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Gentechnisch veränderte Pflanzen: Resultiert mehr Risikoforschung in mehr Sicherheit?

Der Appell der 31 EU-Parlamentarier [1, 2] an die Kommission, Mittel für die Forschung zu Risiken von gentechnisch veränderten Pflanzen bzw. von genomeditierten Pflanzen (Organismen) und zu deren analytischen Nachweis bereitzustellen, ist grundsätzlich zu begrüßen.

In den letzten 25 Jahren hat die Kommission Studien zu möglichen Gefährdungen der Gesundheit von Mensch und Tier sowie der Umwelt durch gentechnisch veränderte Pflanzen mit ca. 300 Millionen Euro unterstützt und mit einem ähnlichen Betrag haben sich zusätzlich die Mitgliedsstaaten in nationalen Projekten beteiligt. In keiner der wissenschaftlichen Untersuchungen konnten reale Gefährdungen nachgewiesen werden und alle Untersuchungen kamen zum Schluss, dass gentechnisch veränderte Pflanzen genau sicher sind wie die aus der konventionellen Züchtung [3,4]. Allerdings sollte man von der Forderung einer 100%igen Sicherheit abrücken. In der belebten Natur gibt es eine solche Sicherheit nicht! Die Wissenschaft kann system- und methodenbedingt nie eine 100%ige Sicherheit beweisen!

Das Vorgehen und die Methoden der Sicherheitsbewertung haben sich weiterentwickelt und verfeinert. Man sollte sie durchaus nun auch auf neu gezüchtete Pflanzen (Organismen) anwenden, auf ihre Eignung überprüfen und dann auch gewillt sein, entsprechende Konsequenzen aus den Forschungsergebnissen zu ziehen.

In der Tat, die Kommission hat seit 2016 keine Forschungsprojekte für Untersuchungen / zur Auffindung möglicher Risiken gentechnisch veränderter Pflanzen mehr gefördert. Kleinere Vorhaben wurden von nationalen Fördereinrichtung an Universitäten unterstützt oder Interessensvertreter haben bestimmte Projekte gezielt finanziert. Ja, es besteht ein Manko an staatlich geförderten Untersuchungen. Die notorisch unterfinanzierten Universitäten und sonstige Forschungseinrichtungen können solche Forschungen nicht eigenständig stemmen.

Soweit überblickbar gibt es in der EU keine Untersuchungen zur Sicherheit genomeditierter Pflanzen. Über die Sinnhaftigkeit solcher Projekte soll hier nicht diskutiert werden, insbesondere nicht über Untersuchungen zu solchen Pflanzen, die sich nicht von natürlich mutierten unterscheiden. Unabhängig von Aspekten der Sicherheit erweitern sie aber generell unser Wissen über diese Pflanzen und ihren Interaktionen mit der Umwelt. Allerdings bedingen solche Untersuchungen stets auch Freisetzungsversuche. Solche Freisetzungen werden von diesen 31 Unterzeichner im Allgemeinen abgelehnt und unterschwellig bis offen in ihren Ländern verhindert (siehe hier das Beispiel Baden-Württemberg, Deutschland [5]). Wer mehr Risikoforschung fordert, sollte sich auch offen zu Freisetzungsversuchen bekennen!

Die Förderung der Entwicklung von Nachweisverfahren von genomeditierten Pflanzen und daraus gewonnenen Erzeugnisse ist schon aus gesellschaftspolitischen Gründen zu begrüßen. Transparenz ist für Akzeptanz und Vertrauen ungemein wichtig! Bei der Methodenentwicklung darf es nicht um den Nachweis einer oder weniger Mutationen gehen; dies ist bereits „state of the art“, sondern um den Nachweis wie die Mutation erzeugt wurde bzw. entstanden ist. Mit der Förderung solcher Forschungsarbeiten können die Möglichkeiten aber auch die Grenzen einer gerichtsfesten Nachweisbarkeit genomeditierter Pflanzen aufgezeigt werden. Auch hier gilt wieder die Politik sollte die Forschungsergebnisse zur Kenntnis nehmen und sie entsprechend umsetzen.

Genetically Modified Plants: Does More Risk Research Result in More Safety?

The appeal of the 31 EU parliamentarians [1,2] to the Commission to provide funds for research on the risks of genetically modified plants or genome-edited plants (organisms) and for their analytical proof to be welcomed in principle.

In the last 25 years, the Commission has supported studies on possible risks to human and animal health as well as to the environment from genetically modified plants with about 300 million euros, and member states have also contributed a similar amount to national projects. None of the scientific studies could prove real hazards and all studies came to the conclusion that genetically modified plants are just as safe as those from conventional breeding [3,4]. However, one should move away from the demand for 100% safety. There is no such certainty in living nature! Science can never prove 100% safety due to the system and methods used!

The procedure and methods of safety assessment have been further evolved and refined. They should certainly now be applied also to newly bred plants (organisms), checked for their suitability and then also be willing to draw appropriate conclusions from the research results.

In fact, since 2016, the Commission has no longer funded research projects for studies to identify possible risks of genetically modified plants. Smaller projects have been supported by national funding bodies at universities or stakeholders have specifically funded certain projects. Yes, there is a shortage of government-funded research. The notoriously underfunded universities and other research institutions cannot carry out such research on their own.

As far as can be seen, there are no studies on the safety of genome-edited plants in the EU. The usefulness of such projects should not be discussed here, especially not studies on plants that do not differ from naturally mutated plants. Regardless of safety aspects, however, these investigations generally increase our knowledge about these plants and their interactions with the environment. However, such studies always require field releases. Such releases are generally rejected by these 31 signatories and are subliminally to openly prevented in their countries (see here the example of Baden-Württemberg, Germany [5]). Those who call for more risk research should also openly declare their support for release trials!

The funding of the development of detection methods for genome-edited plants and products derived from them is to be welcomed for socio-political reasons alone. Transparency is extremely important for acceptance and trust! The development of methods should not be about the detection of one or a few mutations; this is already "state of the art", but about the detection of how the mutation was produced or arose. With the promotion of such research, the possibilities but also the limits of a forensic proof of genome-edited plants can be demonstrated. Here again, politics should take note of the research results and implement them accordingly.

[1] Foote N. (15.02.2022): **MEPs demand EU funding for research into gene editing surveillance**
https://www.euractiv.com/section/agriculture-food/news/meps-demand-eu-funding-for-research-into-gene-editing-surveillance/?_ga=2.202658708.409956239.1644936378-791110242.1644936378

[2] Häusling M. (08.02.2022): EU research on risks and detection methods related to new GM plants
https://www.martin-haeusling.eu/images/220208_Letter_Haeusling_to_COM_Mariya_Gabriel_about_new_GM_research_final.pdf

EU research on risks and detection methods related to new GM plants
https://www.martin-haeusling.eu/images/EU_research_on_risks_and_detection_methods_related_to_new_GM_plants.pdf

[3] Norero D. (21.01.2022): **GMO 25-year safety endorsement: 280 science institutions, more than 3,000 studies**
https://geneticliteracyproject.org/2022/01/21/gmo-20-year-safety-endorsement-280-science-institutions-more-3000-studies/

[4] Jany Kl.-D. (19.02.2022): **Sicherheitsforschung zu gentechnisch veränderten Pflanzen und Lebensmitteln**
https://www.biotech-gm-food.com/wissen/sicherheit-von-gv-pflanzen-25-jahre-forschung-280-wissenschaftliche-einrichtungen-mehr-als-3000-studien

Meetings – Conferences / Veranstaltungen - Tagungen

Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG): Genomeditierung: Neue Techniken für bessere Lebensmittelprodukte
ANUGA Food Tec: Köln 26.04.2022 | 13:40 - 15:10Uhr
https://www.anugafoodtec.de/event/genomeditierung_neue_techniken_fuer_bessere_lebensmittelprodukte?from=tracks

Press Releases – Media Reports / Pressemeldungen und Medienberichte

Kommission: **Pestizide: EU erleichtert Zugang zu biologischen Alternativen und fordert EU-Staaten auf, bei Einschränkungen von Sulfoxaflor voranzukommen**
https://germany.representation.ec.europa.eu/news/pestizide-eu-erleichtert-zugang-zu-biologischen-alternativen-und-fordert-eu-staaten-auf-bei-2022-02-10_de

Foote N.: **EU-Abgeordnete fordern EU-finanzierte Forschung und Überwachung von Gen-Editing**
https://www.euractiv.de/section/landwirtschaft-und-ernahrung/news/eu-abgeordnete-fordern-eu-finanzierte-forschung-und-ueberwachung-von-gen-editing/

Englisch version: https://www.euractiv.com/section/agriculture-food/news/meps-demand-eu-funding-for-research-into-gene-editing-surveillance/?_ga=2.202658708.409956239.1644936378-791110242.1644936378

Stainthorpe S.: **Norwich scientists lead the way in gene editing**
https://www.edp24.co.uk/news/business/norwich-research-park-gene-editing-boost-8692904

Informationsdienst Gentechnik: **In der EU drängen die ersten Crispr-Pflanzen ins Freiland**
https://www.keine-gentechnik.de/nachricht/34550?cHash=937a0c983da1bcdd39e755cf23071cc7

American Society for Microbiology: **Engineered bacterial strains could fertilize crops, reduce waterways pollution**
https://www.eurekalert.org/news-releases/943922

GM Watch: **GM Watch publishes the full definitive account of the Pusztai affair**
https://gmwatch.org/en/106-news/latest-news/19987

see also: Ammann K. (2012): Arpad Pusztai's Feeding experiments of GM potatoes with lectins to rats: Anatomy of a controversy 1998 - 2009
http://www.ask-force.org/web/AF-2-Pusztai/AF-2-Pusztai-Food-Safety-20121127-opensource.pdf

Jany Kl.-D. (2022): Die Pusztai Affäre – Fütterungsversuche an Ratten mit Schneeglöckchen-Lektin-Kartoffeln
https://www.biotech-gm-food.com/komm/pusztai-affaere-um-fuetterungsstudie-an-ratten-mit-schneegloeckchen-lektin-kartoffeln

Analyse der Fütterungsstudie von Pusztai an Ratten mit Schneeglöckchen-Lektin-Kartoffeln
https://www.biotech-gm-food.com/Komm/pusztai-ratten-fuetterungsstudien-mit-schneegloeckchen-lektin-kartoffeln_gv-kartoffeln-lassen-das-gehirn-schrumpfen

Only some selected press releases or media reports are listed here. The daily up-date of the press releases and media reports are ►here: (February week 07)

Publications – Publikationen

Bruckner, B., Hubacek, K., Shan, Y. et al. (2022): **Impacts of poverty alleviation on national and global carbon emissions**. Nat Sustain | https://doi.org/10.1038/s41893-021-00842-z
Wealth and income are disproportionately distributed among the global population. This has direct consequences on consumption patterns and consumption-based carbon footprints, resulting in carbon inequality. Due to persistent inequality, millions of people still live in poverty today. On the basis of global expenditure data, we compute country- and expenditure-specific per capita carbon footprints with unprecedented details. We show that they can reach several hundred tons of CO_2 per year, while the majority of people living below poverty lines have yearly carbon footprints of less than 1 tCO_2. Reaching targets under United Nations Sustainable Development Goal 1, lifting more than one billion people out of poverty, leads to

only small relative increases in global carbon emissions of 1.6–2.1% or less. Nevertheless, carbon emissions in low- and lower-middle-income countries in sub-Saharan Africa can more than double as an effect of poverty alleviation. To ensure global progress on poverty alleviation without overshooting climate targets, high-emitting countries need to reduce their emissions substantially.
https://www.nature.com/articles/s41893-021-00842-z.pdf

Matveeva, T. (2021): **New naturally transgenic plants: 2020 update.** Bio. Comm. 66 (1): 36–46 | https://doi.org/10.21638/spbu03.2021.105
Agrobacterium-mediated gene transfer leads to crown gall or hairy roots disease, due to expression of transferred T-DNA genes. Spontaneous plant regeneration from the transformed tissues can produce natural transformants carrying cellular T-DNA (cT-DNA) sequences of agrobacterial origin. In 2019, based on genomic sequencing data, cT-DNA horizontally transferred from *Agrobacterium* were found in two dozen species of angiosperms. This made it possible to evaluate the spread of this phenomenon, as well as make some generalizations regarding the diversity of horizontally transferred genes. The presented research is a continuation of work in this field. It resulted in the description of new naturally occurring transgenic species *Aeschynomene evenia* C. Wright, *Eperua falcata* Aubl., *Eucalyptus cloeziana* F.Muell., *Boswellia sacra* Flueck., *Kewa caespitosa* (Friedrich) Christenh., *Pharnaceum exiguum* Adamson, *Silene noctiflora* L., *Nyssa sinensis* Oliv., *Vaccinium corymbosum* L., *Populus alba* L. × *Populus glandulosa* Moench. The previously identified patterns regarding the frequency of the occurrence of natural transformants and the general properties of the cT-DNAs were confirmed in this study.
https://biocomm.spbu.ru/article/view/9176

Silva-Pinheiro, P., Nash, P.A., Van Haute, L. et al. (2022): **In vivo mitochondrial base editing via adeno-associated viral delivery to mouse post-mitotic tissue**. Nat Commun 13, 750 | https://doi.org/10.1038/s41467-022-28358-w
Mitochondria host key metabolic processes vital for cellular energy provision and are central to cell fate decisions. They are subjected to unique genetic control by both nuclear DNA and their own multi-copy genome - mitochondrial DNA (mtDNA). Mutations in mtDNA often lead to clinically heterogeneous, maternally inherited diseases that display different organ-specific presentation at any stage of life. For a long time, genetic manipulation of mammalian mtDNA has posed a major challenge, impeding our ability to understand the basic mitochondrial biology and mechanisms underpinning mitochondrial disease. However, an important new tool for mtDNA mutagenesis has emerged recently, namely double-stranded DNA deaminase (DddA)-derived cytosine base editor (DdCBE). Here, we test this emerging tool for in vivo use, by delivering DdCBEs into mouse heart using adeno-associated virus (AAV) vectors and show that it can install desired mtDNA edits in adult and neonatal mice. This work provides proof-of-concept for use of DdCBEs to mutagenize mtDNA in vivo in post-mitotic tissues and provides crucial insights into potential translation to h
https://www.nature.com/articles/s41467-022-28358-w.pdf

Ferreira da Silva, J., Oliveira, G.P., Arasa-Verge, E.A. et al. (2022): **Prime editing efficiency and fidelity are enhanced in the absence of mismatch repair**. Nat Commun 13, 760 | https://doi.org/10.1038/s41467-022-28442-1
Prime editing (PE) is a powerful genome engineering approach that enables the introduction of base substitutions, insertions and deletions into any given genomic locus. However, the efficiency of PE varies widely and depends not only on the genomic region targeted, but also on the genetic background of the edited cell. Here, to determine which cellular factors affect PE efficiency, we carry out a focused genetic screen targeting 32 DNA repair factors, spanning all reported repair pathways. We show that, depending on cell line and type of edit, ablation of mismatch repair (MMR) affords a 2–17 fold increase in PE efficiency, across several human cell lines, types of edits and genomic loci. The accumulation of the key MMR factors MLH1 and MSH2 at PE sites argues for direct involvement of MMR in PE control. Our results shed new light on the mechanism of PE and suggest how its efficiency might be optimised.
https://www.nature.com/articles/s41467-022-28442-1.pdf

Li, S., Lin, D., Zhang, Y. et al.(2022): **Genome-edited powdery mildew resistance in wheat without growth penalties.** Nature | https://doi.org/10.1038/s41586-022-04395-9
Disruption of susceptibility (*S*) genes in crops is an attractive breeding strategy for conferring disease resistance[1,2]. However, *S* genes are implicated in many essential biological functions and deletion of these genes typically results in undesired pleiotropic effects[1]. Loss-of-function mutations in one such *S* gene, *Mildew resistance locus O* (*MLO*), confers durable and broad-spectrum resistance to powdery mildew in various plant species[2,3]. However, *mlo*-associated resistance is also accompanied by growth penalties and yield losses[3,4], thereby limiting its widespread use in agriculture. Here we describe *Tamlo-R32*, a mutant with a 304-kilobase pair targeted deletion in the *MLO-B1* locus of wheat that retains crop growth and yields while conferring robust powdery mildew resistance. We show that this deletion results in an altered local chromatin landscape, leading to the ectopic activation of *Tonoplast monosaccharide transporter 3* (*TaTMT3B*), and that this activation alleviates growth and yield penalties associated with *MLO* disruption. Notably, the function of *TMT3* is conserved in other plant species such as *Arabidopsis thaliana*. Moreover, precision genome editing facilitates the rapid introduction of this *mlo* resistance allele (*Tamlo-R32)* into elite wheat varieties. This work demonstrates the ability to stack genetic changes to rescue growth defects caused by recessive alleles, which is critical for developing high-yielding crop varieties with robust and durable disease resistance.

https://www.nature.com/articles/s41586-022-04395-9

Chanda B., Gilliard A., Jaiswal N., Ling K.-S. (2021): **Comparative Analysis of Host Range, Ability to Infect Tomato Cultivars with Tm-22 Gene, and Real-Time Reverse Transcription PCR Detection of Tomato Brown Rugose Fruit Virus,** Plant Disease 115 (11) |https://doi.org/10.1094/PDIS-05-20-1070-RE

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables in the world. However, tomato is also susceptible to many viral diseases. Several tobamoviruses, including tomato mosaic virus (ToMV), tomato mottle mosaic virus (ToMMV), and tomato brown rugose fruit virus (ToBRFV), are highly contagious pathogens that could result in significant economic losses if not controlled effectively. Tobamoviruses have been managed relatively well with broad adaptation of tomato cultivars with resistance genes. However, emergence of ToBRFV was shown to break down resistance conferred by the common resistance genes, resulting in serious outbreaks in many countries in Asia, Europe, and North America. The objective of this study was to conduct a comparative analysis of biological properties, including host range and disease resistance of ToMV, ToMMV, and ToBRFV. Results showed that despite many similarities in the host range, there were some unique host plant responses for each of the three viruses. In a comparative evaluation of disease resistance using the same tomato cultivars with or without *Tm-2*^{2} gene, there was a striking difference in responses from tomato plants with *Tm-2*^{2} gene inoculated with ToBRFV, ToMV, or ToMMV. Whereas these test plants were resistant to ToMV or ToMMV infection, all test plants were susceptible to ToBRFV. Further, for ToBRFV detection, a sensitive and reliable multiplex real-time reverse transcription (RT)-PCR assay using TaqMan probe with an internal 18S rRNA control was also developed. With simple modifications to RNA extraction and seed soaking, real-time RT-PCR could consistently detect the virus in single infested seed in varied levels of contamination, suggesting its usefulness for seed health assay.
https://apsjournals.apsnet.org/doi/10.1094/PDIS-05-20-1070-RE

Samantara K., Bohra A., Mohapatra S.R., Prihatini R. et al. (2022): **Breeding More Crops in Less Time: A Perspective on Speed Breeding**. Biology 11 (2),275. | https://doi.org/10.3390/biology11020275

Breeding crops in a conventional way demands considerable time, space, inputs for selection, and the subsequent crossing of desirable plants. The duration of the seed-to-seed cycle is one of the crucial bottlenecks in the progress of plant research and breeding. In this context, speed breeding (SB), relying mainly on photoperiod extension, temperature control, and early seed harvest, has the potential to accelerate the rate of plant improvement. Well demonstrated in the case of long-day plants, the SB protocols are being extended to short-day plants to reduce the generation interval time. Flexibility in SB protocols allows them to align and integrate with diverse research purposes including population development, genomic selection, phenotyping, and genomic editing. In this review, we discuss the different SB methodologies and their application to hasten future plant improvement. Though SB has been extensively used in plant phenotyping and the pyramiding of multiple traits for the development of new crop varieties, certain challenges and limitations hamper its widespread application across diverse crops. However, the existing constraints can be resolved by further optimization of the SB protocols for critical food crops and their efficient integration in plant breeding pipelines.
https://www.mdpi.com/2079-7737/11/2/275/htm

Landau, C., Bernards, M., Hager, A., & Williams, M. (2022): **Significance of application timing, formulation, and cytochrome P450 genotypic class on sweet corn response to dicamba.** Weed Science, 1-7 | doi:10.1017/wsc.2022.5

Sweet corn (Zea mays L.) tolerance to dicamba and several other herbicides is due to cytochrome P450 (CYP)-mediated metabolism and is conferred by a single gene (Nsf1). Tolerance varies by CYP genotypic class, with hybrids homozygous for functional CYP (Nsf1Nsf1) being the most tolerant and hybrids homozygous for mutant CYP alleles (nsf1nsf1) being the least tolerant. The herbicide safener cyprosulfamide (CSA) increases tolerance to dicamba by stimulating the expression of several CYPs. However, the extent to which CSA improves the tolerance of different sweet corn CYP genotypic classes to dicamba is poorly understood. Additionally, the effect of growth stage on sweet corn sensitivity to dicamba is inadequately described. The objective of this work was to quantify the significance of application timing, formulation, and CYP genotypic class on sweet corn response to dicamba. Hybrids representing each of the three CYP genotypes (Nsf1Nsf1, Nsf1nsf1, nsf1nsf1), were treated with dicamba or dicamba + CSA at one of three growth stages: V3, V6, or V9. Across all timings, the nsf1nsf1 hybrid was the least tolerant to dicamba, displaying 16% higher crop injury levels 2 wk after treatment and 2,130 kg ha−1 lower ear mass yields compared with the Nsf1Nsf1 hybrid. The V9 growth stage was the most susceptible time for dicamba injury regardless of genotypic class, with 1.89 and 1,750 kg ha−1 lower ear mass yields compared with the V3 and V6 application timings, respectively. The addition of CSA to dicamba V9 applications reduced the injury from dicamba for all three genotypic classes; however, it did not eliminate the injury. The use of Nsf1Nsf1 or Nsf1nsf1 sweet corn hybrids along with herbicide safeners will reduce the frequency and severity of injury from dicamba and other CYP-metabolized herbicides.
https://www.cambridge.org/core/journals/weed-science/article/abs/significance-of-application-timing-formulation-and-cytochrome-p450-genotypic-class-on-sweet-corn-response-to-dicamba/9E2D2F09FE98E3F3C12F0A77EBB7B533

Giovannini, I., Boothby, T.C., Cesari, M. et al.(2022): **Production of reactive oxygen species and involvement of bioprotectants during anhydrobiosis in the tardigrade *Paramacrobiotus spatialis*.** *Sci Rep* 12, 1938 | https://doi.org/10.1038/s41598-022-05734-6
Water unavailability is an abiotic stress causing unfavourable conditions for life. Nevertheless, some animals evolved anhydrobiosis, a strategy allowing for the reversible organism dehydration and suspension of metabolism as a direct response to habitat desiccation. Anhydrobiotic animals undergo biochemical changes synthesizing bioprotectants to help combat desiccation stresses. One stress is the generation of reactive oxygen species (ROS). In this study, the eutardigrade *Paramacrobiotus spatialis* was used to investigate the occurrence of ROS associated with the desiccation process. We observed that the production of ROS significantly increases as a function of time spent in anhydrobiosis and represents a direct demonstration of oxidative stress in tardigrades. The degree of involvement of bioprotectants, including those combating ROS, in the *P. spatialis* was evaluated by perturbing their gene functions using RNA interference and assessing the successful recovery of animals after desiccation/rehydration. Targeting the glutathione peroxidase gene compromised survival during drying and rehydration, providing evidence for the role of the gene in desiccation tolerance. Targeting genes encoding glutathione reductase and catalase indicated that these molecules play roles during rehydration. Our study also confirms the involvement of aquaporins 3 and 10 during rehydration. Therefore, desiccation tolerance depends on the synergistic action of many different molecules working together.
https://www.nature.com/articles/s41598-022-05734-6.pdf

Linster E., Forero Ruiz F.L., Miklankova P. et al. (2022): **Cotranslational N-degron masking by acetylation promotes proteome stability in plants.** Nat Commun 13, 810 | https://doi.org/10.1038/s41467-022-28414-5
N-terminal protein acetylation (NTA) is a prevalent protein modification essential for viability in animals and plants. The dominant executor of NTA is the ribosome tethered N^{α}-acetyltransferase A (NatA) complex. However, the impact of NatA on protein fate is still enigmatic. Here, we demonstrate that depletion of NatA activity leads to a 4-fold increase in global protein turnover via the ubiquitin-proteasome system in Arabidopsis. Surprisingly, a concomitant increase in translation, actioned via enhanced Target-of-Rapamycin activity, is also observed, implying that defective NTA triggers feedback mechanisms to maintain steady-state protein abundance. Quantitative analysis of the proteome, the translatome, and the ubiquitome reveals that NatA substrates account for the bulk of this enhanced turnover. A targeted analysis of NatA substrate stability uncovers that NTA absence triggers protein destabilization via a previously undescribed and widely conserved nonAc/N-degron in plants. Hence, the imprinti
https://www.nature.com/articles/s41467-022-28414-5.pdf

Fliege, C.E., Ward, R.A., Vogel, P., Nguyen, H., Quach, T.et al. (2022): **Fine mapping and cloning of the major seed protein quantitative trait loci on soybean chromosome 20.** Plant J. | https://doi.org/10.1111/tpj.15658
Soybean [*Glycine max* (L.) Merr.] is a unique crop species because it has high levels of both protein and oil in its seed. Of the many quantitative trait loci (QTL) controlling soybean seed protein content, alleles of the cqSeed protein-003 QTL on chromosome 20 exert the greatest additive effect. The high-protein allele exists in both cultivated and wild soybean (*Glycine soja* Siebold & Zucc.) germplasm. Our objective was to fine map this QTL to enable positional-based cloning of its underlying causative gene(s). Fine mapping was achieved by developing and testing a series of populations in which the chromosomal region surrounding the segregating high- versus low-protein alleles was gradually narrowed, using marker-based detection of recombinant events. The resultant 77.8 kb interval was directly sequenced from a *G. soja* source and compared with the reference genome to identify structural and sequence polymorphisms. An insertion/deletion variant detected in *Glyma.20G85100* was found to have near-perfect +/− concordance with high/low-protein allele genotypes inferred for this QTL in parents of published mapping populations. The indel structure was concordant with an evolutionarily recent insertion of a TIR transposon into the gene in the low-protein lineage. Seed protein was significantly greater in soybean expressing an RNAi hairpin downregulation element in two independent events relative to control null segregant lineages. We conclude that a transposon insertion within the CCT domain protein encoded by the *Glyma.20G85100* gene accounts for the high/low seed protein alleles of the cqSeed protein-003 QTL.
https://onlinelibrary.wiley.com/doi/10.1111/tpj.15658

Tanaka W. Ohmori S., Kawakami N., Hirano H.-Y. (2021): **Flower meristem maintenance by TILLERS ABSENT 1 is essential for ovule development in rice, *Development*** (2021) 148 (24): dev199932 | https://doi.org/10.1242/dev.199932
Plant development depends on the activity of pluripotent stem cells in meristems, such as the shoot apical meristem and the flower meristem. In *Arabidopsis thaliana*, *WUSCHEL* (*WUS*) is essential for stem cell homeostasis in meristems and integument differentiation in ovule development. In rice (*Oryza sativa*), the *WUS* ortholog *TILLERS ABSENT 1* (*TAB1*) promotes stem cell fate in axillary meristem development, but its function is unrelated to shoot apical meristem maintenance in vegetative development. In this study, we examined the role of *TAB1* in flower development. The ovule, which originates directly from the flower meristem, failed to differentiate in *tab1* mutants, suggesting that *TAB1* is required for ovule formation. Expression of a stem cell marker was completely absent in the flower meristem at the ovule initiation stage, indicating that *TAB1* is essential for stem cell maintenance in the 'final' flower meristem. The ovule defect in

tab1 was partially rescued by *floral organ number 2* mutation, which causes overproliferation of stem cells. Collectively, it is likely that *TAB1* promotes ovule formation by maintaining stem cells at a later stage of flower development.
https://journals.biologists.com/dev/article-abstract/148/24/dev199932/273695/Flower-meristem-maintenance-by-TILLERS-ABSENT-1-is?redirectedFrom=fulltext

Colantonio V., Ferrão L.F.V., Tieman D.M., Bliznyuk N. (2022): **Metabolomic selection for enhanced fruit flavor**, PNAS 119 (7) e2115865119 | https://doi.org/10.1073/pnas.2115865119
Although they are staple foods in cuisines globally, many commercial fruit varieties have become progressively less flavorful over time. Due to the cost and difficulty associated with flavor phenotyping, breeding programs have long been challenged in selecting for this complex trait. To address this issue, we leveraged targeted metabolomics of diverse tomato and blueberry accessions and their corresponding consumer panel ratings to create statistical and machine learning models that can predict sensory perceptions of fruit flavor. Using these models, a breeding program can assess flavor ratings for a large number of genotypes, previously limited by the low throughput of consumer sensory panels. The ability to predict consumer ratings of liking, sweet, sour, umami, and flavor intensity was evaluated by a 10-fold cross-validation, and the accuracies of 18 different models were assessed. The prediction accuracies were high for most attributes and ranged from 0.87 for sourness intensity in blueberry using XGBoost to 0.46 for overall liking in tomato using linear regression. Further, the best-performing models were used to infer the flavor compounds (sugars, acids, and volatiles) that contribute most to each flavor attribute. We found that the variance decomposition of overall liking score estimates that 42% and 56% of the variance was explained by volatile organic compounds in tomato and blueberry, respectively. We expect that these models will enable an earlier incorporation of flavor as breeding targets and encourage selection and release of more flavorful fruit varieties.
https://www.pnas.org/content/119/7/e2115865119

Cannon, C.H., Piovesan, G. & Munné-Bosch, S. (2022): **Old and ancient trees are life history lottery winners and vital evolutionary resources for long-term adaptive capacity**. Nat. Plants | https://doi.org/10.1038/s41477-021-01088-5
Trees can live for many centuries with sustained fecundity and death is largely stochastic. We use a neutral stochastic model to examine tree demographic patterns that emerge over time, across a range of population sizes and empirically observed mortality rates. A small proportion of trees (~1% at 1.5% mortality) are life-history 'lottery winners', achieving ages >10–20× the median age. Maximum age increases with bigger populations and lower mortality rates. One-quarter of trees (~24%) achieve ages that are three to four times greater than the median age. Three age classes (mature, old and ancient) contribute unique evolutionary diversity across complex environmental cycles. Ancient trees are an emergent property of forests that requires many centuries to generate. They radically change variance in generation time and population fitness, bridging centennial environmental cycles. These life-history 'lottery' winners are vital to long-term forest adaptive capacity and provide invaluable data about environmental history and individual longevity. Old and ancient trees cannot be replaced through restoration or regeneration for many centuries. They must be protected to preserve their invaluable diversity.
https://www.nature.com/articles/s41477-021-01088-5

Chanderbali, A.S., Jin, L., Xu, Q. et al. (2022): ***Buxus* and *Tetracentron* genomes help resolve eudicot genome history.** Nat Commun 13, 643 | https://doi.org/10.1038/s41467-022-28312-w
Ancient whole-genome duplications (WGDs) characterize many large angiosperm lineages, including angiosperms themselves. Prominently, the core eudicot lineage accommodates 70% of all angiosperms and shares ancestral hexaploidy, termed *gamma*. *Gamma* arose via two WGDs that occurred early in eudicot history; however, the relative timing of these is unclear, largely due to the lack of high-quality genomes among early-diverging eudicots. Here, we provide complete genomes for *Buxus sinica* (Buxales) and *Tetracentron sinense* (Trochodendrales), representing the lineages most closely related to core eudicots. We show that *Buxus* and *Tetracentron* are both characterized by independent WGDs, resolve relationships among early-diverging eudicots and their respective genomes, and use the RACCROCHE pipeline to reconstruct ancestral genome structure at three key phylogenetic nodes of eudicot diversification. Our reconstructions indicate genome structure remained relatively stable during early eudicot diversification, and reject hypotheses of *gamma* arising via inter-lineage hybridization between ancestral eudicot lineages, involving, instead, only stem lineage core eudicot ancestors.
https://www.nature.com/articles/s41467-022-28312-w.pdf

Colgan T.J., Arce A.N., Gill R.J., Rodrigues A.R. et al. (2022): **Genomic signatures of recent adaptation in a wild bumblebee.** Molecular Biology and Evolution 39 (2), msab366 | https://doi.org/10.1093/molbev/msab366
Environmental changes threaten insect pollinators, creating risks for agriculture and ecosystem stability. Despite their importance, we know little about how wild insects respond to environmental pressures. To understand the genomic bases of adaptation in an ecologically important pollinator, we analyzed genomes of *Bombus terrestris* bumblebees collected across Great Britain. We reveal extensive genetic diversity within this population, and strong signatures of recent adaptation throughout the genome affecting key processes

including neurobiology and wing development. We also discover unusual features of the genome, including a region containing 53 genes that lacks genetic diversity in many bee species, and a horizontal gene transfer from a *Wolbachia* bacteria. Overall, the genetic diversity we observe and how it is distributed throughout the genome and the population should support the resilience of this important pollinator species to ongoing and future selective pressures. Applying our approach to more species should help understand how they can differ in their adaptive potential, and to develop conservation strategies for those most at risk.
https://academic.oup.com/mbe/article/39/2/msab366/6521030

Industrial Biotechnology – White gene engineering Industrielle Biotechnologie – Weiße Gentechnik

Fraiture M.-A., Gobbo A., Papazova N., Roosens N.H.C. (2022): **Development of a Taxon-Specific Real-Time PCR Method Targeting the Bacillus subtilis Group to Strengthen the Control of Genetically Modified Bacteria in Fermentation Products.** Fermentation 8 (2), 78 | https://doi.org/10.3390/fermentation8020078
Most of the bacteria that are used to produce fermentation products, such as enzymes, additives and flavorings, belong to the Bacillus subtilis group. Recently, unexpected contaminations with unauthorized genetically modified (GM) bacteria (viable cells and associated DNA) that were carrying antimicrobial resistance (AMR) genes was noticed in several microbial fermentation products that have been commercialized on the food and feed market. These contaminations consisted of GM Bacillus species belonging to the B. subtilis group. In order to screen for the potential presence of such contaminations, in this study we have developed a new real-time PCR method targeting the B. subtilis group, including B. subtilis, B. licheniformis, B. amyloliquefaciens and B. velezensis. The method's performance was successfully assessed as specific and sensitive, complying with the Minimum Performance Requirements for Analytical Methods of GMO Testing that is used as a standard by the GMO enforcement laboratories. The method's applicability was also tested on 25 commercial microbial fermentation products. In addition, this method was developed to be compatible with the PCR-based strategy that was recently developed for the detection of unauthorized GM bacteria. This taxon-specific method allows the strengthening of the set of screening markers that are targeting key sequences that are frequently found in GM bacteria (AMR genes and shuttle vector), reinforcing control over the food and feed chain in order to guarantee its safety and traceability.
https://www.mdpi.com/2311-5637/8/2/78

Bhutada G., Menard G., Bhunia R.K., Piotr P. Hapeta P.P. et al. (2022): **Production of human milk fat substitute by engineered strains of Yarrowia lipolytica,** Metabolic Engineering Communications 14, e00192 | https://doi.org/10.1016/j.mec.2022.e00192.
Human milk fat has a distinctive stereoisomeric structure where palmitic acid is esterified to the middle (sn-2) position on the glycerol backbone of the triacylglycerol and unsaturated fatty acids to the outer (sn-1/3) positions. This configuration allows for more efficient nutrient absorption in the infant gut. However, the fat used in most infant formulas originates from plants, which exclude palmitic acid from the sn-2 position. Oleaginous yeasts provide an alternative source of lipids for human nutrition. However, these yeasts also exclude palmitic acid from the sn-2 position of their triacylglycerol. Here we show that *Yarrowia lipolytica* can be engineered to produce triacylglycerol with more than 60% of the palmitic acid in the sn-2 position, by expression of lysophosphatidic acid acyltransferases with palmitoyl-Coenzyme A specificity. The engineered *Y. lipolytica* strains can be cultured on glycerol, glucose, palm oil or a mixture of substrates, under nitrogen limited condition, to produce triacylglycerol with a fatty acid composition that resembles human milk fat, in terms of the major molecular species (palmitic, oleic and linoleic acids). Culture on palm oil or a mixture of glucose and palm oil produced the highest lipid titre and a triacylglycerol composition that is most similar with human milk fat. Our data show that an oleaginous yeast can be engineered to produce a human milk fat substitute (β-palmitate), that could be used as an ingredient in infant formulas.
https://www.sciencedirect.com/science/article/pii/S2214030122000013?via%3Dihub

Gao H.-Y., Zhao H., Hu T.-Y., Jiang Z.-Q. (2022**): Metabolic Engineering of *Saccharomyces cerevisiae* for High-Level Friedelin *via* Genetic Manipulation.** Front. Bioeng. Biotechnol., 07 February 2022 | https://doi.org/10.3389/fbioe.2022.805429
Friedelin, the most rearranged pentacyclic triterpene, also exhibits remarkable pharmacological and anti-insect activities. In particular, celastrol with friedelin as the skeleton, which is derived from the medicinal plant *Tripterygium wilfordii*, is a promising drug due to its anticancer and antiobesity activities. Although a previous study achieved friedelin production using engineered *Saccharomyces cerevisiae*, strains capable of producing high-level friedelin have not been stably engineered. In this study, a combined strategy was employed with integration of endogenous pathway genes into the genome and knockout of inhibiting genes by CRISPR/Cas9 technology, which successfully engineered multiple strains. After introducing an efficient *Tw*OSC1^{T502E}, all strains with genetic integration (*tHMG1*, *ERG1*, *ERG20*, *ERG9*, *POS5*, or *UPC2.1*) showed a 3.0~6.8-fold increase in friedelin production compared with strain BY4741. Through further double knockout of inhibiting genes, only strains GD1 and GD3 produced higher yields. Moreover, strains GQ1 and GQ3 with quadruple mutants (*bts1*; *rox1*; *ypl062w*; *yjl064w*) displayed similar increases. Finally, the dominant strain GQ1 with *Tw*OSC1^{T502E} was cultured in an optimized medium in shake flasks, and the final yield of friedelin reached 63.91 ± 2.45 mg/L, which was approximately 65-fold higher than that of the wild-type strain BY4741 and 229% higher than that in ordinary SD-His-Ura medium. It was the highest titer for friedelin production to date. Our work provides a good

example for triterpenoid production in microbial cell factories and lays a solid foundation for the mining, pathway analysis, and efficient production of valuable triterpenoids with friedelin as the skeleton.
https://www.frontiersin.org/articles/10.3389/fbioe.2022.805429/full

Menon N., Richmond D., Rahman M.R., K. Menon B.R.K. (2022): **Versatile and Facile One-Pot Biosynthesis for Amides and Carboxylic Acids in *E. coli* by Engineering Auxin Pathways of Plant Microbiomes.** ACS Catal. 12, XXX, 2309–2319, | https://doi.org/10.1021/acscatal.1c04901
The development of enzymatic routes toward amide and carboxylic acid bond formation in bioactive molecular scaffolds using aqueous conditions is a major challenge for biopharmaceutical and fine chemical industrial sectors. We report biocatalytic and kinetic characterization of two indole-3-acetamide (IAM) pathway enzymes, tryptophan-2-monooxygenase (iaaM) and indole-3-acetamide hydrolase (iaaH), present in plant microbiomes that produce indole-3-acetic acid (IAA). In this pathway, tryptophan is converted to indole-3-acetamide by the monooxygenase activity of iaaM, followed by its hydrolysis to form carboxylic acid by iaaH enzyme. Since IAA or auxin is an essential natural plant hormone and an important synthon for fine chemicals, the developed monooxygenase-based bioconversion route has a wider scope compared to currently available synthetic and biocatalytic methods to produce synthetic auxins and a range of amides and carboxylic acids for agrochemical and pharmaceutical applications. To display this, one-pot multienzyme biosynthetic cascades for preparative-scale production of IAA derivatives were performed by incorporating tryptophan synthase and tryptophan halogenase enzymes. We also report the creation of an efficient *de novo* biosynthesis for IAA and its derivatives from glucose or indoles via a reconstructed IAM pathway in *Escherichia coli*.
https://pubs.acs.org/doi/10.1021/acscatal.1c04901

Oreb, M., Tripp, J. (2022**): Maßgeschneiderte Hefezellen für biotechnologische Anwendungen.** Biospektrum 28, 14–17 | https://doi.org/10.1007/s12268-022-1704-y

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

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