Sunday Evening News No 265

Week 05 (2022-01-31 – 2022-06-02)

Selected and edited by ***BGF*** Jany

White Genetic Engineering (Industrial Biotechnology) - Food Enzymes

Weiße Gentechnik (Industrielle Biotechnologie) - Lebensmittelenzyme

Hanlon P. & Sewalt V. (2021): **GEMs: genetically engineered microorganisms and the regulatory oversight of their uses in modern food production**, Critical Reviews in Food Science and Nutrition, 61:6, 959-970, DOI: 10.1080/10408398.2020.1749026
Over the past several decades, the use of genetically engineered microorganisms (GEMs, often referred to as Genetically Modified Microorganisms or GMMs) has become widespread in the production of food processing aids and other food ingredients. GEMs are advancing food production by increasing efficiency, reducing waste and resource requirements, and ultimately enabling beneficial innovations such as the cost-effective fortification of food with essential nutrients, vitamins, and amino acids, and delivery of tailored enzymes to achieve unique food processing capabilities. Regulatory agencies, including those in the European Union, United States, and Canada review the safety of GEMs when evaluating food substances produced using GEMs to ensure that both the microorganism and the resulting food substance are safe. This paper provides a summary of historical and current use of GEMs in food manufacture, an overview of frameworks that regulate their use, and a description of the safety assessment of both GEMs and food substances produced with GEMs. The paper encourages regulatory agencies around the globe to take a more aligned approach to the safety evaluation and regulatory oversight of GEM-produced food ingredients and enzymes, a category of food substances that enables more sustainable consumer food choices.
https://www.tandfonline.com/doi/full/10.1080/10408398.2020.1749026
https://www.tandfonline.com/doi/pdf/10.1080/10408398.2020.1749026?needAccess=true

Stemke D.J. (2004): **Genetically Modified Microorganisms**. In: Parekh S.R. (eds) The GMO Handbook. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-59259-801-4_4
https://link.springer.com/content/pdf/10.1007%2F978-1-59259-801-4_4.pdf

Dederer H.-G., Hamburger D. (2022**): Are genome-edited micro-organisms covered by Directive 2009/41/EC?—implications of the CJEU's judgment in the case C-528/16 for the contained use of genome-edited micro-organisms.** Journal of Law and the Biosciences 9 (1), lsab033, | https://doi.org/10.1093/jlb/lsab033
In its judgement of July 25, 2018, the Court of Justice of the European Union (CJEU) in the case C-528/16, *Confédération paysanne and Others*, held that organisms obtained by techniques of mutagenesis are 'genetically modified organisms' (GMOs). It follows from the Court's reasoning that genome-edited organisms, ie organisms resulting from techniques of directed mutagenesis, are GMOs as well and are fully regulated by Directive 2001/18/EC. However, Directive 2001/18/EC only stipulates rules for the deliberate release and placing on the market of GMOs. By contrast, the European Union (EU) has adopted a separate set of rules laid down in Directive 2009/41/EC, which apply to the so-called 'contained use' of 'genetically modified micro-organisms' (GMMs). Whether also genome-edited micro-organisms are GMMs and, thus, subject to Directive 2009/41/EC is of crucial importance since contained use activities with genome-edited micro-organisms are currently carried out extensively, eg in laboratories and research facilities. An in-depth legal analysis shows that the CJEU's interpretation of Directive 2001/18/EC can be extended to Directive 2009/41/EC which means that, in the end, genome-edited micro-organisms are GMMs invariably subject to Directive 2009/41/EC.
https://academic.oup.com/jlb/article/9/1/lsab033/6513430

see also /siehe auch (in German only)
https://www.biotech-gm-food.com/kommentare/eugh-urteil-mutagenese-geschlossene-systeme-rl-2009-49-EG

Dederer H.-G. (2021): **rDNA Traces in Fermentation Products Using Genetically Modified Microorganisms (GMMs),** Zeitschrift für Stoffrecht 18 (3), 135 - 147 | https://doi.org/10.21552/stoffr/2021/3/6
Certain products such as amino acids, flavourings, oligosaccharides, organic acids, or vitamins are obtained by fermentation using genetically modified microorganisms (GMMs). Such fermentation products may be used as or in food and feed. Although the GMMs are separated from the fermentation products during downstream processing these products may, nevertheless, contain traces of rDNA originating from the GMMs. The European Commission holds the view that fermentation products obtained by using GMMs are subject to Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed to the extent that rDNA is still present in the fermentation product irrespective of the amount of rDNA sequences. However, it follows from the travaux préparatoires as well as early discussions on the scope of Regulation (EC) No 1829/2003 starting immediately after its entry into force

that it was neither designed nor framed to be applicable to fermentation products obtained by the use of GMMs. Accordingly, Regulation (EC) No 1829/2003 cannot be considered to be fit for purpose as regards regulation of such products. In particular, it is clear from the regulation's wording and context that it does not apply to food or feed products obtained by fermentation using GMMs if the GMMs have been removed during downstream processing. In this case, the GMMs are mere 'processing aids' within the meaning of Recital 16, sentences 3 and 4, of Regulation (EC) No 1829/2003. Therefore, such food or feed products obtained by fermentation using GMMs are excluded from the scope of the regulation since, first, these products are not produced 'from' but produced 'with' GMMs (cf. Artt. 2(6), (7) and (10), 3(1)(c), 15(1)(c) of Regulation (EC) No 1829/2003 as construed in light of Recital 16, sentences 1, 3 and 4) and, second, the GMMs are not 'source material' of the fermentation products (cf. Art. 2(8) and (9), Art. 3(1)(a), Art. 15(1)(a) of Regulation (EC) No 1829/2003 as construed in light of Recital 16, sentences 1, 3 and 4). As GMMs are 'processing aids' within the meaning of sentences 3 and 4 of Recital 16 and the fermentation products are, therefore, produced 'with' the GMMs within the meaning of sentence 1 of Recital 16, sentence 2 of the recital has, logically, no relevance as regards the distinction between food or feed produced 'from' or 'with' GMMs. rDNA traces in fermentation products obtained by the use of GMMs are not 'ingredients' either. Rather, they constitute mere 'residues'. Any health safety concerns related to the presence of rDNA traces are addressed by other Union legislation, e.g., on food additives, food enzymes and food flavourings or feed additives and other feed materials, respectively, or, as the case may be, on novel foods.
https://stoffr.lexxion.eu/data/article/17593/pdf/stoffr_2021_03-008.pdf

Fraiture, M.A., Bogaerts, B., Winand, R. et al. (2020): **Identification of an unauthorized genetically modified bacteria in food enzyme through whole-genome sequencing.** Sci Rep 10, 7094 | https://doi.org/10.1038/s41598-020-63987-5
Recently, the unexpected presence of a viable unauthorized genetically modified bacterium in a commercialized food enzyme (protease) product originating from a microbial fermentation process has been notified at the European level (RASFF 2019.3332). This finding was made possible thanks to the use of the next-generation sequencing technology, as reported in this study. Whole-genome sequencing was used to characterize the genetic modification comprising a sequence from the pUB110 shuttle vector (GenBank: M19465.1), harbouring antimicrobial resistance genes conferring a resistance to kanamycine, neomycin and bleomycin, flanked on each side by a sequence coding for a protease (GenBank: WP_032874795.1). In addition, based on these data, two real-time PCR methods, that can be used by enforcement laboratories, specific to this unauthorized genetically modified bacterium were developed and validated. The present study emphasizes the key role that whole-genome sequencing can take for detection of unknown and unauthorized genetically modified microorganisms in commercialized microbial fermentation products intended for the food and feed chain. Moreover, current issues encountered by the Competent Authorities and enforcement laboratories with such unexpected contaminations and the importance of performing official controls were highlighted.
https://www.nature.com/articles/s41598-020-63987-5

D'aes, J.; Fraiture, M.-A.; Bogaerts, B.; De Keersmaecker, S.C.J.; Roosens, N.H.C.; Vanneste, K. (2021): **Characterization of Genetically Modified Microorganisms Using Short- and Long-Read Whole-Genome Sequencing Reveals Contaminations of Related Origin in Multiple Commercial Food Enzyme Products.** Foods, 10, 2637 | https://doi.org/10.3390/foods10112637
Despite their presence being unauthorized on the European market, contaminations with genetically modified (GM) microorganisms have repeatedly been reported in diverse commercial microbial fermentation produce types. Several of these contaminations are related to a GM Bacillus velezensis used to synthesize a food enzyme protease, for which genomic characterization remains currently incomplete, and it is unknown whether these contaminations have a common origin. In this study, GM B. velezensis isolates from multiple food enzyme products were characterized by short- and long-read whole-genome sequencing (WGS), demonstrating that they harbor a free recombinant pUB110-derived plasmid carrying antimicrobial resistance genes. Additionally, single-nucleotide polymorphism (SNP) and whole-genome based comparative analyses showed that the isolates likely originate from the same parental GM strain. This study highlights the added value of a hybrid WGS approach for accurate genomic characterization of GMM (e.g., genomic location of the transgenic construct), and of SNP-based phylogenomic analysis for source-tracking of GMM.
https://www.mdpi.com/2304-8158/10/11/2637

EFSA - Lebensmittelenzyme

Die Publikation von Dederer (2022) hat mich veranlasst meine „Enzymwebseite" (https://www.biotech-enzymes.com/) mit dem Schwerpunkt Lebensmittelenzyme zu aktualisieren. Hierbei wurden die abgeschlossenen Sicherheitsbewertungen der Lebensmittel durch die EFSA auf den Stand von 30.01.2022 gebracht und den Fortgang der Bewertungen der Anträge, die bis zum 11.03.2015 bei der Kommission eingegangen sind, zu analysieren. Erst wenn alle diese Lebensmittelenzyme auf ihre Sicherheit bewertet worden sind, wird die Kommission die Unionsliste erstellen. Bislang hat die ►EFSA je nach Zählungsart (Enzyme oder EFSA-Questionnumber) 123 bzw. 106 Enzyme auf ihre Sicherheit bewertet. Vier von ihnen wurden als nicht sicher eingestuft und bei 8 konnte aufgrund der

eingereichten Daten keine abschließende Aussage zur Sicherheit gemacht werden. Aus der Liste der Anträge, die bis März 2015 eingegangen sind, stehen 114 im Bewertungsverfahren und 43 stehen noch in der Warteschleife (https://www.biotech-enzymes.com/unionsliste-lebensmittelenzyme-ausstehende-sicherheitsbewertungen-durch-efsa)
Von den 49 Anträgen, die nach März 2015 eingegangen sind (EFSA-Question-Nr. ab 2019-xxxx) stehen 26 im Bewertungsverfahren und 23 warten auf ihre Bewertung (https://www.biotech-enzymes.com/unionsliste-lebensmittelenzyme-ausstehende-sicherheitsbewertungen-durch-efsa-antraege-nach-stichtag-maerz-2015)

Soweit aufgrund der zugänglichen Datenlagelage (und der Zeit) unterliegt keines der Lebensmittelenzyme den Auswirkungen des EuGH-Urteils C-528/16 zu den Mutageneseverfahren. Auch wenn bei einigen Lebensmittelenzymen gezielt Aminosäuren mit gentechnischen Verfahren ausgetauscht wurden, so wurden diese Verfahren bereits vor 2001 angewandt. Bestehen bleibt aber, dass für Arbeiten mit genomeditierten Mikroorganismen (Verfahren nach 2001) im geschlossenen System auch das EuGH-Urteil Anwendung findet.

Die Problematik des Vorhandenseins von rDNA besteht natürlich auch bei Lebensmittelenzymen. Dies hat zwar nicht unbedingt etwas mit der gesundheitlichen Unbedenklichkeit zu tun, aber berührt das Zulassungsverfahren. Solche Lebensmittelenzyme müssten wahrscheinlich nach Verordnung (EU) 1829/2003 zugelassen werden. Für zwei Lebensmittelenzyme wurde im Bewertungsverfahren das Vorhandensein von rDNA explizit erwähnt.

EFSA-Food Enzymes

The publication by Dederer (2022) prompted me to update my "enzyme website" (https://www.biotech-enzymes.com/) This involved updating the closed safety assessments by EFSA of food enzymes up to 30.01.2022 and analysing the progress of the assessments of the applications received by the Commission by 11.03.2015. Only when all these food enzymes have been evaluated for their safety the Commission will establish the Union list. So far, the ► EFSA has assessed the safety of 123 or 106 food enzymes, depending on the type of counting (enzymes or EFSA question number). Four of them were classified as unsafe and for 8 ones no conclusive statement on safety could be made on the basis of the data submitted.
From the list of applications received by March 2015, 114 are in the evaluation process and 43 are still on hold (https://www.biotech-enzymes.com/unionsliste-lebensmittelenzyme-ausstehende-sicherheitsbewertungen-durch-efsa).

Of the 49 applications received after March 2015 (EFSA-Question-Nr. as of 2019-xxxx and higher), 26ones are in the evaluation process and 23 ones are awaiting evaluation (https://www.biotech-enzymes.com/unionsliste-lebensmittelenzyme-ausstehende-sicherheitsbewertungen-durch-efsa-antraege-nach-stichtag-maerz-2015)

As far as based on the accessible data (and time), none of the food enzymes are subject to the impact of the ECJ ruling C-528/16 on mutagenesis procedures. Even if amino acids were specifically exchanged with genetic engineering methods for some food enzymes, these methods were already used before 2001. However, it remains the case that the ECJ ruling also applies on working with genome-edited microorganisms (procedures after 2001) in a closed system.

The problem of the presence of rDNA naturally also exists with food enzymes. This does not necessarily have anything to do with health safety, but it does affect the authorisation procedure. Such food enzymes would probably have to be approved under Regulation (EU) 1829/2003. For two food enzymes, the presence of rDNA was explicitly mentioned in the opinion.

Meetings – Conferences

EFSA: **ONE Conference 2022 registration now open!**

https://www.efsa.europa.eu/en/news/one-conference-2022-registration-now-open

8. Jahrestreffen der Seniorexperten Chemie: **Chemie – vielseitig, spannend, unentbehrlich!** Wernigerode/Harz, 02. -04.05.2022
www.gdch.de/SEC2022

Press Releases – Media Reports / Pressemeldungen und Medienberichte

Kornly J.: **EU-Kommission deklariert Atomkraft und Erdgas als „grün" – was das für dich bedeutet**
https://utopia.de/news/was-es-fuer-dich-bedeutet-wenn-die-eu-atomkraft-und-erdgas-als-gruen-deklariert/?utm_source=Interessenten&utm_campaign=9bdbada495-Newsletter_DO_22KW05&utm_medium=email&utm_term=0_af58dac727-9bdbada495-264788737

Commission: EU taxonomy for sustainable activities
What the EU is doing to create an EU-wide classification system for sustainable activities
https://ec.europa.eu/info/business-economy-euro/banking-and-finance/sustainable-finance/eu-taxonomy-sustainable-activities_en

Hefferon K.L. and Miller H.I.**: Nitrogen-Fixing Bacteria Are the Latest Genetic Engineering Breakthrough**
https://henrymillermd.org/25981/nitrogen-fixing-bacteria-are-the-latest-genetic
https://www.europeanscientist.com/en/features/nitrogen-fixing-bacteria-are-the-latest-genetic-engineering-breakthrough/
Publication: https://pubs.acs.org/doi/10.1021/acssynbio.1c00049

Cudis C.: **Massive production of 'Golden Rice' seeds to start this year**
https://www.pna.gov.ph/articles/1166915

Only some selected press releases or media reports are listed here. The daily up-date of the press releases and media reports are ▶here: (January week 04)

Publications – Publikationen

Merbach W.: (2021): **Grüne Gentechnik – Chance und Herausforderung:** Evangelische Verantwortung; Das Magazin des Evangelischen Arbeitskreises der CDU/CSU | Ausgabe 11+12/2021
https://www.eak-cducsu.de/evangelische-verantwortung

BfN Schriften 622: **Analyse von Nachweismethoden für genomeditierte und klassische GV-Pflanzen**
https://www.bfn.de/sites/default/files/2022-02/Skript622.pdf

Paarlberg R. (2022): **The trans-Atlantic conflict over "green" farming**. Food Policy 108, April 2022, 102229 | https://doi.org/10.1016/j.foodpol.2022.102229
With its new Farm to Fork (F2F) strategy, the EU plans to expand organic farming, an approach that rules out both synthetic chemicals and modern biotechnology, and it intends to use trade and assistance policies to pursue this strategy not just at home but also through Green Alliances abroad. The United States, by contrast, is emphasizing agricultural innovations based on the latest science—including gene-editing—and is now organizing with other countries a Coalition for Productivity Growth as a counter to European influence. Environmentalists in Europe believe their new vision is "green," but on closer inspection it is not. If organic farming scaled up to replace 25 percent of conventional farming in Europe, much more land would have to be converted to food production, with damaging results for wildlife habitat and the climate. In its earlier rejection of GMOs, Europe caused environmental harm by foregoing options to cut insecticide use and adopt no-till practices. Europe's regulatory example also discouraged the adoption of GMO food crops around the world. Europe is now inviting similar harms by classifying and regulating gene-edited crops as GMOs, but this most recent aversion to agricultural science is less likely to enjoy global influence.
https://www.sciencedirect.com/science/article/pii/S0306919222000124?dgcid=author

Vesprini F., Whelan A.I., Goberna M.F., Murrone M.L. et al. (2022): **Update of Argentina's Regulatory Policies on the Environmental Risk Assessment.** Front. Bioeng. Biotechnol| https://doi.org/10.3389/fbioe.2021.834589
The Environmental Risk Assessment (ERA) of genetically modified (GM) crops in Argentina is carried out by the National Advisory Commission on Agricultural Biotechnology (CONABIA) and the Innovation and Biotechnology Coordination (CIyB). Both have a large experience with this assessment, since 1991, when CONABIA was created. The continuous support to biotechnology as a state policy and as part of the decision to encourage

developers in the regulatory process has helped make progress in the revision of the regulations. The experience gained during the last 30 years and the worldwide scientific advances supported the bases to update the regulatory framework. Focusing on the biosafety strengthening and the improvement of the applicant's experience in the GM crops evaluation process, during 2020 and 2021, the ERA went through a reviewing process. Some important modifications were made, such as (i) the assessment of stacked GM crops with focus on the possible interactions between transgenes and the expression products, (ii) the strengthening of the ERA taking into account the transportability of data and conclusions from the Confined Field Trials (CFTs), (iii) the adoption of Familiarity and History of Safe Use (HOSU) concepts on the risk assessment of the expression products, (iv) the special considerations for the unintended effects of insertional sites, and (v) as a post commercial release of GM crops, the Insect Resistance Management Plan (IRMP) was reformulated. These novel approaches enhance the ERA; they make it more efficient by applying the science criteria and the accumulated experience and scientific bibliography on the topic.
https://www.frontiersin.org/articles/10.3389/fbioe.2021.834589/full

Yang Y., Xu C, Shen Z., Yan C. (2022): **Crop Quality Improvement Through Genome Editing Strategy.** Front. Genome Ed| https://doi.org/10.3389/fgeed.2021.819687
Good quality of crops has always been the most concerning aspect for breeders and consumers. However, crop quality is a complex trait affected by both the genetic systems and environmental factors, thus, it is difficult to improve through traditional breeding strategies. Recently, the CRISPR/Cas9 genome editing system, enabling efficiently targeted modification, has revolutionized the field of quality improvement in most crops. In this review, we briefly review the various genome editing ability of the CRISPR/Cas9 system, such as gene knockout, knock-in or replacement, base editing, prime editing, and gene expression regulation. In addition, we highlight the advances in crop quality improvement applying the CRISPR/Cas9 system in four main aspects: macronutrients, micronutrients, anti-nutritional factors and others. Finally, the potential challenges and future perspectives of genome editing in crop quality improvement is also discussed.
https://www.frontiersin.org/articles/10.3389/fgeed.2021.819687/full

Wada N., Osakabe K., Osakabe Y. (2022): **Expanding the plant genome editing toolbox with recently developed CRISPR-Cas systems**. Plant Physiology, kiac027 | https://doi.org/10.1093/plphys/kiac027
Since its first appearance, CRISPR-Cas9 has been developed extensively as a programmable genome editing tool, opening a new era in plant genome engineering. However, CRISPR-Cas9 still has some drawbacks, such as limitations of the PAM sequence, target specificity, and the large size of the *cas9* gene. To combat invading bacterial phages and plasmid DNAs, bacteria and archaea have diverse and unexplored CRISPR-Cas systems, which have the potential to be developed as a useful genome editing tools. Recently, discovery and characterization of additional CRISPR-Cas systems have been reported. Among them, several CRISPR-Cas systems have been applied successfully to plant and human genome editing. For example, several groups have achieved genome editing using CRISPR-Cas type I-D and type I-E systems, which had never been applied for genome editing previously. In addition to higher specificity and recognition of different PAM sequences, recently developed CRISPR-Cas systems often provide unique characteristics that differ from well-known Cas proteins such as Cas9 and Cas12a. For examples, type I CRISPR-Cas10 induces small indels and bi-directional long-range deletions ranging up to 7.2 kb in tomatoes (*Solanum lycopersicum* L.). Type IV CRIPSR-Cas13 targets RNA, not dsDNA, enabling highly specific knockdown of target genes. In this article, we review the development of CRISPR-Cas systems, focusing especially on their application to plant genome engineering. Recent
https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiac027/6517796

Zhang Y., Cheng Y., Fang H., Roberts N. et al. (2022): **Highly Efficient Genome Editing in Plant Protoplasts by Ribonucleoprotein Delivery of CRISPR-Cas12a Nucleases.** Front. Genome Ed., https://doi.org/10.3389/fgeed.2022.780238
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) mediated genome editing is a powerful approach for crop improvement. Traditional transformation methods based on plasmid delivery pose concerns associated with transgene integration and off-target effects. CRISPR delivered as ribonucleoproteins (RNPs) can prevent exogenous DNA integration, minimize off-target effects, and reduce cellular toxicity. Although RNP delivered CRISPR genome editing has been demonstrated in many plant species, optimization strategies that yield high editing efficiencies have not been thoroughly investigated. Using rice and citrus protoplast systems we demonstrated highly efficient genome editing using Cas12a delivered as RNPs. Four Cas12a variants, including LbCas12a, LbCas12a-E795L, AsCas12a, and AsCas12a Ultra, were investigated. Nearly 100% editing efficiency was observed for three out of four target sites by LbCas12a, LbCas12a-E795L, and AsCas12a Ultra, as measured by restriction fragment length polymorphism (RFLP) and verified by next generation sequencing of PCR amplicons. RNP delivery resulted in higher editing efficiencies than plasmid delivery at 32°C and 25°C. LbCas12a and LbCas12a-E795L demonstrated increased editing efficiencies in comparison to AsCas12a and AsCas12a Ultra, especially when used at lower RNP concentrations. In addition, we discovered that a 1:1 Cas12a:crRNA molar ratio is sufficient to achieve efficient genome editing. Nuclear localization signals (NLSs) are essential for efficient RNP-based genome editing. However, the different crRNA modifications tested did not significantly improve genome editing efficiency. Finally, we applied the Cas12a RNP system in citrus protoplasts and obtained similarly high editing efficiencies at the target site. Our study provides a comprehensive guideline for Cas12a-mediated genome editing using RNP delivery in plant cells, setting the foundation for the generation of transgene-free genome edited plants.
https://www.frontiersin.org/articles/10.3389/fgeed.2022.780238/full

Tiwari J.K., Buckseth T., Challam C., Zinta R. et al. (2022): **CRISPR/Cas Genome Editing in Potato: Current Status and Future Perspectives.** Front. Genet. | https://doi.org/10.3389/fgene.2022.827808
https://www.frontiersin.org/articles/10.3389/fgene.2022.827808/full

Lin C.-S., Hsu C.-T., Yuan, Y.-H., Zheng P.-X. et al. (2022): **DNA-free CRISPR-Cas9 gene editing of wild tetraploid tomato *Solanum peruvianum* using protoplast regeneration.** Plant Physiology, kiac022 | https://doi.org/10.1093/plphys/kiac022
Wild tomatoes (*Solanum peruvianum*) are important genomic resources for tomato research and breeding. Development of a foreign DNA-free CRISPR-Cas delivery system has potential to mitigate public concern about genetically modified organisms. Here, we established a DNA-free CRISPR-Cas9 genome editing system based on an optimized protoplast regeneration protocol of *S. peruvianum*, an important resource for tomato introgression breeding. We generated mutants for genes involved in small interfering RNAs (siRNA) biogenesis, *RNA-DEPENDENT RNA POLYMERASE 6* (*SpRDR6*) and *SUPPRESSOR OF GENE SILENCING 3* (*SpSGS3*); pathogen-related peptide precursors, *PATHOGENESIS-RELATED PROTEIN-1* (*SpPR-1*) and *PROSYSTEMIN* (*SpProSys*); and fungal resistance (*MILDEW RESISTANT LOCUS O, SpMlo1*) using diploid or tetraploid protoplasts derived from *in vitro*-grown shoots. The ploidy level of these regenerants was not affected by PEG-Ca^{2+}-mediated transfection, CRISPR reagents, or the target genes. By karyotyping and whole genome sequencing analysis, we confirmed that CRISPR-Cas9 editing did not introduce chromosomal changes or unintended genome editing sites. All mutated genes in both diploid and tetraploid regenerants were heritable in the next generation. *spsgs3* null T_0 regenerants and *sprdr6* null T_1 progeny had wiry, sterile phenotypes in both diploid and tetraploid lines. The sterility of the *spsgs3* null mutant was partially rescued, and fruits were obtained by grafting to wild-type stock and pollination with wild-type pollen. The resulting seeds contained the mutated alleles. Tomato yellow leaf curl virus proliferated at higher levels in *spsgs3* and *sprdr6* mutants than in the wild type. Therefore, this protoplast regeneration technique should greatly facilitate tomato polyploidization and enable the use of CRISPR-Cas for *S. peruvianum* domestication and tomato breeding.
https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiac022/6516539?login=false

Fetters, A.M., Cantalupo, P.G., Wei, N. et al. (2022): **The pollen virome of wild plants and its association with variation in floral traits and land use**. Nat Commun 13, 523 | https://doi.org/10.1038/s41467-022-28143-9
Pollen is a unique vehicle for viral spread. Pollen-associated viruses hitchhike on or within pollen grains and are transported to other plants by pollinators. They are deposited on flowers and have a direct pathway into the plant and next generation via seeds. To discover the diversity of pollen-associated viruses and identify contributing landscape and floral features, we perform a species-level metagenomic survey of pollen from wild, visually asymptomatic plants, located in one of four regions in the United States of America varying in land use. We identify many known and novel pollen-associated viruses, half belonging to the Bromoviridae, Partitiviridae, and Secoviridae viral families, but many families are represented. Across the regions, species harbor more viruses when surrounded by less natural and more human-modified environments than the reverse, but we note that other region-level differences may also covary with this. When examining the novel connection between virus richness and floral traits, we find that species with multiple, bilaterally symmetric flowers and smaller, spikier pollen harbored more viruses than those with opposite traits. The association of viral diversity with floral traits highlights the need to incorporate plant-pollinator interactions as a driver of pollen-associated virus transport into the study of plant-viral interactions.
https://www.nature.com/articles/s41467-022-28143-9.pdf

Hu Y,, Linghu L., Li M., Mao D. et al. (2022): **Nutritional components and protein quality analysis of genetically modified phytase maize.** GM Crops & Food, DOI: 10.1080/21645698.2021.2009418
The nutritional components and protein quality of genetically modified maize expressing phytase gene (GM) were analyzed and evaluated in this study. The nutritional components were analyzed by Chinese national standard methods. The ileostomy Bama miniature pigs were utilized to analyze the true digestibility of protein and amino acids. The digestible indispensable amino acid score (DIAAS) was adopted to evaluate the protein quality of GM, its parental maize (PM) and commercial available maize Zhengdan 958 (ZD). Meanwhile, the widely used protein digestibility corrected amino acid score (PDCAAS) was also calculated and compared with DIAAS. The content of protein, fat, vitamins, and minerals of all the strains of maize are in the normal ranges of OECD and/or ILSI. The DIAAS of GM, PM, and ZD were 54.57, 31.75, and 33.91, respectively, and the first limiting amino acid for GM, PM, and ZD was lysine. In conclusion, the introduction of *phyA2* gene in GM maize does not disturb the digestion of protein/amino acid, but has the ability to promote the digestion of amino acids.
https://www.tandfonline.com/doi/full/10.1080/21645698.2021.2009418

Then C., Miyazaki J., Bauer-Panskus A. (2022): **Deficiencies in the Risk Assessment of Genetically Engineered Bt Cowpea Approved for Cultivation in Nigeria: A Critical Review.** Plants *11*(3), 380; https://doi.org/10.3390/plants11030380

We analyze the application filed for the marketing and cultivation of genetically engineered Bt cowpea (event AAT 709A) approved in Nigeria in 2019. Cowpea (Vigna ungiguiculata) is extensively grown throughout sub-Saharan Africa and consumed by around two hundred million people. The transgenic plants produce an insecticidal, recombinant Bt toxin meant to protect the plants against the larvae of Maruca vitrata, which feed on the plants and are also known as pod borer. Our analysis of the application reveals issues of concern regarding the safety of the Bt toxins produced in the plants. These concerns include stability of gene expression, impact on soil organisms, effects on non-target species and food safety. In addition, we show deficiencies in the risk assessment of potential gene flow and uncontrolled spread of the transgenes and cultivated varieties as well as the maintenance of seed collections. As far as information is publicly available, we analyze the application by referring to established standards of GMO risk assessment. We take the provisions of the Cartagena Protocol on Biosafety (CPB) into account, of which both Nigeria and the EU are parties. We also refer to the EU standards for GMO risk assessment, which are complementary to the provisions of the CPB.
https://www.mdpi.com/2223-7747/11/3/380

Clark M., Tepper K., Petroll K., Kumar S,, Sunna A., Maselko M. (2022): **Bioremediation of Industrial Pollutants by Insects Expressing a Fungal Laccase.** ACS Synthetic Biology 11 (1), 308-316 | DOI: 10.1021/acssynbio.1c00427
Inadequate management of household and industrial wastes poses major challenges to human and environmental health. Advances in synthetic biology may help address these challenges by engineering biological systems to perform new functions such as biomanufacturing of high-value compounds from low-value waste streams and bioremediation of industrial pollutants. The current emphasis on microbial systems for biomanufacturing, which often requires highly preprocessed inputs and sophisticated infrastructure, is not feasible for many waste streams. Furthermore, concerns about transgene biocontainment have limited the release of engineered microbes or plants for bioremediation. Engineering of animals may provide opportunities for utilizing various waste streams that are not suitable for microbial biomanufacturing while effective transgene biocontainment options should enable *in situ* bioremediation. Here, we engineer the model insect *Drosophila melanogaster* to express a functional laccase from the fungus *Trametes trogii*. Laccase-expressing flies reduced concentrations of the endocrine disruptor bisphenol A by more than 50% when present in their growth media. A lyophilized powder prepared from engineered adult flies retained substantial enzymatic activity, degrading more than 90% of bisphenol A and the textile dye indigo carmine in aqueous solutions. Our results demonstrate that transgenic animals may be used to bioremediate environmental contaminants *in vivo* and serve as novel production platforms for industrial enzymes. These results support further development of insects, and possibly other animals, as bioproduction platforms and their potential use in bioremediation.
https://pubs.acs.org/doi/10.1021/acssynbio.1c00427

Zhang, Q., Bastard, P. et al. (2022): **Human genetic and immunological determinants of critical COVID-19 pneumonia**. *Nature* | https://doi.org/10.1038/s41586-022-04447-0
SARS-CoV-2 infection is benign in most individuals but, in ~10% of cases, it triggers hypoxemic COVID-19 pneumonia, which becomes critical in ~3% of cases. The ensuing risk of death (~1%) doubles every five years from childhood onward and is ~1.5 times greater in men than in women. What are the molecular and cellular determinants of critical COVID-19 pneumonia? Inborn errors of type I IFNs, including autosomal TLR3 and X-linked TLR7 deficiencies, are found in ~1-5% of patients with critical pneumonia under 60 years old, and a lower proportion in older patients. Pre-existing autoantibodies neutralizing IFN-α, –β, and/or –ω, which are more common in men than in women, are found in ~15-20% of patients with critical pneumonia over 70 years old, and a lower proportion in younger patients. Thus, at least 15% of cases of critical COVID-19 pneumonia can apparently be explained. The TLR3- and TLR7-dependent production of type I IFNs by respiratory epithelial cells and plasmacytoid dendritic cells, respectively, is essential for host defense against SARS-CoV-2. In ways that can depend on age and sex, insufficient type I IFN immunity in the respiratory tract during the first few days of infection may account for the spread of the virus, leading to pulmonary and systemic inflammation.
https://www.nature.com/articles/s41586-022-04447-0
https://www.nature.com/articles/s41586-022-04447-0_reference.pdf

---

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 .

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

Klaus-Dieter Jany
Nelkenstrasse 36
D-76351 Linkenheim-Hochstetten
jany@biotech-gm-food.com

1. Vorsitzender des WGG e.V.
Postfach 120721
D-60114 Frankfurt/Main
jany@wgg-ev.de