for nuclease introduction (*encoded* genome insertion of DNA encoding the nuclease, *NS* null-segregant selection) and the resulting edit achieved in the genome.

Thus far, public discussion focused on genome editing as a technology that can be used for the public good has not occurred because of the tendency for both scientific and general media outlets to emphasize the technology as a means to avoid regulation (*e.g.*, "CRISPR foods dodge USDA" [Science 2016] and "Gene-edited CRISPR mushroom escapes US regulation" [Waltz 2016]). Communication must reflect the desire to achieve regulation appropriate to the degree of concern and not as an attempt to circumvent appropriate regulatory oversight.

Transparency to improve public understanding The scientists who are engaged in developing and advancing applications of plant genome editing bear a great deal of responsibility for framing public understanding of this technology. Many current publications in the field lack clearly defined descriptions of processes, which contributes to misunderstanding as to areas of genome editing that may be of relatively greater or lesser concern in terms of regulatory oversight. Additionally, the thrust of publications for this emerging technology largely focuses on obtaining the edit and on demonstrating proof of concept. Items of public and regulatory concern, such as off-target effects within the genome and their downstream implications to the derived phenotype, receive lesser consideration. Scientists studying therapeutic applications of genome editing have highlighted unbiased genome-wide analyses for off-target effects and rearrangements that could contribute to downstream toxicity as a research need (Corrigan-Curay *et al.* 2015). Furthermore, scientists considering gene drives that are enabled through genome editing have established guidelines for their responsible investigation (Akbari *et al.* 2015). While these issues are of lesser concern for genome editing as applied to crops (Weber *et al.* 2012; Schnell *et al.* 2015; Anderson *et al.* 2016), the public will not easily distinguish a difference and so addressing general concerns regarding genome editing is important in anticipation of public questions. Scientific stakeholder outreach and education will be important for communicating to the public at large that genome editing advances the public good by yielding fundamental advances in knowledge and providing useful traits in crops.

Conclusions

Understanding the current and future landscape surrounding the regulation of crops developed using genome editing is complicated by the tendency of regulators and the public to view products derived through bioengineering processes as distinctly different from those developed using selective breeding or conventional mutagenesis. Clarity is lacking regarding the process of GEEN and the outcome of genome editing (a unique trait or phenotype) as a regulatory paradigm. Transparent communication is needed to clarify that not all gene edits are the same in terms of the approach used and the result obtained. To date, regulatory opinions offered on a case-by-case basis have provided somewhat varied views concerning genome editing and its application. Regulatory views will remain in flux until public opinion gels regarding the technology. Transparency in communication by scientists and regulators to clearly define the processes used and products obtained may forestall overregulation of crops developed by genome editing. Ultimately, it will be important for regulators to forego process-based regulation and focus on regulating the products intended for environmental release (Conko *et al.* 2016; Wolt *et al.* 2016).

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References

Akbari OS, Bellen HJ, Bier E, Bullock SL, Burt A, Church GM, Cook KR, Duchek P, Edwards OR, Esvelt KM, Gantz VM, Golic KG, Gratz SJ, Harrison MM, Hayes KR, James AA, Kaufman TC, Knoblich J, Malik HS, Matthews KA, O'Connor-Giles KM, Parks AL, Perrimon N, Port F, Russell S, Ueda R, Wildonge J (2015) Safeguarding gene drive experiments in the laboratory. Science 349:927–929

Anderson JE, Michno JM, Kono TJY, Stec AO, Campbell BW, Curtin SJ, Stupar RM (2016) Genomic variation and DNA repair associated with soybean transgenesis: a comparison to cultivars and mutagenized plants. BMC Biotechnol 16:1. doi:10.1186/s12896-016-0271-z

Beetham PR, Kipp PB, Sawycky XL, Arntzen CJ, May GD (1999) A tool for functional plant genomics: chimeric RNA/DNA oligonucleotides cause *in vivo* gene-specific mutations. Proc Natl Acad Sci U S A 96:8774–8778

Breyer D, Herman P, Brandenburger A, Gheysen G, Remaut E, Soumillion P, Van Doorsselaere J, Custers R, Pauwels K, Sneyers M, Reheul D (2009) Commentary: genetic modification through oligonucleotide-mediated mutagenesis. A GMO regulatory challenge? Environ Biosafety Res 8:57–64

BVL (German Federal Office of Consumer Protection and Food Safety) (2015) Opinion on the legal classification of New Plant Breeding Techniques, in particular ODM and CRISPR-Cas9. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Braunschweig

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

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 (2016) Improving cold storage and processing traits in potato through targeted gene knockout. Plant Biotechnol J 14:169–176

Conko G, Kershen DL, Miller H, Parrott WA (2016) A risk-based approach to the regulation of genetically engineered organisms. Nat Biotechnol 34:493–503

Corrigan-Curay J, O'Reilly M, Kohn DB, Cannon PM, Bao G, Bushman FD, Carroll D, Cathomen T, Joung JK, Roth D, Sadelain M, Scharenberg AM, von Kalle C, Zhang F, Jambou R, Rosenthal E, Hassani M, Singh A, Porteus MH (2015) Genome editing technologies: defining a path to clinic. Mol Ther 23:796–806

European Food Safety Authority Panel on Genetically Modified Organisms (2012) Scientific opinion addressing the safety assessment of plants developed using zinc finger nuclease 3 and other site-directed nucleases with similar function. EFSA J 10:2943

Gao F, Shen XZ, Jiang F, Wu Y, Han C (2016) DNA-guided genome editing using the *Natronobacterium gregoryi* Argonaute. Nat Biotechnol 34:768–773

Gocal G, Avissar P, Knuth M, Beetham P, Walker K (2002) Non-transgenic herbicide resistant plants. US Patent 20,030,084,473 A1, Washington, DC

Gruère GP, Rao S (2007) A review of international labeling policies of genetically modified food to evaluate India's proposed rule. AgBioForum 10:51–64

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

International Life Sciences Institute (2013) ILSI Workshop on New Breeding Technologies (NBT). International Life Sciences Institute SEA Region Australasia, Canberra

Kershen DL (2015) Sustainability Council of New Zealand Trust v The Environmental Protection Authority: gene editing technologies and the law. GM Crops Food 6:216–222

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

Lusser M, Davies HV (2013) Comparative regulatory approaches for groups of new plant breeding techniques. N Biotechnol 30:437–446

Lusser M, Parisi C, Plan D, Rodríguez-Cerezo E (2011) New plant breeding techniques: state-of-the-art and prospects for commercial development. Joint Research Centre Technical Report EUR 24760. Publications Office of the European Union, Luxembourg

Lusser M, Parisi C, Plan D, Rodríguez-Cerezo E (2012) Deployment of new biotechnologies in plant breeding. Nat Biotechnol 30:231–239

Lusser M, Rodríguez-Cerezo E (2012) Comparative regulatory approaches for new plant breeding techniques. Workshop proceedings. European Commission. JRC Technical Report EUR, vol 25237. Publications Office of the European Union, Luxembourg

NASEM (National Academies of Sciences, Engineering, and Medicine) (2016) Gene drives on the horizon: advancing science, navigating uncertainty, and aligning research with public values. National Academies Press, Washington, DC

Pauwels K, Podevin N, Breyer D, Carroll D, Herman P (2014) Engineering nucleases for gene targeting: safety and regulatory considerations. N Biotechnol 31:18–27

Podevin N, Davies HV, Hartung F, Nogue F, Casacuberta JM (2013) Site-directed nucleases: a paradigm shift in predictable, knowledge-based plant breeding. Trends Biotechnol 31:375–383

Podevin N, Devos Y, Davies HV, Nielsen KM (2012) Transgenic or not? No simple answer! EMBO Rep 13:1057–1061

Science (2016) CRISPR foods dodge USDA. Science 352:388

Schnell J, Steele M, Bean J, Neuspiel M, Girard C, Dormann N, Pearson C, Savoie A, Bourbonnière L, Macdonald P (2015) A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. Transgenic Res 24:1–17

Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM, Rock JM, Wu YY, Katibah GE, Zhifang G, McCaskill D, Simpson MA, Blakeslee B, Greenwalt SA, Butler HJ, Hinkley SJ, Zhang L, Rebar EJ, Gregory PD, Urnov FD (2009) Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases. Nature 459:437–441

Smyth S, McHughen A (2008) Regulating innovative crop technologies in Canada: the case of regulating genetically modified crops. Plant Biotechnol J 6:213–225

Smyth SJ, McDonald J, Falck-Zepeda J (2014) Investment, regulation, and uncertainty: managing new plant breeding techniques. GM Crops Food 5:44–57

Swedish Board of Agriculture (2015) Is regulatory oversight needed for field trials of plants produced using genome editing technology? Jordbruksverket, Jönköping

Waltz E (2016) Gene-edited CRISPR mushroom escapes US regulation. Nature 532:293

Weber N, Halpin C, Hannah LC, Kough J, Parrott W (2012) Editor's choice: crop genome plasticity and its relevance to food and feed safety of genetically engineered breeding stacks. Plant Physiol 160: 1842–1853

Whelan AI, Lema MA (2015) Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina. GM Crops Food 6:253–265

Wolt JD, Wang K, Yang B (2016) The regulatory status of genome-edited crops. Plant Biotechnol J 14:510–518

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