Published online: 5 June 2019
https://doi.org/10.1038/s41565-019-0468-0

References
1. Conti, J., Satterfield, T. & Harthorn, B. H. *Risk Anal.* **31**, 1734–1748 (2011).
2. Vandermoere, F., Blanchemanche, S., Bieberstein, A., Marette, S. & Roosen, J. *Public Underst. Sci.* **20**, 195–206 (2011).
3. Chen, H. & Yada, R. *Trends Food Sci. Technol.* **22**, 585–594 (2011).
4. Parisi, C., Vigani, M. & Rodríguez-Cerezo, E. *Nano Today* **10**, 124–127 (2015).
5. Yawson, R. M. & Kuzma, J. *J. Consum. Policy* **33**, 299–322 (2010).
6. Gupta, N., Fischer, A. R. H., van der Lans, I. A. & Frewer, L. J. *J. Nanopart. Res.* **14**, 857 (2012).
7. Landry, M. P. & Mitter, N. *Nat. Nanotechnol.* https://doi.org/10.1038/s41565-019-0463-5 (2019).
8. Rip, A. *Sci. Cult.* **15**, 349–365 (2006).
9. Beumer, K. Nanotechnology and Development: Styles of Governance in India, South Africa, and Kenya. PhD thesis, Maastricht Univ. (2016).
10. Felt, U. & Fochler, M. *Minerva* **48**, 219–238 (2010).
11. Laurent, B. *Sci. Eng. Ethics* **17**, 649–666 (2011).
12. Mitzschke, A. *Elusive Publics: Understanding Techno-Scientific Controversy and Democratic Governance in the GM Crops Debate.* PhD thesis, Maastricht Univ. (2018).
13. Beumer, K. & Bhattacharya, S. *Sci. Public Policy* **40**, 628–643 (2013).
14. Agriculture & rural development. *The World Bank* https://data.worldbank.org/topic/agriculture-and-rural-development?locations=IN&view=chart (2019).
15. Indian vice president inaugurates the "Bangalore Nano 2008". *AzoNano* http://www.azonano.com/news.aspx?newsID=9152 (2008).
16. India ranks number one in Google search volume on topics related to nanotechnology. *Newswire Today* http://www.newswiretoday.com/news/6765/ (2006).
17. Romig, A. D. Jr et al. *Technol. Forecast Soc. Change* **74**, 1634–1642 (2007).
18. Wickson, F., Delgado, A. & Kjølberg, K. L. *Nat. Nanotechnol.* **5**, 757–758 (2010).
19. Irwin, A. *Soc. Stud. Sci.* **36**, 299–320 (2006).
20. Dewey, J. *The Public and its Problems* (Swallow, 1927).
21. Marres, N. S. *No Issue, No Public: Democratic Deficits after the Displacement of Politics.* PhD thesis, University of Amsterdam (2005).
22. Capon, A., Gillespie, J., Rolfe, M. & Smith, W. *BMC Public Health* **15**, 424 (2015).
23. Priest, S. H., Bonfadelli, H. & Rusanen, M. *Risk Anal.* **23**, 751–766 (2003).
24. Macoubrie, J. *Public Underst. Sci.* **15**, 221–241 (2006).

# How nanocarriers delivering cargos in plants can change the GMO landscape

Genetically modified organisms as foods are a globally contested topic. What dictates the regulatory oversight of genetically modified crops could be redefined by advances in nanotechnology and genome editing.

Markita P. Landry and Neena Mitter

Few issues are as polarizing as genetically modified organisms (GMOs) as foods, with substantial variability in what constitutes a GMO across different countries. In the advent of new technologies such as genome editing, the landscape of how plant genetic manipulation is accomplished is bound to shift both regulatory purview and public acceptance of GMOs. Today, the development of a genetically modified (GM) crop is estimated to take 13 years of research and development at a cost of US$136 million[1,2], whereby the regulatory definition of a GM crop is defined by the incorporation of foreign DNA into the plant host genome or the use of a bacterial pathogen, features of plant genetic manipulation that are difficult to avoid with current plant transformation technologies. The emerging world of nanotechnology in agriculture has the potential to change the existing paradigm of plant genetic modification by leveraging nanocarriers as shifters of the current GMO landscape. Partnership of nanotechnology and biology has already started to change the contours of this new landscape and to challenge existing legislations.

## Plant transformation standards

Genetic engineering of plants is increasingly important to generate pathogen-resistant and high-yielding crops amid a growing population and changing global climate. The workflow for generating a genetically engineered plant varietal involves delivery of DNA to plants, followed by selection of successful transformants and regeneration of the GM progeny. The former of the two, biomolecule delivery, is uniquely challenging to accomplish in plants, due to the presence of a rigid and multilayered cell wall. Consequently, conventional approaches for biomolecule delivery to most cells, for which the dominant barrier is the lipid membrane, cannot be used for delivery in plants. Two predominant methods for biomolecule delivery in plants are *Agrobacterium* and biolistic delivery, the former of which is only amenable for delivery of DNA targeting nuclear transformations. *Agrobacterium*-mediated delivery is tractable for a limited range of plant species, can only target the nuclear genome, and results in random DNA integration and constitutive expression, which may disrupt endogenous plant genes and limits temporal control over transgene expression. Biolistic delivery of DNA using gold particles involves a high-pressure gene gun and relies on physical disruption of the plant cell wall and membranes, which can yield tissue damage and multiple transgene insertions into random portions of the plant genome. With both delivery modes, integration of transgenic DNA into the plant genome triggers GMO labelling of the transformed plant, if it is to be sold as a consumable.

## Nanocarriers for GM crops

Despite delivery limitations, there are success stories in the generation of GM crops. GM cotton, corn, soybean, canola and sorghum are examples of food and feed crops adding value to the US$5 trillion global agribusiness industry[3]. The economic gains from GM cotton in India[4], GM canola in Australia[5] and recent acceptance of Golden Rice in Bangladesh[6] highlight need-based considerations to meet the challenges of food and nutritional security. However, generation of such crops with current tools, as described above, is laborious and largely subject to GM regulatory purview. Approaches that enable finer control over biomolecule delivery to plants with unassisted internalization through the cell wall could challenge how GM crops are both produced and subsequently regulated. Compared with the approximately 500 nm size exclusion limit of the cell membrane, the plant cell wall excludes particles larger than approximately 5–20 nm (ref. [7]). Nanomaterials, defined as having at least one dimension measuring under 100 nm, thus present a unique opportunity for biomolecule delivery to plants. While nanomaterials have been studied for gene delivery into animal cells, their potential for plant systems is a more recent undertaking. In a pioneering study, mesoporous silica nanoparticles measuring a few hundred nanometres in size were biolistically delivered to plant tissues with a gene gun for delivery of DNA and chemicals[8]. Developments in plant transformation also include delivery of DNA using polyethyleneimine-coated $Fe_3O_4$ magnetic nanoparticles (MNP) as carriers, and applying magentic force to direct the MNP–DNA complexes into the pollen of cotton before pollination[9]. Another key development is the possibility of plant delivery devoid of external force: carbon nanotubes (CNTs) have been shown to diffuse into plant cells and can be chemically modified for DNA delivery to either the nuclear[10] or chloroplast[11] genome. Genetic engineering of the chloroplast genome could assist in transgene containment, as plastid genomes are maternally inherited, and is an exciting prospect for high-yield production of heterologous generation of protein products. CNTs, DNA nanostructures and DNA origami have also shown successful unassisted internalization into plant cells, and can deliver an RNA interference (RNAi) payload for transgene-free gene-silencing applications[12,13]. The latter four studies have shown that nanomaterials can offer the additional advantage of protecting DNA and RNA cargoes from nuclease degradation, extending cargo lifetime once intracellular.

## New non-GM opportunities

Genome-editing tools, such as zinc finger nucleases, transcription activator-like effector nucleases and, more recently, clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9), have greatly advanced genetic engineering. CRISPR–Cas9 has shown success in creating desired traits in plants, including rice, tomato, sorghum, wheat and corn[14], several of which have shown edits can be conserved through multiple plant generations. The ease and throughput of CRISPR–Cas9 have also enabled genome editing to become a popular tool to screen genotype–phenotype relationships in plant breeding programmes. However, CRISPR-based genome editing in plants faces the same issues as traditional plant genetic transformations, in that the DNA (if delivering a CRISPR plasmid) or RNA + protein (if delivering a ribonucleoprotein complex, RNP) delivery into plant cells remains limited by the issue of transport across the cell wall. RNP delivery is largely limited by protein fragility and large size, although a few key studies have demonstrated RNP delivery to cell wall-free protoplasts[15] and plant embryos[16] with biolistic delivery. The more tractable and efficient *Agrobacterium*-mediated delivery of DNA, even if for expression of CRISPR plasmids, triggers GM oversight. As such, nanotechnologies enabling force-independent DNA delivery without transgene integration suggest that transient expression of CRISPR constructs could be leveraged for permanent genome editing without GM labelling in most nations. Importantly, CNT-based DNA delivery has been shown to yield transient transgene expression, where Demirer and colleagues confirmed no integration of the transgene into the nuclear plant genome, producing an enticing opportunity to combine nanotechnology delivery approaches with genome-editing tools[10]. However, what constitutes a GMO is a regulatory semantic that is either product based or process based: for instance, the product-based US GMO pipeline has granted a few dozen CRISPR-based gene knockouts exemption from GM labelling, such as the 'non-browning' mushroom in which nucleases such as CRISPR were used to knock out an oxidase gene that causes pigmentation[17]. Conversely, process-based GM regulations implemented in the European Union are agnostic to how a plant genome is modified (aside from natural recombination or breeding), and have established that CRISPR-edited plants will be regulated as GM foods[18]. It remains to be seen how most nations decide how to classify non-transgenic genome-edited plants, with several countries concurring with product-based regulatory stances, and most remaining undecided[7]. Nonetheless, nanocarriers to deliver plant genetic cargo are likely to play a key role in shaping this dynamic changing regulatory landscape.

## Shifting RNAi applications

Despite much hype, the use of RNAi as a pest management tool is limited to a few successful transgenic crops mainly due to the limitations posed by regulation and acceptance of GM crops. Researchers and industry are seeking innovations in leveraging and exploiting the potential of RNAi for crop protection. Topical application of double-stranded RNAs as the key trigger molecule of RNAi, as direct control agents, as resistance-factor repressors or as developmental disruptors, is gaining momentum. 'RNAi in a drum' as a spray-on technology is being actively pursued by many large, well-established agrochemical companies as a replacement or alternative to chemicals with potential 'green' credentials. However, a major bottleneck for RNAi application in crops is that naked double-stranded RNA, when sprayed on plants, provides a limited protection window of only five to seven days[19–21]. Nanocarriers are emerging as effective translational tools in realizing the commercial viability of topical RNAi application, making nanotechnology and RNAi combined a game changer for the crop protection industry. Examples such as 'BioClay' — layered double hydroxide clay nanoparticles that deliver RNAi as a stable application with increased longevity — are progressing towards translation from the lab to the field, and highlight the significance of nanoparticles as delivery vehicles[22].

As with any technology, RNAi and nanocarriers both have to pass through appropriate regulatory hurdles. The Office of the Gene Technology Regulator in Australia has proposed to categorize the application of RNA molecules to induce RNAi as a technique that is not GM technology provided that the RNA cannot give rise to changes to the genomic sequence and cannot be translated into protein. In the United States, RNAi-based technology regulation falls outside of what constitutes GM and rests with the Environmental Protection Agency[23]. The non-GM status of topical RNAi reduces the costs of segregating GM and conventional produce destined for overseas markets. Global trade of food and agricultural commodities derived from non-GM crops becomes more attainable, and, in this respect, some importing countries maintain 'zero tolerance' policies for commodities derived from GM crops[24]. Topical RNAi is timely in light of a call in Europe for a transition to chemical pesticide-free agriculture[25], which resonates with the European Union Directive 2009/128/EC. Therefore, RNAi-based biopesticides are expected to reach the market in the form of non-GM strategies such as sprays, stem injection, root drenching, seed treatment or other applications. However, the regulatory framework for nanocarriers will have to be addressed on a case-by-case basis and needs particular attention in light of the increasingly prominent use of nanotechnology in plant science. A robust regulatory framework, based on global best practice, is critical to build confidence and certainty; it underpins public investment and ensures collective efforts to address global challenges in agriculture.

Agricultural biotechnology is at the core of global food security. With its central role in our collective futures, it is unsurprising that the optimization of plant health and crop yields has accrued enthusiasm; however, the approach of genetic modification of consumable crops has been met with incommensurate scepticism from the public. Recent advances in genome editing and parallel developments in nanotechnology stand to improve the precision and throughput of generating GM plants, which could serve the dual purpose of expediting crop research and bypassing GM regulatory oversight, while also assuaging public concern over pathogen-based approaches to creating GM crops. Non-integrating delivery of DNA plasmids to crops can enable non-GM gene knockouts, whereas RNAi technology assisted by nanocarriers could operationally constitute a non-GM approach. The general public is now seeking a transition to chemical pesticide-free agriculture, a view that is gaining traction globally and for which both GM and non-GM approaches can contribute significant benefits. Nanotechnology applications in agriculture could enable the next transition in the food and agribusiness sector. ❐

Markita P. Landry[1,2,3]* and Neena Mitter[4]*
[1]*Department of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA, USA.* [2]*Innovative Genomics Institute, Berkeley, CA, USA.* [3]*Chan-Zuckerberg Biohub, San Francisco, CA, USA.* [4]*Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Queensland, Australia.*
**e-mail: landry@berkeley.edu; n.mitter@uq.edu.au*

Published online: 5 June 2019
https://doi.org/10.1038/s41565-019-0463-5

## References

1. Smyth, S. J., Falck-Zepeda, J. & Ludlow, K. *Estey J. Int. Law Trade Policy* **17**, 173–195 (2016).
2. *CropLinks* Spring Edition (CropLife Australia, 2018); https://www.croplife.org.au/resources/crop-links/croplinks-spring-edition-2018/
3. Agribusiness. *The World Bank* http://www.worldbank.org/en/topic/agriculture/brief/help-farmers-reach-markets (2017).
4. Burkitbayeva, S., Qaim, M. & Swinnen, J. *Trends Biotechnol.* **34**, 260–263 (2016).
5. Biden, S., Smyth, S. J. & Hudson, D. *GM Crops Food* **9**, 13–20 (2018).
6. Owens, B. *Nat. Biotechnol.* **36**, 559–560 (2018).
7. Cunningham, F. J., Goh, N. S., Demirer, G. S., Matos, J. L. & Landry, M. P. *Trends Biotechnol.* **36**, 882–897 (2018).
8. Torney, F., Trewyn, B. G., Lin, V. S. Y. & Wang, K. *Nat. Nanotechnol.* **2**, 295–300 (2007).
9. Zhao, X. et al. *Nat. Plants* **3**, 956–964 (2017).
10. Demirer, G. S. et al. *Nat. Nanotechnol.* https://doi.org/10.1038/s41565-019-0382-5 (2019).
11. Kwak, S. Y. et al. *Nat. Nanotechnol.* https://doi.org/10.1038/s41565-019-0375-4 (2019).
12. Zhang, H. et al. *Proc. Natl Acad. Sci. USA* **116**, 7543–7548 (2019).
13. Demirer, G. S., Zhang, H., Goh, N., Chang, R. & Landry, M. P. *SSRN* https://doi.org/10.2139/ssrn.3352632 (2019).
14. Bortesi, L. & Fischer, R. *Biotechnol. Adv.* **33**, 41–52 (2015).
15. Woo, J. W. et al. *Nat. Biotechnol.* **33**, 1162–1164 (2015).
16. Svitashev, S., Schwartz, C., Lenderts, B., Young, J. K. & Mark Cigan, A. *Nat. Commun.* **7**, 13274 (2016).
17. Waltz, E. *Nature* **532**, 293 (2016).
18. Callaway, E. *Nature* **560**, 16 (2018).
19. Tenllado, F., Martínez-García, B., Vargas, M. & Díaz-Ruíz, J. R. *BMC Biotechnol.* **3**, 3 (2003).
20. Mitter, N., Worrall, E. A., Robinson, K. E., Xu, Z. P. & Carroll, B. J. *Curr. Opin. Virol.* **26**, 49–55 (2017).
21. Wang, M. et al. *Nat. Plants* **2**, 16151 (2016).
22. Mitter, N. et al. *Nat. Plants* **3**, 16207–16207 (2017).
23. Acosta, L. *Restrictions on Genetically Modified Organisms: United States* (Library of Congress, 2014).
24. Gray, P. et al. *Synthetic Biology in Australia: An Outlook to 2030* (Australian Council of Learned Academies, 2018).
25. Albouy, I., Huyghe, C. & Ewert, F. *Towards Chemical Pesticide-Free Agriculture* (INRA, 2018); https://inra-dam-front-resources-cdn.brainsonic.com/ressources/afile/442690-5075f-resource-priroites-scientifiques-horizon-europe-food-2030-pesticide.pdf