

# Sunday Evening News No 336

2023-07-24 – 2023-07-30

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## **Press Releases -Media / Presse- und Medienberichte**

**AFC: Issue der Woche: Gentechnik – gern gesehen...?!**

<https://www.afc.net/blog/issue-der-woche-gentechnik-gern-gesehen>

**Mönch A.: Neue genomische Techniken (NGT) - Zustimmung im EU-Agrarrat**

<https://www.agrarzeitung.de/nachrichten/politik/neue-genomische-techniken-ngt-zustimmung-im-eu-agrarrat-108080>

Informationsdienst Gentechnik: **Neue Gentechnik: viel Kritik am Kommissionsentwurf im Agrarrat**

<https://www.keine-gentechnik.de/nachricht/34804?cHash=7aa6859810d054c52310e258b15cb8b3>

Testbiotech Basis-Text 25-07-2023: **Unterschiede: Neue Gentechnik und Mutagenese**

<https://www.testbiotech.org/sites/default/files/Tabelle%20Vergleich%20Neue%20Gentechnik%20%26%20Mutagenese.pdf>

**Remarks by Commissioner Stella Kyriakides at the Agrifish Council - New Genomic Techniques**

<https://www.kurzy.cz/tema/8262359.html>

**ARRIGE: STATEMENT ABOUT EC PROPOSAL ON NEW GENOMIC TECHNIQUES REGULATION IN PLANTS**

[https://www.arrige.org/wp-content/uploads/2023/07/ARRIGE\\_SC\\_statement\\_NGTs.pdf](https://www.arrige.org/wp-content/uploads/2023/07/ARRIGE_SC_statement_NGTs.pdf)

**Kovak E.: Want to Make EU Agriculture More Sustainable?**

<https://thebreakthrough.org/issues/food-agriculture-environment/want-to-make-eu-agriculture-more-sustainable>

**Dahm J.: EU ministers split on risks, potential of looser gene editing rules**

<https://www.euractiv.com/section/agriculture-food/news/eu-ministers-split-on-risks-potential-of-looser-gene-editing-rules/>

**Meunier E.: The European Commission wants to put an end to GMOs**

<https://www.infogm.org/7834-european-commission-wants-to-put-an-end-to-gmos?lang=fr>

Only some selected press releases or media reports are listed here. The daily up-date of the press releases and media reports are [▶ here](#): June week 30

## **Publications – Publikationen**

Liang Y., Chen F., Xue J.-Y. (2023): **Genomics and Biotechnology Empower Plant Science Research Horticulturae** 9 (8), 863; <https://doi.org/10.3390/horticulturae9080863>  
<https://www.mdpi.com/2311-7524/9/8/863>

Ahmar S., Gruszka D. (2023): **CRISPR/Cas9 boosts wheat yield by reducing brassinosteroid signaling.** Trends in Biochemical Sciences | <https://doi.org/10.1016/j.tibs.2023.07.005>

A modern green revolution is needed to ensure global food security. Recently, [Song et al.](#) reported a new strategy to create high-yielding, semi-dwarf wheat varieties with improved nitrogen-use efficiency by inhibiting brassinosteroid (BR) signaling through clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein9 (Cas9)-mediated knockout of the *ZnF-B* gene encoding a zinc-finger RING-type E3 ligase.

[https://www.cell.com/trends/biochemical-sciences/fulltext/S0968-0004\(23\)00171-8](https://www.cell.com/trends/biochemical-sciences/fulltext/S0968-0004(23)00171-8)

Wu, T., Lu, S., Cai, Y. et al. (2023): **Molecular breeding for improvement of photothermal adaptability in soybean.** *Mol Breeding* **43**, 60 | <https://doi.org/10.1007/s11032-023-01406-z>

Soybean (*Glycine max* (L.) Merr.) is a typical short-day and temperate crop that is sensitive to photoperiod and temperature. Responses of soybean to photothermal conditions determine plant growth and development, which affect its architecture, yield formation, and capacity for geographic adaptation. Flowering time, maturity, and other traits associated with photothermal adaptability are controlled by multiple major-effect and minor-effect genes and genotype-by-environment interactions. Genetic studies have identified at least 11 loci (*E1-E4*, *E6-E11*, and *J*) that participate in photoperiodic regulation of flowering time and maturity in soybean. Molecular cloning and characterization of major-effect flowering genes have clarified the photoperiod-dependent flowering pathway, in which the photoreceptor gene *phytochrome A*, circadian evening complex (EC) components, central flowering repressor *E1*, and *FLOWERING LOCUS T* family genes play key roles in regulation of flowering time, maturity, and adaptability to photothermal conditions. Here, we provide an overview of recent progress in genetic and molecular analysis of traits associated with photothermal adaptability, summarizing advances in molecular breeding practices and tools for improving these traits. Furthermore, we discuss methods for breeding soybean varieties with better adaptability to specific ecological regions, with emphasis on a novel strategy, the Potalaization model, which allows breeding of widely adapted soybean varieties through the use of multiple molecular tools in existing elite widely adapted varieties.

<https://link.springer.com/article/10.1007/s11032-023-01406-z>

Su, H., Wang, Y., Xu, J. et al. (2023): **Generation of the transgene-free canker-resistant *Citrus sinensis* using Cas12a/crRNA ribonucleoprotein in the T0 generation.** *Nat Commun* **14**, 3957 (2023). <https://doi.org/10.1038/s41467-023-39714-9>

Citrus canker caused by *Xanthomonas citri* subsp. *citri* (*Xcc*) is a destructive citrus disease worldwide. Generating disease-resistant cultivars is the most effective, environmentally friendly and economic approach for disease control. However, citrus traditional breeding is lengthy and laborious. Here, we develop transgene-free canker-resistant *Citrus sinensis* lines in the T0 generation within 10 months through transformation of embryogenic protoplasts with Cas12a/crRNA ribonucleoprotein to edit the canker susceptibility gene *CsLOB1*. Among the 39 regenerated lines, 38 are biallelic/homozygous mutants, demonstrating a 97.4% biallelic/homozygous mutation rate. No off-target mutations are detected in the edited lines. Canker resistance of the *cslob1*-edited lines results from both abolishing canker symptoms and inhibiting *Xcc* growth. The transgene-free canker-resistant *C. sinensis* lines have received regulatory approval by USDA APHIS and are exempted from EPA regulation. This study provides a sustainable and efficient citrus canker control solution and presents an efficient transgene-free genome-editing strategy for citrus and other crops.

<https://www.nature.com/articles/s41467-023-39714-9>

Sajad Majeed Zargar, Ammarah Hami, Madhiya Manzoor, Rakeeb Ahmad Mir et al., (2023): **Buckwheat OMICS: present status and future prospects** *Critical Reviews in Biotechnology*, DOI: [10.1080/07388551.2023.2229511](https://doi.org/10.1080/07388551.2023.2229511)

Buckwheat (*Fagopyrum spp.*) is an underutilized resilient crop of North Western Himalayas belonging to the family Polygonaceae and is a source of essential nutrients and therapeutics. Common Buckwheat and Tatar Buckwheat are the two main cultivated species used as food. It is the only grain crop possessing rutin, an important metabolite with high nutraceutical potential. Due to its inherent tolerance to various biotic and abiotic stresses and a short life cycle, Buckwheat has been proposed as a model crop plant. Nutritional security is one of the major concerns, breeding for a nutrient-dense crop such as Buckwheat will provide a sustainable solution. Efforts toward improving Buckwheat for nutrition and yield are limited due to the lack of available: genetic resources, genomics, transcriptomics and metabolomics. In order to harness the agricultural importance of Buckwheat, an integrated breeding and OMICS platforms needs to be established that can pave the way for a better understanding of crop biology and developing commercial varieties. This, coupled with the availability of the genome sequences of both Buckwheat species in the public domain, should facilitate the identification of alleles/QTLs and candidate genes. There is a need to further our understanding of the molecular basis of the genetic regulation that controls various economically important traits. The present review focuses on: the food and nutritional importance of Buckwheat, its various omics resources, utilization of omics approaches in understanding Buckwheat biology and, finally, how an integrated platform of breeding and omics will help in developing commercially high yielding nutrient rich cultivars in Buckwheat.

<https://www.tandfonline.com/doi/abs/10.1080/07388551.2023.2229511?src=&journalCode=ibty20>

Nonaka S., Ito M., Ezura H. (2023): **Targeted modification of CmACO1 by CRISPR/Cas9 extends the shelf-life of Cucumis melo var. reticulatus melon** *Frontiers in Genome Editing* | DOI: [10.3389/fgeed.2023.1176125](https://doi.org/10.3389/fgeed.2023.1176125)

The gaseous plant hormone ethylene is a regulator of fruit shelf-life, one of the essential traits in fruits. Extending fruit shelf-life reduces food loss, thereby expected to contribute to food security. The enzyme 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) is the final step of the ethylene production pathway. Its suppression via antisense technology has been demonstrated to extend the shelf-life of melon, apple, and papaya. Genome editing technology is an innovative technique for plant breeding. Because the genome editing technology would not leave the exogenous genes in the final crop products, the crops via genome editing can be considered non-genetically modified yields; compared to conventional breeding, such as mutation breeding, the breeding term would be expected to be relatively short. These points include the advantage of this technique in utilization for commercial applications. We attempted to extend the shelf-life of the Japanese luxury melon (*Cucumis melo* var. *reticulatus*, 'Harukei-3') via modification of the ethylene synthesis pathway with the genome editing technology, CRISPR/Cas9 system. The Melonet-DB (<https://melonet-db.dna.affrc.go.jp/ap/top>) showed that the melon genome had the five *CmACOs* and the gene *CmACO1* predominantly expressed in harvested fruits. From this information, *CmACO1* was expected to be a key gene for shelf-life in melons. Based on this information, the *CmACO1* was selected as the target of the CRISPR/Cas9 system and introduced the mutation. The final product of this melon did not have any exogenous genes. The mutation was inherited for at least two generations. In the T<sub>2</sub> generation, the fruit phenotypes 14 days after harvest were as follows: ethylene production was reduced to one-tenth that of the wild type, pericarp colour remained green, and higher fruit firmness. Early fermentation of the fresh fruit was observed in the wild-type fruit but not in the mutant. These results show that *CmACO1* knockout via CRISPR/Cas9 extended the melon's shelf-life. Moreover, our results suggest that genome editing technology would reduce food loss and contribute to food security.

<https://www.frontiersin.org/articles/10.3389/fgeed.2023.1176125/full>

Gann P.J.I., Dharwadker D., Cherati S.R., Kari Vinzant K. et al. (2023). **Targeted mutagenesis of the vacuolar H<sup>+</sup> translocating pyrophosphatase gene reduces grain chalkiness in rice**, *The Plant Journal* | DOI: [10.1111/tpj.16317](https://doi.org/10.1111/tpj.16317)

Grain chalkiness is a major concern in rice production because it impacts milling yield and cooking quality, eventually reducing market value of the rice. A gene encoding vacuolar H<sup>+</sup> translocating pyrophosphatase (*V-PPase*) is a major quantitative trait locus in *indica* rice, controlling grain chalkiness. Higher transcriptional activity of this gene is associated with increased chalk content. However, whether the suppression of *V-PPase* could reduce chalkiness is not clear. Furthermore, natural variation in the chalkiness of *japonica* rice has not been linked with *V-PPase*. Here, we describe promoter targeting of the *japonica* *V-PPase* allele that led to reduced grain chalkiness and the development of more translucent grains. Disruption of a putative GATA element by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 suppressed *V-PPase* activity, reduced grain chalkiness and impacted post-germination growth that could be rescued by the exogenous supply of sucrose. The mature grains of the targeted lines showed a much lower percentage of large or medium chalk. Interestingly, the targeted lines developed a significantly lower chalk under heat stress, a major inducer of grain chalk. Metabolomic analysis showed that pathways related to starch and sugar metabolism were affected in the developing grains of the targeted lines that correlated with higher inorganic pyrophosphate and starch contents and upregulation of starch biosynthesis genes. In summary, we show a biotechnology approach of reducing grain chalkiness in rice by downregulating the transcriptional activity of *V-PPase* that presumably leads to altered metabolic rates, including starch biosynthesis, resulting in more compact packing of starch granules and formation of translucent rice grains.

<https://onlinelibrary.wiley.com/doi/10.1111/tpj.16317>

Uchida M., Konishi T., Fujigasaki A. et al. (2023): **Dysfunctional Pro1 leads to female sterility in rice blast fungi**. *iScience* 26, 107020 | <https://doi.org/10.1016/j.isci.2023.107020>

Although sexual reproduction is widespread in eukaryotes, some fungal species can only reproduce asexually. In the rice blast fungus *Pyricularia (Magnaporthe) oryzae*, several isolates from the region of origin retain mating ability, but most isolates are female sterile. Therefore, female fertility may have been lost during its spread from the origin. Here, we show that functional mutations of Pro1, a global transcriptional regulator of mating-related genes in filamentous fungi, is one cause of loss of female fertility in this fungus. We identified the mutation of Pro1 by backcrossing analysis between female-fertile and female-sterile isolates. The dysfunctional Pro1 did not affect the infection processes but conidial release was increased. Furthermore, various mutations in Pro1 were detected in geographically distant *P. oryzae*, including pandemic isolates of wheat blast fungus. These results provide the first evidence that loss of female fertility may be advantageous to the life cycle of some plant pathogenic fungi.

<https://www.cell.com/action/showPdf?pii=S2589-0042%2823%2901097-0>

Esser, S.P., Rahlff, J., Zhao, W. et al. (2023): **A predicted CRISPR-mediated symbiosis between uncultivated archaea**. *Nat Microbiol* | <https://doi.org/10.1038/s41564-023-01439-2>

CRISPR–Cas systems defend prokaryotic cells from invasive DNA of viruses, plasmids and other mobile genetic elements. Here, we show using metagenomics, metatranscriptomics and single-cell genomics that CRISPR systems of widespread, uncultivated archaea can also target chromosomal DNA of archaeal episymbionts of the DPANN superphylum. Using meta-omics datasets from Crystal Geyser and Horonobe Underground Research Laboratory, we find that CRISPR spacers of the hosts *Candidatus* Altiaarchaeum crystalense and *Ca.* A. horonobense, respectively, match putative essential genes in their episymbionts' genomes of the genus *Ca.* Huberiaarchaeum and that some of these spacers are expressed in situ. Metabolic interaction modelling also reveals complementation between host–episymbiont systems, on the basis of which we propose that

episymbionts are either parasitic or mutualistic depending on the genotype of the host. By expanding our analysis to 7,012 archaeal genomes, we suggest that CRISPR–Cas targeting of genomes associated with symbiotic archaea evolved independently in various archaeal lineages.  
<https://www.nature.com/articles/s41564-023-01439-2>

Wei Sun et al (2023): **AcrIIIC4 inhibits type II-C Cas9 by preventing R-loop formation**, PNAS 120 (31) e2303675120 | <https://doi.org/10.1073/pnas.2303675120>

Anti-CRISPR (Acr) proteins are encoded by phages and other mobile genetic elements and inhibit host CRISPR–Cas immunity using versatile strategies. AcrIIIC4 is a broad-spectrum Acr that inhibits the type II-C CRISPR–Cas9 system in several species by an unknown mechanism. Here, we determined a series of structures of *Haemophilus parainfluenzae* Cas9 (HpaCas9)–sgRNA in complex with AcrIIIC4 and/or target DNA, as well as the crystal structure of AcrIIIC4 alone. We found that AcrIIIC4 resides in the crevice between the REC1 and REC2 domains of HpaCas9, where its extensive interactions restrict the mobility of the REC2 domain and prevent the unwinding of target double-stranded (ds) DNA at the PAM-distal end. Therefore, the full-length guide RNA:target DNA heteroduplex fails to form in the presence of AcrIIIC4, preventing Cas9 nuclease activation. Altogether, our structural and biochemical studies illuminate a unique Acr mechanism that allows DNA binding to the Cas9 effector complex but blocks its cleavage by preventing R-loop formation, a key step supporting DNA cleavage by Cas9.

<https://www.pnas.org/doi/10.1073/pnas.2303675120>

Chen W, Amir M.B., Liao Y., Yu H., He W., Lu Z. (2023): **New Insights into the *Plutella xylostella* Detoxifying Enzymes: Sequence Evolution, Structural Similarity, Functional Diversity, and Application Prospects of Glucosinolate Sulfatases**, J. Agric. Food Chem. 71, 29, 10952–10969 | <https://doi.org/10.1021/acs.jafc.3c03246>

Brassica plants have glucosinolate (GLs)–myrosinase defense mechanisms to deter herbivores. However, *Plutella xylostella* specifically feeds on Brassica vegetables. The larvae possess three glucosinolate sulfatases (PxGSS1–3) that compete with plant myrosinase for shared GLs substrates and produce nontoxic desulfo-GLs (deGLs). Although PxGSSs are considered potential targets for pest control, the lack of a comprehensive review has hindered the development of PxGSSs-targeted pest control methods. Recent advances in integrative multi-omics analysis, substrate–enzyme kinetics, and molecular biological techniques have elucidated the evolutionary origin and functional diversity of these three PxGSSs. This review summarizes research progress on PxGSSs over the past 20 years, covering sequence properties, evolution, protein modification, enzyme activity, structural variation, substrate specificity, and interaction scenarios based on functional diversity. Finally, we discussed the potential applications of PxGSSs-targeted pest control technologies driven by artificial intelligence, including CRISPR/Cas9-mediated gene drive, transgenic plant-mediated RNAi, small-molecule inhibitors, and peptide inhibitors. These technologies have the potential to overcome current management challenges and promote the development and field application of PxGSSs-targeted pest control.

<https://pubs.acs.org/doi/10.1021/acs.jafc.3c03246?ref=PDF#>

Jansson, J.K. (2023): **Microorganisms, climate change, and the Sustainable Development Goals: progress and challenges**. Nat Rev Microbiol | <https://doi.org/10.1038/s41579-023-00953-8>

<https://www.nature.com/articles/s41579-023-00953-8>

Teng Y, Jiang T., Yan Y. (2023): **The expanded CRISPR toolbox for constructing microbial cell factories** Trends in Biotechnology | <https://doi.org/10.1016/j.tibtech.2023.06.012>

Microbial cell factories (MCFs) convert low-cost carbon sources into valuable compounds. The CRISPR/Cas9 system has revolutionized MCF construction as a remarkable genome editing tool with unprecedented programmability. Recently, the CRISPR toolbox has been significantly expanded through the exploration of new CRISPR systems, the engineering of Cas effectors, and the incorporation of other effectors, enabling multi-level regulation and gene editing free of double-strand breaks. This expanded CRISPR toolbox powerfully promotes MCF construction by facilitating pathway construction, enzyme engineering, flux redistribution, and metabolic burden control. In this article, we summarize different CRISPR tool designs and their applications in MCF construction for gene editing, transcriptional regulation, and enzyme modulation. Finally, we also discuss future perspectives for the development and application of the CRISPR toolbox.

[https://www.cell.com/trends/biotechnology/fulltext/S0167-7799\(23\)00204-4?rss=yes](https://www.cell.com/trends/biotechnology/fulltext/S0167-7799(23)00204-4?rss=yes)

## EFSA

CEP Panel (2023): Safety evaluation of the food enzyme inulinase from the genetically modified *Aspergillus oryzae* strain MUCL 44346. *EFSA Journal*, 21( 7), 1–17.

<https://doi.org/10.2903/j.efsa.2023.8148>

<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.8148>

CEP Panel (2023). Safety evaluation of a food enzyme containing endo-polygalacturonase and pectin lyase activities from the non-genetically modified *Aspergillus tubingensis* strain NZYM-PE. *EFSA Journal*, 21 ( 7), 1–17. <https://doi.org/10.2903/j.efsa.2023.8151>  
<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.8151>

CEP Panel (2023): Safety evaluation of the food enzyme ribonuclease P from the non-genetically modified *Penicillium citrinum* strain AE-RP-4. *EFSA Journal*, 21( 7), 1–14. <https://doi.org/10.2903/j.efsa.2023.8153>  
<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.8153>

CEP Panel (2023): **Food manufacturing processes and technical data used in the exposure assessment of food enzymes.** *EFSA Journal*, 21( 7), 1–31. <https://doi.org/10.2903/j.efsa.2023.8094>  
<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.8094>

Jaskiewicz, K, Taylor, O, Senior, B, and Maestre, M. (2023): **Communication of food-related health risks and benefits – a systematic review (2018-2022).** EFSA supporting publication 20 ( 7):EN-8203. 49 pp. doi:[10.2903/sp.efsa.2023.EN-8203](https://doi.org/10.2903/sp.efsa.2023.EN-8203)  
<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2023.EN-8203>

Álvarez, F., Arena, M., Auteri, D., Binaglia, M., Castoldi, A. F., Chiusolo, A., Crivellente, F., Egsmose, M., Fait, G., Ferilli, F., Gouliarmou, V., Nogareda, L. H., Ippolito, A., Istace, F., Jarrah, S., Kardassi, D., Kienzler, A., Lanzoni, A., ... Villamar-Bouza, L. (2023). **Peer review of the pesticide risk assessment of the active substance glyphosate.** *EFSA Journal*, 21( 7), 1–52. <http://doi.org/10.2903/j.efsa.2023.8164>  
<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.8164>

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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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