

# Sunday Evening News No 347

2023-10-09 – 2023-10-15

Compiled and edited by **BGF** Jany



## Meetings – Conferences / Tagungen - Konferenzen

EU-Kommission: **Neue Züchtungstechniken als Beitrag zur Bewältigung multipler Krisen des 21. Jahrhunderts**

Mittwoch, 8. November 2023, 18:00 - 20:30 (CET)

Europäisches Haus

Unter den Linden 78, 10117 Berlin, Deutschland

[https://germany.representation.ec.europa.eu/events/neue-zuechtungstechniken-als-beitrag-zur-bewaeltigung-multipler-krisen-des-21-jahrhunderts-2023-11-08\\_de](https://germany.representation.ec.europa.eu/events/neue-zuechtungstechniken-als-beitrag-zur-bewaeltigung-multipler-krisen-des-21-jahrhunderts-2023-11-08_de)

## Press Releases -Media / Presse- und Medienberichte

EU-Commission: **Commission authorises three genetically modified maize varieties as food and animal feed but not for cultivation, and renews the authorisation of another**

(MON 89034 x 1507 x MIR 162xNK 603xDAS-40278-9; GA21 x T25; MON 87419; MIR 162 (renew))

[https://ec.europa.eu/commission/presscorner/detail/en/mex\\_23\\_4963](https://ec.europa.eu/commission/presscorner/detail/en/mex_23_4963)

Think-Thank Commission: **BRIEFING - EU Legislation in Progress: Plants produced by new genomic techniques**

[https://www.europarl.europa.eu/RegData/etudes/BRIE/2023/754549/EPRS\\_BRI\(2023\)754549\\_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/BRIE/2023/754549/EPRS_BRI(2023)754549_EN.pdf)

Bertoli D., Miller H.: **Viewpoint: Crop biotechnology skeptics — who insist that elusive ‘consumer acceptance’ should trump science — block agricultural innovation**

<https://geneticliteracyproject.org/2023/10/10/viewpoint-crop-biotechnology-skeptics-who-insist-that-elusive-consumer-acceptance-should-trump-science-block-agricultural-innovation/>

Informationsdienst Gentechnik: **Neue Gentechnik: Politik und Verbände fordern Patentregelung**

<https://www.keine-gentechnik.de/nachricht/34827?cHash=17041ed3a7479ebf7130f5893931e3bb>

Expert:innenkonferenz: **Kritik an EU-Plänen zur Neuen Gentechnik**

[https://www.bmk.gv.at/service/presse/gewessler/20231011\\_gentechnik.html](https://www.bmk.gv.at/service/presse/gewessler/20231011_gentechnik.html)

Bundesrat: TOP 30: [328/23 Vorschlag für eine Verordnung des Europäischen Parlaments und des Rates über mit bestimmten neuen genomischen Techniken gewonnene Pflanzen und die aus ihnen gewonnenen Lebens- und Futtermittel sowie zur Änderung der Verordnung \(EU\) 2017/625](#)

(TOP 30):

<https://www.bundesrat.de/SharedDocs/TO/1037/to-node.html>

Empfehlungen sind unter folgendem link eingestellt: <https://dserver.bundestag.de/brd/2023/0328-1-23.pdf>

Only some selected press releases or media reports are listed here. The daily up-date of the press releases and media reports are [▶ here](#): October week 41

## Publications – Publikationen

N.O. Brain (2023): Letter to the editor to the article: ‘**The presence of carcinogenic compounds in gene-edited NGOs causes cancer in humans**’. Arch Toxicol **97**, 3303 <https://doi.org/10.1007/s00204-023-03594-1>

**Conflict of interest**

This article was written under the influence (WUI). The mental capacity of the author may have been affected by a bacterial infection and medication.

<https://link.springer.com/article/10.1007/s00204-023-03594-1>

Idoko-Akoh, A., Goldhill, D.H., Sheppard, C.M. et al. (2023): **Creating resistance to avian influenza infection through genome editing of the ANP32 gene family**. Nat Commun **14**, 6136 | <https://doi.org/10.1038/s41467-023-41476-3>

Chickens genetically resistant to avian influenza could prevent future outbreaks. In chickens, influenza A virus (IAV) relies on host protein ANP32A. Here we use CRISPR/Cas9 to generate homozygous gene edited (GE) chickens containing two ANP32A amino acid substitutions that prevent viral polymerase interaction. After IAV challenge, 9/10 edited chickens remain uninfected. Challenge with a higher dose, however, led to breakthrough infections. Breakthrough IAV virus contained IAV polymerase gene mutations that conferred adaptation to the edited chicken ANP32A. Unexpectedly, this virus also replicated in chicken embryos edited to remove the entire ANP32A gene and instead co-opted alternative ANP32 protein family members, chicken ANP32B and ANP32E. Additional genome editing for removal of ANP32B and ANP32E eliminated all viral growth in chicken cells. Our data illustrate a first proof of concept step to generate IAV-resistant chickens and show that multiple genetic modifications will be required to curtail viral escape.

<https://www.nature.com/articles/s41467-023-41476-3>

Dima O., Custers R.; De Veirman L., Inzé D. (2023): EU legal proposal for genome-edited crops hints at a science-based approach. Trends in Plant Science |

<https://doi.org/10.1016/j.tplants.2023.09.014>

The European Commission (EC) recently published a legislative proposal that hints at a science-based approach to the regulation of genome-editing applications in crops in the EU. This would be in line with legislation in an increasing number of countries worldwide, but further science-based advice on implementation will be essential.

<https://www.cell.com/action/showPdf?pii=S1360-1385%2823%2900307-2>

Guyomard, H., Soler, LG., Détang-Dessendre, C. et al. (2023): **The European Green Deal improves the sustainability of food systems but has uneven economic impacts on consumers and farmers.** Commun Earth Environ 4, 358 | <https://doi.org/10.1038/s43247-023-01019-6>

The European Green Deal aims notably to achieve a fair, healthy, and environmentally friendly food system in the European Union. We develop a partial equilibrium economic model to assess the market and non-market impacts of the three main levers of the Green Deal targeting the food chain: reducing the use of chemical inputs in agriculture, decreasing post-harvest losses, and shifting toward healthier average diets containing lower quantities of animal-based products. Substantially improving the climate, biodiversity, and nutrition performance of the European food system requires jointly using the three levers. This allows a 20% reduction in greenhouse gas emissions of food consumption and a 40–50% decrease in biodiversity damage. Consumers win economically thanks to lower food expenditures. Livestock producers lose through quantity and price declines. Impacts on revenues of food/feed field crop producers are positive only when the increase in food consumption products outweighs the decrease in feed consumption.

<https://www.nature.com/articles/s43247-023-01019-6>

Gojon, A., Nussaume, L., Luu, D. T., Murchie, E. H., Baekelandt, A. et al. (2023): **Approaches and determinants to sustainably improve crop production.** Food and Energy Security, 12, e369. |

<https://doi.org/10.1002/fes3.369>

Plant scientists and farmers are facing major challenges in providing food and nutritional security for a growing population, while preserving natural resources and biodiversity. Moreover, this should be done while adapting agriculture to climate change and by reducing its carbon footprint. To address these challenges, there is an urgent need to breed crops that are more resilient to suboptimal environments. Huge progress has recently been made in understanding the physiological, genetic and molecular bases of plant nutrition and environmental responses, paving the way towards a more sustainable agriculture. In this review, we present an overview of these progresses and strategies that could be developed to increase plant nutrient use efficiency and tolerance to abiotic stresses. As illustrated by many examples, they already led to promising achievements and crop improvements. Here, we focus on nitrogen and phosphate uptake and use efficiency and on adaptation to drought, salinity and heat stress. These examples first show the necessity of deepening our physiological and molecular understanding of plant environmental responses. In particular, more attention should be paid to investigate stress combinations and stress recovery and acclimation that have been largely neglected to date. It will be necessary to extend these approaches from model plants to crops, to unravel the relevant molecular targets of biotechnological or genetic strategies directly in these species. Similarly, sustained efforts should be done for further exploring the genetic resources available in these species, as well as in wild species adapted to unfavourable environments. Finally, technological developments will be required to breed crops that are more resilient and efficient. This especially relates to the development of multiscale phenotyping under field conditions and a wide range of environments, and use of modelling and big data management to handle the huge amount of information provided by the new molecular, genetic and phenotyping techniques.

<https://onlinelibrary.wiley.com/doi/10.1002/fes3.369>

Tsakirpaloglou N., Septiningsih E., Thomson M. (2023): **Guidelines for Performing CRISPR/Cas9 Genome Editing for Gene Validation and Trait Improvement in Crops.** *Plants* 2023, 12(20), 3564; <https://doi.org/10.3390/plants12203564>

With the rapid advances in plant genome editing techniques over the past 10 years, more efficient and powerful crop genome editing applications are now possible. Candidate genes for key traits can be validated using CRISPR/Cas9-based knockouts and through the up- and down-regulation of gene expression. Likewise, new trait improvement approaches can take advantage of targeted editing to improve stress tolerance, disease

resistance, and nutritional traits. However, several key steps in the process can prove tricky for researchers who might be new to plant genome editing. Here, we present step-by-step guidelines and best practices for a crop genome editing pipeline that should help to improve the rate of success. Important factors in the process include proper target sequence analysis and single guide RNA (sgRNA) design, sequencing of the target site in the genotypes of interest, performing an in vitro CRISPR/Cas9 ribonucleoprotein (RNP) assay to validate the designed sgRNAs, preparing the transformation constructs, considering a protoplast editing step as further validation, and, finally, stable plant transformation and mutation detection by Sanger and/or next-generation sequencing. With these detailed guidelines, a new user should be able to quickly set up a genome editing pipeline in their crop of interest and start making progress with the different CRISPR/Cas-based editing variants for gene validation and trait improvement purposes.

<https://www.mdpi.com/2223-7747/12/20/3564>

Salse J., Barnard R.L., Veneault-Fourrey C., Rouached H. (2023): **Strategies for breeding crops for future environments.** Trends in Plant Science |

DOI:<https://doi.org/10.1016/j.tplants.2023.08.007>

The green revolution successfully increased agricultural output in the early 1960s by relying primarily on three pillars: plant breeding, irrigation, and chemical fertilization. Today, the need to reduce the use of chemical fertilizers, water scarcity, and future environmental changes, together with a growing population, requires innovative strategies to adapt to a new context and prevent food shortages. Therefore, scientists from around the world are directing their efforts to breed crops for future environments to sustainably produce more nutritious food. Herein, we propose scientific avenues to be reinforced in selecting varieties, including crop wild relatives, either for monoculture or mixed cropping systems, taking advantage of plant–microbial interactions, while considering the diversity of organisms associated with crops and unlocking combinatorial nutritional stresses.

[https://www.cell.com/trends/plant-science/pdf/S1360-1385\(23\)00269-8.pdf](https://www.cell.com/trends/plant-science/pdf/S1360-1385(23)00269-8.pdf)

Cichońska, P., Bryś, J. & Ziarno, M. (2023): **Use of natural biotechnological processes to modify the nutritional properties of bean-based and lentil-based beverages.** *Sci Rep* **13**, 16976 (2023). <https://doi.org/10.1038/s41598-023-44239-8>

The market for plant-based beverages (PBBs) is relatively new; hence, to enable its further development, it is important to use new raw materials and improve production technology. The use of natural biotechnological processes can diversify the segment of PBBs, which may offer products with better functionality than those available in the market. Therefore, the present study aimed to determine the effects of fermentation and germination on the nutritional properties of bean-based beverages (BBs) and lentil-based beverages (LBs). The applied processes significantly ( $p \leq 0.05$ ) influenced the characteristics of PBBs. Fermentation improved the antioxidant properties (e.g., by increasing the level of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity by 2–6% and 3–7% for BBs and LBs, respectively) and modified the fatty acid (FA) profile of PBBs. This process increased the share of polyunsaturated FAs in the sn2 position in triacylglycerols, which may promote its absorption in the intestine. The simultaneous use of germination and fermentation was most effective in decreasing oligosaccharide content ( $< 1.55$  mg/kg), which may reduce digestive discomfort after consuming PBBs. We recommend that the designing of innovative legume-based beverages should include the application of fermentation and germination to obtain products with probiotic bacteria and improved nutritional properties.

<https://www.nature.com/articles/s41598-023-44239-8>

Forner, J., Kleinschmidt, D., Meyer, E.H. et al. (2023): **Targeted knockout of a conserved plant mitochondrial gene by genome editing.** *Nat. Plants* | <https://doi.org/10.1038/s41477-023-01538-2>

Fusion proteins derived from transcription activator-like effectors (TALEs) have emerged as genome editing tools for mitochondria. TALE nucleases (TALENs) have been applied to delete chimaeric reading frames and duplicated (redundant) genes but produced complex genomic rearrangements due to the absence of non-homologous end-joining. Here we report the targeted deletion of a conserved mitochondrial gene, *nad9*, encoding a subunit of respiratory complex I. By generating a large number of TALEN-mediated mitochondrial deletion lines, we isolated, in addition to mutants with rearranged genomes, homochondriomic mutants harbouring clean *nad9* deletions. Characterization of the knockout plants revealed impaired complex I biogenesis, male sterility and defects in leaf and flower development. We show that these defects can be restored by expressing a functional Nad9 protein from the nuclear genome, thus creating a synthetic cytoplasmic male sterility system. Our data (1) demonstrate the feasibility of using genome editing to study mitochondrial gene functions by reverse genetics, (2) highlight the role of complex I in plant development and (3) provide proof-of-concept for the construction of synthetic cytoplasmic male sterility systems for hybrid breeding by genome editing.

<https://www.nature.com/articles/s41477-023-01538-2>

Wold-McGimsey F., Krosch C., Alarcón-Reverte R., Ravet K. et al. (2023): **Multi-target genome editing reduces polyphenol oxidase activity in wheat (*Triticum aestivum* L.) grains** *Front. Plant Sci.*, 15 ; Sec. Plant Biotechnology Volume 14 - 2023 |

<https://doi.org/10.3389/fpls.2023.1247680>

**Introduction:** Polyphenol oxidases (PPO) are dual activity metalloenzymes that catalyse the production of quinones. In plants, PPO activity may contribute to biotic stress resistance and secondary metabolism but is undesirable for food producers because it causes the discolouration and changes in flavour profiles of products during post-harvest processing. In wheat (*Triticum aestivum* L.), PPO released from the aleurone layer of the grain during milling results in the discolouration of flour, dough, and end-use products, reducing their value. Loss-of-function mutations in the PPO1 and PPO2 paralogous genes on homoeologous group 2 chromosomes confer reduced PPO activity in the wheat grain. However, limited natural variation and the proximity of these genes complicates the selection of extremely low-PPO wheat varieties by recombination. The goal of the current study was to edit all copies of PPO1 and PPO2 to drive extreme reductions in PPO grain activity in elite wheat varieties.

**Results:** A CRISPR/Cas9 construct with one single guide RNA (sgRNA) targeting a conserved copper binding domain was used to edit all seven PPO1 and PPO2 genes in the spring wheat cultivar 'Felder'. Five of the seven edited T1 lines exhibited significant reductions in PPO activity, and T2 lines had PPO activity up to 86.7% lower than wild-type. The same construct was transformed into the elite winter wheat cultivars 'Guardian' and 'Steamboat', which have five PPO1 and PPO2 genes. In these varieties PPO activity was reduced by >90% in both T1 and T2 lines. In all three varieties, dough samples from edited lines exhibited reduced browning.

**Discussion:** This study demonstrates that multi-target editing at late stages of variety development could complement selection for beneficial alleles in crop breeding programs by inducing novel variation in loci inaccessible to recombination.

<https://www.frontiersin.org/articles/10.3389/fpls.2023.1247680/full>

Zhu, Q., Deng, L., Chen, J. et al. (2023): **Redesigning the tomato fruit shape for mechanized production.** Nat. Plants | <https://doi.org/10.1038/s41477-023-01522-w>

Crop breeding for mechanized harvesting has driven modern agriculture. In tomato, machine harvesting for industrial processing varieties became the norm in the 1970s. However, fresh-market varieties whose fruits are suitable for mechanical harvesting are difficult to breed because of associated reduction in flavour and nutritional qualities. Here we report the cloning and functional characterization of *fs8.1*, which controls the elongated fruit shape and crush resistance of machine-harvestable processing tomatoes. *FS8.1* encodes a non-canonical GT-2 factor that activates the expression of cell-cycle inhibitor genes through the formation of a transcriptional module with the canonical GT-2 factor SGT-16. The *fs8.1* mutation results in a lower inhibitory effect on the cell proliferation of the ovary wall, leading to elongated fruits with enhanced compression resistance. Our study provides a potential route for introducing the beneficial allele into fresh-market tomatoes without reducing quality, thereby facilitating mechanical harvesting.

<https://www.nature.com/articles/s41477-023-01522-w>

Agarwal T., Wang X., Mildenhall F., Ibrahim I.M. et al. (2023): **Chilling stress drives organ-specific transcriptional cascades and dampens diurnal oscillation in tomato.** Horticulture Research, 10, Issue 8, uhad137 | <https://doi.org/10.1093/hr/uhad137>

Improving chilling tolerance in cold-sensitive crops, e.g. tomato, requires knowledge of the early molecular response to low temperature in these under-studied species. To elucidate early responding processes and regulators, we captured the transcriptional response at 30 minutes and 3 hours in the shoots and at 3 hours in the roots of tomato post-chilling from 24°C to 4°C. We used a pre-treatment control and a concurrent ambient temperature control to reveal that majority of the differential expression between cold and ambient conditions is due to severely compressed oscillation of a large set of diurnally regulated genes in both the shoots and roots. This compression happens within 30 minutes of chilling, lasts for the duration of cold treatment, and is relieved within 3 hours of return to ambient temperatures. Our study also shows that the canonical ICE1/CAMTA-to-CBF cold response pathway is active in the shoots, but not in the roots. Chilling stress induces synthesis of known cryoprotectants (trehalose and polyamines), in a CBF-independent manner, and induction of multiple genes encoding proteins of photosystems I and II. This study provides nuanced insights into the organ-specific response in a chilling sensitive plant, as well as the genes influenced by an interaction of chilling response and the circadian clock.

<https://academic.oup.com/hr/article/10/8/uhad137/7222428>

Tamizi, A.A., Md-Yusof, A.A., Mohd-Zim, N.A. et al. (2023): **Agrobacterium-mediated in planta transformation of cut coleoptile: a new, simplified, and tissue culture-independent method to deliver the CRISPR/Cas9 system in rice.** Mol Biol Rep | <https://doi.org/10.1007/s11033-023-08842-2>

**Background:** *Agrobacterium*-mediated transformation and particle bombardment are the two common approaches for genome editing in plant species using CRISPR/Cas9 system. Both methods require careful manipulations of undifferentiated cells and tissue culture to regenerate the potentially edited plants. However, tissue culture techniques are laborious and time-consuming.

**Methods and results:** In this study, we have developed a simplified, tissue culture-independent protocol to deliver the CRISPR/Cas9 system through *in planta* transformation in Malaysian rice (*Oryza sativa* L. subsp. *indica* cv. MR 219). Sprouting seeds with cut coleoptile were used as the target for the infiltration by *Agrobacterium tumefaciens* and we achieved 9% transformation efficiency. In brief, the dehusked seeds were surface-sterilised and imbibed, and the coleoptile was cut to expose the apical meristem. Subsequently, the cut coleoptile was inoculated with *A. tumefaciens* strain EHA105 harbouring CRISPR/Cas9 expression vector. The co-cultivation was conducted for five to six days in a dark room (25 ± 2 °C) followed by rooting, acclimatisation, and growing phases. Two-month-old plant leaves were then subjected to a hygromycin selection, and hygromycin-resistant



plants were identified as putative transformants. Further validation through the polymerase chain reaction verified the integration of the *Cas9* gene in four putative T<sub>0</sub> lines. During the fruiting stage, it was confirmed that the *Cas9* gene was still present in three randomly selected tillers from two 4-month-old transformed plants.

Conclusion: This protocol provides a rapid method for editing the rice genome, bypassing the need for tissue culture. This article is the first to report the delivery of the CRISPR/Cas9 system for *in planta* transformation in rice.

<https://link.springer.com/article/10.1007/s11033-023-08842-2>

Huang, X., Jia, H., Xu, J. et al. (2023): **Transgene-free genome editing of vegetatively propagated and perennial plant species in the T<sub>0</sub> generation via a co-editing strategy.** *Nat. Plants* (<https://doi.org/10.1038/s41477-023-01520-y>)

Transgene-free plant genome editing in the T<sub>0</sub> generation is highly desirable but challenging<sup>1,2</sup>. Here we achieved such a goal using a co-editing strategy via *Agrobacterium*-mediated transient expression of cytosine base editor to edit *ALS* encoding acetolactate synthase to confer herbicide chlorsulfuron resistance as a selection marker, Cas12a/CRISPR RNA for editing gene(s) of interest, and green fluorescent protein for selecting transgene-free transformants. The biallelic/homozygous transgene-free mutation rates for target genes among herbicide-resistant transformants ranged from 1.9% to 42.1% in tomato, tobacco, potato and citrus. This co-editing strategy is particularly useful for transgene-free genome editing of vegetatively propagated and perennial plant species in the T<sub>0</sub> generation.

<https://www.nature.com/articles/s41477-023-01520-y>

Ren Y., Liu B., Jiang H., Cheng W. et al. (2023): **Precision editing of GLR1 confers glufosinate resistance without yield penalty in rice,** *Plant Biotechnology Journal* | DOI: 10.1111/pbi.14168

<https://onlinelibrary.wiley.com/doi/10.1111/pbi.14168>

Yart, L., Wijaya, A.W., Lima, M.J. et al. (2023): **Cellular agriculture for milk bioactive production.** *Nat Rev Bioeng* | <https://doi.org/10.1038/s44222-023-00112-x>

Cellular agriculture, that is, the cell culture-based production of food, may provide an alternative to traditional farming, minimizing the environmental effect, while ensuring nutritional quality, ingredient safety and security of food products. In addition to the cell-based production of animal-based products (meat, poultry and seafood), cellular agriculture of dairy, such as milk, is being increasingly explored. Cellular agriculture could drive specific applications of new and functional ingredients tailored to infant needs, particularly in the dairy industry. Given the known long-term benefits of exclusive breastfeeding, this technology has the potential to supply functional milk bioactives for infants who cannot be breastfed. In this Review, we discuss cell-based biotechnology approaches, applying synthetic biology or precision fermentation, for the production of functional and personalized milk bioactives. We highlight cell culture-based techniques, including mammary gland organoids, and microbial-based approaches to produce milk bioactives, such as human milk oligosaccharides and human milk lactoferrin. Finally, we emphasize challenges of scale, costs and socio-political aspects with regard to human milk production and outline the key future milestones of this field.

<https://www.nature.com/articles/s44222-023-00112-x>

## EFSA

CEP Panel 2023): Safety evaluation of the food enzyme  $\alpha$ -amylase from the non-genetically modified *Bacillus amyloliquefaciens* strain LMG-S 32676. *EFSA Journal*, 21(10), 1–14.

<https://doi.org/10.2903/j.efsa.2023.8255> |

<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.8255>

CEP Panel(2023): Safety evaluation of the food enzyme asparaginase from the genetically modified *Aspergillus oryzae* strain NZYM-SP. *EFSA Journal*, 21(10), 1–15. |

<https://doi.org/10.2903/j.efsa.2023.8254>

<https://efsa.onlinelibrary.wiley.com/action/showCitFormats?doi=10.2903%2Fj.efsa.2023.8254&mobileUi=0>

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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.

*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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