

# Sunday Evening News No 269

Week 10 (2022-07-03 – 2022-13-03)

Selected and edited by **BGF** Jany

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**Mr. Putin stop this war - show moral greatness and end the suffering of the population and the death of civilians**

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## Press Releases – Media Reports / Pressemeldungen und Medienberichte

**BUND: Neue Gentechnik: Leere Versprechungen für Klima und Landwirtschaft**

<https://www.bund.net/service/presse/pressemitteilungen/detail/news/neue-gentechnik-leere-versprechungen-fuer-klima-und-landwirtschaft/>

**Theresa D.: What is stopping gene-edited food from saving our planet?**

Not science, apparently

<https://interestingengineering.com/gene-edited-food-saving-our-planet>

**Choi A.: Gene-edited beef cattle receive regulatory clearance in U.S.**

<https://www.latimes.com/science/story/2022-03-08/gene-edited-beef-cattle-get-regulatory-clearance-in-us>

**McNamara P.: New genomic techniques – saviour or nemesis?**

<https://www.newfoodmagazine.com/article/161750/new-genomic-techniques-saviour-or-nemesis/>

**RiskMonger: The Green Death: How the EU's Farm2Famine Strategy will Affect a Post-Ukraine World**

<https://risk-monger.com/2022/03/06/the-green-death-how-the-eus-farm2famine-strategy-will-affect-a-post-ukraine-world/>

Only some selected press releases or media reports are listed here. The daily up-date of the press releases and media reports are [▶ here](#): March week 09)

## Publications – Publikationen

Sturme M.H.J., van der Berg J.P., Lianne M. S. Bouwman L.M.S., De Schrijver A., Ruud A. de Maagd R.A., Kleter G.A, Battaglia-de Wilde E. (2022): **Occurrence and Nature of Off-Target Modifications by CRISPR-Cas Genome Editing in Plants**. ACS Agric. Sci. Technol |

<https://doi.org/10.1021/acsagscitech.1c00270>

CRISPR-Cas-based genome editing allows for precise and targeted genetic modification of plants. Nevertheless, unintended off-target edits can arise that might confer risks when present in gene-edited food crops. Through an extensive literature review we gathered information on CRISPR-Cas off-target edits in plants. Most observed off-target changes were small insertions or deletions (1–22 bp) or nucleotide substitutions, and large deletions (>100 bp) were rare. One study detected the insertion of vector-derived DNA sequences, which is important considering the risk assessment of gene-edited plants. Off-target sites had few mismatches (1–3 nt) with the target sequence and were mainly located in protein-coding regions, often in target gene homologues. Off-targets edits were predominantly detected via biased analysis of predicted off-target sites instead of unbiased genome-wide analysis. CRISPR-Cas-edited plants showed lower off-target mutation frequencies than conventionally bred plants. This Review can aid discussions on the relevance of evaluating off-target modifications for risk assessment of CRISPR-Cas-edited plants.

<https://pubs.acs.org/doi/pdf/10.1021/acsagscitech.1c00270>

Varghese M., Malhotra B., Bisht N.C. (2022): **Genome Editing in Polyploid Brassica Crops**. In: Kole C., Mohapatra T. (eds) *The Brassica juncea Genome*. Compendium of Plant Genomes. Springer, Cham. [https://doi.org/10.1007/978-3-030-91507-0\\_25](https://doi.org/10.1007/978-3-030-91507-0_25)

[https://doi.org/10.1007/978-3-030-91507-0\\_25](https://doi.org/10.1007/978-3-030-91507-0_25)

Genome editing technology like the CRISPR/Cas9 has emerged as an excellent and rapid plant breeding tool for exploring basic plant biology as well as for increasing agricultural GDPs. Although CRISPR-based genome editing had shown its applicability in different plant genomes since 2013, the genus *Brassica* has come across this precise gene modification tool only since 2015. In polyploid *Brassica* species, CRISPR/Cas9 technology has been

established in two major aspects; firstly, for the functional validation of novel genes and secondly, for improving various agriculturally important traits, like for example the enhancements of seed numbers and oil contents as well as downregulating the susceptibility genes responsible for various abiotic and biotic stresses. With the current chapter, we explicitly bring about all the case studies from the advent of CRISPR/Cas based mutagenesis in all the species so far, of the agriculturally significant genus *Brassica* from a period of half a decade to present. The current chapter not only combines all the CRISPR/Cas9 genome editing reports published exclusively from the *Brassica* species but also succinctly discusses the possibilities and challenges for overcoming its shortcomings in the case of polyploid crops. Additionally, we bring about the opportunities that remain open for targeting the key desired traits towards fulfilling the breeding milestones of the Indian oilseed mustard, *Brassica juncea*.

[https://link.springer.com/chapter/10.1007/978-3-030-91507-0\\_25](https://link.springer.com/chapter/10.1007/978-3-030-91507-0_25)

He, F., Wang, W., Rutter, W.B. et al. (2022): **Genomic variants affecting homoeologous gene expression dosage contribute to agronomic trait variation in allopolyploid wheat.** Nat Commun 13, 826 | <https://doi.org/10.1038/s41467-022-28453-y>

Allopolyploidy greatly expands the range of possible regulatory interactions among functionally redundant homoeologous genes. However, connection between the emerging regulatory complexity and expression and phenotypic diversity in polyploid crops remains elusive. Here, we use diverse wheat accessions to map expression quantitative trait loci (eQTL) and evaluate their effects on the population-scale variation in homoeolog expression dosage. The relative contribution of *cis*- and *trans*-eQTL to homoeolog expression variation is strongly affected by both selection and demographic events. Though *trans*-acting effects play major role in expression regulation, the expression dosage of homoeologs is largely influenced by *cis*-acting variants, which appear to be subjected to selection. The frequency and expression of homoeologous gene alleles showing strong expression dosage bias are predictive of variation in yield-related traits, and have likely been impacted by breeding for increased productivity. Our study highlights the importance of genomic variants affecting homoeolog expression dosage in shaping agronomic phenotypes and points at their potential utility for improving yield in polyploid crops.

<https://www.nature.com/articles/s41467-022-28453-y.pdf>

Sun, H., Jiao, W.B., Krause, K. et al. (2022): **Chromosome-scale and haplotype-resolved genome assembly of a tetraploid potato cultivar.** Nat Genet |

<https://doi.org/10.1038/s41588-022-01015-0>

Potato is the most widely produced tuber crop worldwide. However, reconstructing the four haplotypes of its autotetraploid genome remained an unsolved challenge. Here, we report the 3.1 Gb haplotype-resolved (at 99.6% precision), chromosome-scale assembly of the potato cultivar 'Otava' based on high-quality long reads, single-cell sequencing of 717 pollen genomes and Hi-C data. Unexpectedly, ~50% of the genome was identical-by-descent due to recent inbreeding, which was contrasted by highly abundant structural rearrangements involving ~20% of the genome. Among 38,214 genes, only 54% were present in all four haplotypes with an average of 3.2 copies per gene. Taking the leaf transcriptome as an example, 11% of the genes were differently expressed in at least one haplotype, where 25% of them were likely regulated through allele-specific DNA methylation. Our work sheds light on the recent breeding history of potato, the functional organization of its tetraploid genome and has the potential to strengthen the future of genomics-assisted breeding.

<https://www.nature.com/articles/s41588-022-01015-0.pdf>

Jordan N.R., Kuzma J., Ray D.K., Foot K. et al. (2022): **Should Gene Editing Be Used to Develop Crops for Continuous-Living-Cover Agriculture? A Multi-Sector Stakeholder Assessment Using a Cooperative Governance Approach.** Front. Bioeng. Biotechnol |

<https://doi.org/10.3389/fbioe.2022.843093>

Continuous-living-cover (CLC) agriculture integrates multiple crops to create diversified agroecosystems in which soils are covered by living plants across time and space continuously. CLC agriculture can greatly improve production of many different ecosystem services from agroecosystems, including climate adaptation and mitigation. To go to scale, CLC agriculture requires crops that not only provide continuous living cover but are viable in economic and social terms. At present, lack of such viable crops is strongly limiting the scaling of CLC agriculture. Gene editing (GE) might provide a powerful tool for developing the crops needed to expand CLC agriculture to scale. To assess this possibility, a broad multi-sector deliberative group considered the merits of GE—relative to alternative plant-breeding methods—as means for improving crops for CLC agriculture. The group included many of the sectors whose support is necessary to scaling agricultural innovations, including actors involved in markets, finance, policy, and R&D. In this article, we report findings from interviews and deliberative workshops. Many in the group were enthusiastic about prospects for applications of GE to develop crops for CLC agriculture, relative to alternative plant-breeding options. However, the group noted many issues, risks, and contingencies, all of which are likely to require responsive and adaptive management. Conversely, if these issues, risks, and contingencies cannot be managed, it appears unlikely that a strong multi-sector base of support can be sustained for such applications, limiting their scaling. Emerging methods for responsible innovation and scaling have potential to manage these issues, risks, and contingencies; we propose that outcomes from GE crops for CLC agriculture are likely to be much improved if these emerging methods are used to govern such projects. However, both GE of CLC crops and responsible innovation and scaling are unrefined innovations. Therefore, we suggest that the best pathway for exploring GE of CLC crops is to intentionally couple implementation and refinement of both kinds of innovations. More broadly, we argue that

such pilot projects are urgently needed to navigate intensifying grand challenges around food and agriculture, which are likely to create intense pressures to develop genetically-engineered agricultural products and equally intense social conflict.

<https://www.frontiersin.org/articles/10.3389/fbioe.2022.843093/full>

Zhang D., Dong S., Zhang Z., Yu C. Xu J., Wang C., Liu Y. (2022): **Evaluation of the impact of transgenic maize BT799 on growth, development and reproductive function of Sprague-Dawley rats in three generations.** Food and Chemical Toxicology 160, 112776 |

<https://doi.org/10.1016/j.fct.2021.112776>

BT799 was *Bacillus thuringiensis*-genetic modified (GM) maize, and Sprague-Dawley (SD) rats were treated with different diet formulations containing BT799 maize grain (33% and 66%) or its non-transgenic Zhengdan 958 (ZD958, 33% and 66%). The feeding lasted for 10 (P)/14 (F1 and F2) weeks. The reproductive capacity and pathological responses were detected in each generation of rats fed with BT799 and ZD958. During the growth and development of parental rats, each group showed the same trend in body weight gain and food intake, with a few fluctuations at individual time points. No statistically significant difference was observed in reproductive data (copulation index, fertility index, and live birth rate) of rats fed with transgenic maize compared with non-transgenic maize. We observed some apparent changes in reproductive data (sperm numbers and motility) and pathological responses (organ relative weights, hematological parameters, serum chemistry parameters, and sex hormone levels) among rats fed with BT799 maize grain. However, these differences were within the laboratory's historical normal range of control SD rats and not maize grain dose-dependent. These changes were not considered to be adverse or toxic. No significant difference in macroscopic or histological adverse effects was observed between rats consuming transgenic BT799 diet and non-transgenic diet. In conclusion, the long-term intake of BT799 maize was as safe as the corresponding non-transgenic maize for three-generation SD rats.

<https://www.sciencedirect.com/science/article/abs/pii/S0278691521008097>

de Albuquerque, T.M., Mendes, L.W., Rocha, S.M.B. et al.(2022): **Genetically related genotypes of cowpea present similar bacterial community in the rhizosphere.** Sci Rep 12, 3472 (2022). <https://doi.org/10.1038/s41598-022-06860-x>

Plant breeding reduces the genetic diversity of plants and could influence the composition, structure, and diversity of the rhizosphere microbiome, selecting more homogeneous and specialized microbes. In this study, we used 16S rRNA sequencing to assess the bacterial community in the rhizosphere of different lines and modern cowpea cultivars, to investigate the effect of cowpea breeding on bacterial community assembly. Thus, two African lines (IT85F-2687 and IT82D-60) and two Brazilian cultivars (BRS-Guariba and BRS-Tumucumaque) of cowpea were assessed to verify if the generation advance and genetic breeding influence the bacterial community in the rhizosphere. No significant differences were found in the structure, richness, and diversity of bacterial community structure between the rhizosphere of the different cowpea genotypes, and only slight differences were found at the OTU level. The complexity of the co-occurrence network decreased from African lines to Brazilian cultivars. Regarding functional prediction, the core functions were significantly altered according to the genotypes. In general, African lines presented a more abundance of groups related to chemoheterotrophy, while the rhizosphere of the modern cultivars decreased functions related to cellulolysis. This study showed that the genetic breeding process affects the dynamics of the rhizosphere community, decreasing the complexity of interaction in one cultivar. As these cowpea genotypes are genetically related, it could suggest a new hypothesis of how genetic breeding of similar genotypes could influence the rhizosphere microbiome.

<https://www.nature.com/articles/s41598-022-06860-x.pdf>

Thagun C., Horii Y., Mori M., Fujita S. et al. (2022): **Non-transgenic Gene Modulation via Spray Delivery of Nucleic Acid/Peptide Complexes into Plant Nuclei and Chloroplasts**

ACS-Nano | <https://doi.org/10.1021/acsnano.1c07723>

Genetic engineering of economically important traits in plants is an effective way to improve global welfare. However, introducing foreign DNA molecules into plant genomes to create genetically engineered plants not only requires a lengthy testing period and high developmental costs but also is not well-accepted by the public due to safety concerns about its effects on human and animal health and the environment. Here, we present a high-throughput nucleic acids delivery platform for plants using peptide nanocarriers applied to the leaf surface by spraying. The translocation of sub-micrometer-scale nucleic acid/peptide complexes upon spraying varied depending on the physicochemical characteristics of the peptides and was controlled by a stomata-dependent-uptake mechanism in plant cells. We observed efficient delivery of DNA molecules into plants using cell-penetrating peptide (CPP)-based foliar spraying. Moreover, using foliar spraying, we successfully performed gene silencing by introducing small interfering RNA molecules in plant nuclei *via* siRNA-CPP complexes and, more importantly, in chloroplasts *via* our CPP/chloroplast-targeting peptide-mediated delivery system. This technology enables effective nontransgenic engineering of economically important plant traits in agricultural systems.

<https://pubs.acs.org/doi/10.1021/acsnano.1c07723>

<https://pubs.acs.org/doi/pdf/10.1021/acsnano.1c07723>

Straw E.A., Thompson L.J., Leadbeater L., Brown M.J.F. (2022): **'Inert' ingredients are understudied, potentially dangerous to bees and deserve more research attention.** Proc. R. Soc. B.2892021235320212353 | <https://doi.org/10.1098/rspb.2021.2353>

Agrochemical formulations are composed of two broad groups of chemicals: active ingredients, which confer pest control action, and 'inert' ingredients, which facilitate the action of the active ingredient. Most research into the effects of agrochemicals focusses on the effects of active ingredients. This reflects the assumption that 'inert' ingredients are non-toxic. A review of relevant research shows that for bees, this assumption is without empirical foundation. After conducting a systematic literature search, we found just 19 studies that tested the effects of 'inert' ingredients on bee health. In these studies, 'inert' ingredients were found to cause mortality in bees through multiple exposure routes, act synergistically with other stressors and cause colony level effects. This lack of research is compounded by a lack of diversity in study organism used. We argue that 'inert' ingredients have distinct, and poorly understood, ecological persistency profiles and toxicities, making research into their individual effects necessary. We highlight the lack of mitigation in place to protect bees from 'inert' ingredients and argue that research efforts should be redistributed to address the knowledge gap identified here. If so-called 'inert' ingredients are, in fact, detrimental to bee health, their potential role in widespread bee declines needs urgent assessment.

<https://royalsocietypublishing.org/doi/10.1098/rspb.2021.2353>

Hallerman, E.M., Bredlau, J.P., Camargo, L.S.A. et al. (2022): **Towards progressive regulatory approaches for agricultural applications of animal biotechnology.** Transgenic Res | <https://doi.org/10.1007/s11248-021-00294-3>

Traditional breeding techniques, applied incrementally over thousands of years, have yielded huge benefits in the characteristics of agricultural animals. This is a result of significant, measurable changes to the genomes of those animal species and breeds. Genome editing techniques may now be applied to achieve targeted DNA sequence alterations, with the potential to affect traits of interest to production of agricultural animals in just one generation. New opportunities arise to improve characteristics difficult to achieve or not amenable to traditional breeding, including disease resistance, and traits that can improve animal welfare, reduce environmental impact, or mitigate impacts of climate change. Countries and supranational institutions are in the process of defining regulatory approaches for genome edited animals and can benefit from sharing approaches and experiences to institute progressive policies in which regulatory oversight is scaled to the particular level of risk involved. To facilitate information sharing and discussion on animal biotechnology, an international community of researchers, developers, breeders, regulators, and communicators recently held a series of seven virtual workshop sessions on applications of biotechnology for animal agriculture, food and environmental safety assessment, regulatory approaches, and market and consumer acceptance. In this report, we summarize the topics presented in the workshop sessions, as well as discussions coming out of the breakout sessions. This is framed within the context of past and recent scientific and regulatory developments. This is a pivotal moment for determination of regulatory approaches and establishment of trust across the innovation through-chain, from researchers, developers, regulators, breeders, farmers through to consumers. <https://link.springer.com/content/pdf/10.1007/s11248-021-00294-3.pdf>

## Industrial Biotechnology

Verma A.K., Chettri D., Verma A.K. (2022): **Potential of CRISPR/Cas9-Based Genome Editing in the Fields of Industrial Biotechnology: Strategies, Challenges, and Applications.** In: Verma P. (eds) Industrial Microbiology and Biotechnology. Springer, Singapore. [https://doi.org/10.1007/978-981-16-5214-1\\_23](https://doi.org/10.1007/978-981-16-5214-1_23)

The development of the system clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) represented a major breakthrough in industrial biotechnology. CRISPR/Cas9 is a highly orthogonal and versatile system and has stimulated research in the fields of metabolic engineering, systems biology, and synthetic biology. The CRISPR/Cas9 toolbox includes CRISPR editing, CRISPR activation, CRISPR interference, and CRISPR-mediated protein imaging. These basic tools can be used to model the genome and program the desired gene expression, paving the way for efficient genomic and transcriptomic manipulations to optimize the efficiency of microorganisms. These CRISPR/Cas9 techniques can be further improved for the overproduction of the desired enzymes. The method has been successfully applied to a number of industrially important microorganisms including bacteria, yeast, and filamentous fungi. However, the efficiency of the CRISPR/Cas9 system's gene processing is still unsatisfactory, and several versatile models of this method can be designed for better efficiency.

[https://link.springer.com/chapter/10.1007/978-981-16-5214-1\\_23](https://link.springer.com/chapter/10.1007/978-981-16-5214-1_23)

Löhr N.A. Eisen F., Wiebke Thiele W., Platz L. et al. (2022): **Unprecedented Mushroom Polyketide Synthases Produce the Universal Anthraquinone Precursor,** Angew. Chem. Int. Ed. 2022, e202116142 | <https://doi.org/10.1002/anie.202116142>

(Pre-)anthraquinones are widely distributed natural compounds and occur in plants, fungi, microorganisms, and animals, with atochryson (1) as the key biosynthetic precursor. Chemical analyses established mushrooms of the genus *Cortinarius* –the webcaps– as producers of atochryson-derived octaketide pigments. However, more recent genomic data did not provide any evidence for known atochryson carboxylic acid (4) synthases nor any other PKS producing oligocyclic metabolites. Here, we describe an unprecedented class of non-reducing polyketide synthases (PKS). In vitro assays with recombinant enzyme in

combination with in vivo product formation in the heterologous host *Aspergillus niger* established CoPKS1 and CoPKS4 of *C. odorifer* as members of a new class of atrochrysonic carboxylic acid synthases. CoPKS4 catalyzed both hepta- and octaketide synthesis and yielded 6-hydroxymusizin (6), along with 4. These first oligocyclic mushroom PKSs illustrate how the biosynthesis of bioactive natural metabolites independently evolved in various groups of life.

<https://onlinelibrary.wiley.com/doi/10.1002/anie.202116142>

Zhuo C., Wang X., Docampo-Palacios M., Sanders B. C., Engle N.L. et al. (2022): **Developmental changes in lignin composition are driven by both monolignol supply and laccase specificity**, *Science Advances* 8 (10) | DOI: [10.1126/sciadv.abm8145](https://doi.org/10.1126/sciadv.abm8145)

The factors controlling lignin composition remain unclear. Catechyl (C)-lignin is a homopolymer of caffeyl alcohol with unique properties as a biomaterial and precursor of industrial chemicals. The lignin synthesized in the seed coat of *Cleome hassleriana* switches from guaiacyl (G)- to C-lignin at around 12 to 14 days after pollination (DAP), associated with a rerouting of the monolignol pathway. Lack of synthesis of caffeyl alcohol limits C-lignin formation before around 12 DAP, but coniferyl alcohol is still synthesized and highly accumulated after 14 DAP. We propose a model in which, during C-lignin biosynthesis, caffeyl alcohol noncompetitively inhibits oxidation of coniferyl alcohol by cell wall laccases, a process that might limit movement of coniferyl alcohol to the apoplast. Developmental changes in both substrate availability and laccase specificity together account for the metabolic fates of G- and C-monolignols in the *Cleome* seed coat.

<https://www.science.org/doi/10.1126/sciadv.abm8145>

<https://www.science.org/doi/epdf/10.1126/sciadv.abm8145>

#### EFSA:

GMO Panel (2022): Scientific Opinion on the Statement complementing the EFSA Scientific Opinion on the assessment of genetically modified oilseed rape MS11 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-BE-2016-138). *EFSA Journal* 20 (3):7190, 7 pp. <https://doi.org/10.2903/j.efsa.2022.7190>  
<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2022.7190>

CEP Panel (2022): Scientific Opinion on the safety evaluation of the food enzyme glucan 1,4 $\alpha$ -glucosidase from the genetically modified *Aspergillus niger* strain NZYM-BR. *EFSA Journal* 20 (3): 7191, 13 pp. | <https://doi.org/10.2903/j.efsa.2022.7191>  
<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2022.7191>

CEP Panel (2022). Scientific Opinion on the safety evaluation of the food enzyme non-reducing end  $\alpha$ -L-arabinofuranosidase from the genetically modified *Trichoderma reesei* strain NZYM-GV. *EFSA Journal* 20 (3): 7173, 12 pp. | <https://doi.org/10.2903/j.efsa.2022.7173>  
<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2022.7173>

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Wie immer wird für Hinweise und der Zusendung von Publikationen und sonstigen Informationen gedankt. pdf-Dateien können meist direkt aus den links heruntergeladen werden.

Bitte besuchen sie auch die Webseite des Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG): [www.wgg-ev.de](http://www.wgg-ev.de).

*As always, I thank you all for hints and for publications. Most of the pdf files can be downloaded directly from the links.*

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