Obligatory metabolomic profiling of gene-edited crops is risk disproportionate

Maria Fedorova1* and Rod A. Herman2

1Corteva Agriscience, 7100 NW 62nd Ave, PO Box 1000, Johnston, IA 50131, USA, and
2Corteva Agriscience, 9330 Zionsville Road, Indianapolis, IN 46268, USA

SUMMARY

It has been argued that the application of metabolomics to gene-edited crops would present value in three areas: (i) the detection of gene-edited crops; (ii) the characterization of unexpected changes that might affect safety; and (iii) building on the track record of rigorous government regulation in supporting consumer acceptance of genetically modified organisms (GMOs). Here, we offer a different perspective, relative to each of these areas: (i) metabolomics is unable to differentiate whether a mutation has resulted from gene editing or from traditional breeding techniques; (ii) it is risk-disproportionate to apply metabolomics for regulatory purposes to search for possible compositional differences within crops developed using the least likely technique to generate unexpected compositional changes; and (iii) onerous regulations for genetically engineered crops have only contributed to unwarranted public fears, and repeating this approach for gene-edited crops is unlikely to result in a different outcome. It is also suggested that article proposing the utility of specific analytical techniques to support risk assessment would benefit from the input of scientists with subject matter expertise in risk assessment.

PROPOSED USE OF METABOLOMICS FOR GENE-EDITED CROPS

Recently, a article was published proposing that metabolomics be applied to the oversight of crops developed through modern breeding techniques (Fraser et al., 2020). Unlike another recent articles containing similar arguments (Christ et al., 2018), this new article focused specifically on gene-edited crops. Arguments against the obligatory application of metabolomics to crops produced using modern breeding techniques have been detailed previously (Chassy, 2010; Harrigan and Chassy, 2012; Delaney et al., 2019). Fraser et al. promote the application of metabolomics to gene-edited crops in three areas: (i) the detection of gene-edited crops; (ii) the characterization of unexpected changes that might affect safety; and (iii) building on the track record of rigorous government regulation in supporting consumer acceptance of genetically engineered crops. Here, we offer a different perspective relative to each of these applications of metabolomics to gene-edited crops.

METABOLOMICS AS A DETECTION TOOL

Fraser et al. (2020) recognize the many technical challenges that currently exist in appropriately interpreting intrinsically dynamic and variable metabolomes. Even as such challenges are reduced in the future, the unique identification of a gene-edited crop via metabolomics would face the same fundamental challenge as the DNA-based approaches currently used for the unique identification of genetically engineered crops. As acknowledged by Fraser et al. (2020), gene editing introduces genetic changes that are often indistinguishable from those that could occur in plants spontaneously or through the application of classical mutagenesis techniques. Thus, although metabolomics could detect metabolic consequences of a genetic change, it cannot uniquely identify the origin of the change, i.e. discriminate between gene editing and other forms of mutagenesis, from a potential statutory requirement standpoint. For researchers’ internal tracking purposes, metabolomics might in some cases identify a gene-edited plant versus a
non-edited control plant when both are grown under similar environmental conditions, and only when there is a clear metabolic consequence of the editing. However, identifying such plants by metabolomics appears to be a far less practical method than detecting the DNA mutation itself.

CROP BREEDING AND COMPOSITIONAL CHANGES

Metabolomics can be used as a non-targeted technique to characterize the small-molecule compositional profile of a crop variety but seems to be of little relevance to the safety assessment of gene-edited crops. Developments in our understanding of crop genetics, advancements in genome sequencing and decades of research have clearly demonstrated that modern breeding techniques cause fewer and less significant unexpected changes in crop composition compared with traditional breeding methods (Ricroch et al., 2011; Herman and Price, 2013; Schnell et al., 2015; Louwaars, 2019). Spontaneous and induced genetic variability that result in phenotypic variability are cornerstones of plant breeding. Non-targeted induced mutagenesis using irradiation or mutagenic chemicals has been employed by plant breeders for many decades, resulting in over 3300 officially registered mutant varieties (https://mvd.iaea.org/#!Search, accessed on 23 March 2020). The safety record of products of traditional breeding methods has been exemplary, despite the intended as well as additional unintended metabolic changes that can be caused by collateral mutations that are often numerous and almost always unknown (Sikora et al., 2011; Glenn et al., 2017; Scientific Advice Mechanism, 2017; Louwaars, 2019).

Classical mutagenesis is one of the methods used in traditional breeding, and the resultant varieties are regulated as conventionally bred plants. Gene editing allows crop breeders to introduce mutations in endogenous genes similar to those that could occur spontaneously or through classical mutagenesis, albeit in a targeted manner, and plants can be verified for the absence of transgenic DNA sequences in the final line. The frequency of off-target mutations from gene editing is significantly lower than the mutation frequency from classical mutagenic breeding (EFSA, 2012; FSANZ, 2019; EFSA, 2020; Graham et al., 2020). Furthermore, various approaches can be used to avoid off-target mutations in plants (Hahn and Nekrasov, 2018; Young et al., 2019; Graham et al., 2020), and, even if some still occur initially, they have a high chance of being segregated out in sexually propagated species during subsequent breeding cycles. Finally, if needed, an off-target mutation can be specifically tracked to confirm its absence in the final gene-edited line. The body of literature reporting the generation of off-target free gene-edited plants keeps growing (Braatz et al., 2017; Ueta et al., 2017; Nekrasov et al., 2017; Tang et al., 2018; Han et al., 2019; al Amin et al., 2019; Zheng et al., 2020; Gao et al., 2020, to mention just a few). Hence, gene editing is expected to cause a similar intended metabolic change as could occur in a conventionally bred variety, but with far fewer unintended metabolic changes (in any at all): i.e., unintended metabolic changes are of a lesser magnitude, more preventable and more manageable than those that can occur in conventionally bred varieties, especially those produced through classical mutational breeding. Standard breeding and evaluation practices conducted for each new variety prior to commercialization, regardless of production method, have proven to be a successful mechanism to eliminate unfavorable changes, resulting in the release of market varieties that are safe for humans, livestock and the environment (Scheben and Edwards, 2017; https://www. betterseed.org/wp-content/uploads/Common-Practices-of-Pla nt-Breeders-Formatted.docx-2.pdf). Therefore, additional safety concerns for gene-edited plants compared with traditionally bred varieties are not warranted and will be poorly informed by metabolomic profiling.

SAFETY

For a quarter of a century, compositional assessment has been a regulatory requirement for transgenic crops as part of the risk assessment, and results have consistently demonstrated fewer and less significant unexpected changes compared with traditionally bred varieties (Herman and Price, 2013). This is expected because of the precision of the genetic changes and the greater knowledge of the outcomes of these changes (Herman et al., 2019b). No unexpected compositional changes related to safety concerns have been observed in hundreds of such studies. The even greater precision and smaller genetic alterations associated with gene editing clearly present an even lower risk of unexpected changes that could lead to a safety concern. Thus, the relative risks of an unexpected compositional change resulting in a safety concern are graded as follows: traditional breeding > genetic engineering > gene editing. It is therefore risk-disproportionate to suggest that metabolomics should be specifically applied to gene-edited crops to address any safety concerns associated with new crop varieties. Why focus methods designed to detect unexpected adverse compositional alterations specifically on crop varieties developed by methods least likely to induce unexpected compositional changes?

ACCEPTANCE

Fraser et al. (2020) wrote the following in their article:

“For example, despite the lack of consumer acceptance, it is impressive that the vast body of multidisciplinary outputs have been able to establish first-generation transgenic crops as a safe technology. Given this endorsement of scientific robustness and approaches, is it not sensible to treat the emerging technologies of
gene editing in crop plants with the same rigor as previously carried out with transgenic technologies? In fact, we can learn from previous successful approaches.’

We agree that the use of genetic engineering and gene editing to develop new crop varieties is well understood by the scientific community to be of no higher risk than developing crop varieties through traditional breeding methods (https://www.pewresearch.org/fact-tank/2015/01/29/5-key-findings-science/; https://matthesian.wordpress.com/2015/01/22/the-pile-of-evidence-on-gmo-safely/; Georges and Ray, 2017; Urnov et al., 2018), yet public fear of genetic engineering remains prevalent. However, public fear has likely been driven and exacerbated by risk-disproportionate regulation which can be perceived by the public as appropriate because of some inherent technology risks (Herman et al., 2020). Jurisdictions with the most risk-disproportionate regulation, like the European Union, have seen the least consumer acceptance of modern breeding methods and the most restricted availability of its products to farmers. Therefore, applying metabolomics in a risk-disproportionate manner to gene-edited crops would be inconsistent with the evidence and experience with transgenic crops (Herman et al., 2019a) and counter to public acceptance.

RISK ASSESSMENT

Risk assessment is a scientific discipline with its own specialized concepts, such as ‘problem formulation’ and ‘pathways to harm’ (Tepfer et al., 2013; Raybould, 2019). Risk assessors working with well-defined operational frameworks select technologies best suited to evaluate identified risks, rather than trying to search for hypothetical risks for which a particular technology might be applied. Ever increasing regulatory requirements resulting from the inappropriate application of new technologies to risk assessment, as opposed to starting with problem formulation and the construction of potential pathways to harm, can transform sound risk assessment into a prescribed checklist of studies. This is already exemplified within many regulatory frameworks for genetically engineered crops, despite overwhelming evidence that such crops do not pose higher risks than varieties developed through traditional breeding (Wolt, 2019). Therefore, manuscripts suggesting the utility of specific technologies to support the risk assessment of crops developed with new breeding methods, such as gene editing, would benefit from input from scientists with expertise in risk assessment. In our opinion, the article written by Fraser et al. (2020) did not take into full consideration the established principles for risk assessment and the deep history of the risk-assessment process applied to crop biotechnology when arguing for metabolomics as a technology for safety assessment.

CONFLICT OF INTEREST

The authors are employed by a company that develops and markets genetically engineered seed.

AUTHOR CONTRIBUTIONS

The authors contributed equally to this work.

REFERENCES

al Amin, N., Ahmad, N., Wu, N., Pu, X., Ma, T., Du, Y., Bo, X., Wang, N., Shard, R. and Wang, P. (2019) CRISPR-Cas9 mediated targeted disruption of FAD2-2 microsomal omega-6 desaturase in soybean (Glycine max L.) BMC Biotechnol. 9(9). https://doi.org/10.1186/s12896-019-0501-2

Braatz, J., Harloff, H.-J., Mascher, M., Stein, N., Himmelbach, A. and Jung, C. (2017) CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoelogous gene copies in polyplody oilseed rape (Brassica napus). Plant Physiol. 174, 935–942. https://doi.org/10.1104/pp.17.00426

Chassy, B.M. (2010) Can-omics inform a food safety assessment? Regul. Toxicol. Pharmacol. 58(3S), S52–S70.

Christ, B., Pluskal, T., Aubry, S. and Weng, J.-K. (2018) Contribution of untargeted metabolomics for future assessment of biotech crops. Trends Plant Sci. 23, 1047–1056.

Delaney, B., Hazebroek, J., Herman, R., Juber, D. and Storer, N.P. (2019) Untargeted metabolomics are not useful in the risk assessment of GM Crops. Trends Plant Sci. 24, 383–384.

EFSA. (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(10), 2943. https://doi.org/10.2903/j.efsa.2012.2943.

EFSA. (2020) Public consultation - Applicability of the EFSA opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. http://www.efsa.europa.eu/sites/default/files/consultation/consultation/Scientific_opinion_SDN1_2_Oligonucleotide_Combined.pdf

FSANZ. (2019) Final report. Review of food derived using new breeding techniques. https://www.foodstandards.gov.au/consumer/gmfood/Documents/NBT%20Final%20report.pdf

Fraser, P.D., Aharoni, A., Hall, R.D., Huang, S., Giovannoni, J.J., Sonnewald, U. and Fernie, A.R. (2020) Metabolomics should be deployed in the identification and characterization of gene-edited crops. Plant J. 102(5), 897–902. https://doi.org/10.1111/tpi.14679

Gao, H., Gadlage, M.J., Lafitte, H.R. et al. (2020) Superior field performance of waxy corn engineered using CRISPR-Cas9. Nat. Biotechnol. 38(5), 579–581.

Georges, F. and Ray, H. (2017) Genome editing of crops: a renewed opportunity for food security. GM Crops & Food 8(11), 1–12.

Glenn, K.C., Alosp, B., Bell, E., Goley, M., Jenkinson, J., Liu, B., Martin, C., Parrott, W., Souder, C. and Sparks, O. (2017) Bringing new plant varieties to market: plant breeding and selection practices advance beneficial characteristics while minimizing unintended changes. Crop Sci. 57, 2906–2921.

Graham, N., Gunvant, B.P. and Bubeck, D.M. et al. (2020) Plant genome editing and the relevance of off-target changes. Plant Physiol. https://doi.org/10.1104/pp.19.01194

Hahn, F. and Nekrasov, V. (2018) CRISPR/Cas precision: do we need to worry about off-targeting in plants? Plant Cell Rep. 38(4), 437–441. https://doi.org/10.1007/s00299-018-2359-5

Han, J., Guo, B., Guo, Y., Zhang, B., Wang, X. and Qiu, L.-J. (2019) Creation of early flowering germplasm of soybean by CRISPR/Cas9 technology. Front. Plant Sci. 10. https://doi.org/10.3389/fpls.2019.01446

Harrigan, G.G. and Chassy, B. (2012) Challenges for metabolomics as a tool in safety assessments. In Metabolomics (Roesner, U., ed). St. Louis: Intech, pp. 331–348.

Herman, R.A., Fedorova, M. and Storer, N.P. (2019a) Will following the regulatory script for GMOs promote public acceptance of gene-edited crops? Trends Biotechnol. 37, 1272–1273.

Herman, R.A., Gaffney, J. and Storer, N.P. (2020) Enlightened oversight of genetically engineered crops for the next generation. Agric. Environ. Lett. 5, e20004. https://doi.org/10.1002/ael.20004

© 2020 Corteva Agriscience. The Plant Journal published by Society for Experimental Biology and John Wiley & Sons Ltd., The Plant Journal, (2020), 103, 1985–1988
Herman, R.A. and Price, W.D. (2013) Unintended compositional changes in genetically modified (GM) crops: 20 years of research. J. Agric. Food Chem. 61, 11695–11701.

Herman, R.A., Zhuang, M., Storer, N.P., Cnudde, F. and Delaney, B. (2019b) Risk-only assessment of genetically engineered crops is risky. Trends Plant Sci. 24, 68–68.

Louwaars, N. (2019) Food safety and plant breeding - why are there no problems in practice? In The functional field of food law (Urazbaeva, A., ed). Wageningen, The Netherlands: Wageningen Academic Press Publishers, pp. 89–101.

Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D. and Kamoun, S. (2017) Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Nat. Sci. Rep. 7(482), 1–6. https://doi.org/10.1038/s41598-017-00578-x

Raybould, A. (2019) Problem formulation and phenotypic characterisation for the development of novel crops. Transgenic Res. 28, 135–145.

Ricroch, A.E., Berge, J.B. and Kuntz, M. (2011) Evaluation of genetically engineered crops using transcriptomic, proteomic and metabolomic profiling techniques. Plant Physiol. 155, 1752–1761.

Scientific Advice Mechanism. (2017) Report. New techniques in agricultural biotechnology. https://op.europa.eu/en/publication-detail/-/publication/103eb49f-4047-11e7-a9b0-01aa75ed71a1/language-en/format-PDF/source-94584105

Scheben, A. and Edwards, D. (2017) Genome editors take on crops. Science, 355, 1122–1123.

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

Sikora, P., Chawade, A., Larsson, M., Olsson, J. and Olsson, O. (2011) Mutagenesis as a tool in plant genetics, functional genomics, and breeding. Int. J. Plant Genom. 2011, 1–13.

Tang, X., Liu, G., Zhou, J. et al. (2018) A large-scale whole-genome sequencing analysis reveals highly specific genome editing in both Cas9 and Cpf1 (Cas12a) nucleases in rice. Genome Biol. 19, 84. https://doi.org/10.1186/s13059-018-1458-5

Tepfer, M., Racovita, M. and Craig, W. (2013) Putting problem formulation at the forefront of GMO risk analysis. GM Crops & Food, 4, 10–15.

Ueta, R., Abe, C., Watanabe, T., Sugano, S.S., Ishihara, R., Ezura, H., Osakabe, Y. and Osakabe, K. (2017) Rapid breeding of parthenocarpic tomato plants using CRISPR/Cas9. Nat. Sci. Rep. 7(507), 1–8. https://doi.org/10.1038/s41598-017-00501-4

Urnov, F.D., Ronald, P.C. and Carroll, D. (2018) A call for science-based review of the European court’s decision on gene-edited crops. Nat. Biotechnol. 36, 800–802.

Wolt, J.D. (2019) Current risk assessment approaches for environmental and food and feed safety assessment. Transgenic Res 28, 111–117.

Young, J., Zastrow-Hayes, G., Deschamps, S. et al. (2019) CRISPR-Cas9 editing in maize: systematic evaluation of off-target activity and its relevance in crop improvement. Nat. Sci. Rep. 9(6729), 1–11. https://doi.org/10.1038/s41598-019-43141-6

Zheng, M., Zhang, L., Tang, M., Liu, J., Liu, H., Yang, H., Fan, S., Terzaghi, W., Wang, H. and Hua, W. (2020) Knockout of two BnaMAX1 homologs with CRISPR/Cas9-targeted mutagenesis improves plant architecture and increases yield in rapeseed (Brassica napus L.). Plant Biotechnol. J. 18(3), 644–654.

© 2020 Corteva Agriscience. The Plant Journal published by Society for Experimental Biology and John Wiley & Sons Ltd., The Plant Journal, (2020), 103, 1985–1988