Sunday Evening News No 327

2023-05-15 – 2023-05-21

Compiled and edited by ***BGF*** Jany

Wissenschaftskreis Genomik und Gentechnik e.V.

Meetings – Conferences / Veranstaltungen - Konferenzen

FGV-WGG-Pressekonferenz **„40 Jahre Grüne Gentechnik“**
Redner werden die beiden Nobelpreisträger R. J. Roberts und C. Nüsslein-Vollhard sein.
Freitag, 2. Juni 2023, 14 Uhr
Landesvertretung Sachsen-Anhalt
Luisenstraße 18, 10117 Berlin

Harnessing Genome Editing - Technologies for Viticulture
May 29, 2023 | 2:00 pm - 6:00 pm (CEST/GMT +2)
Registration Link: foodsystems-org.zoom.us/webinar/register/2716802681765/WN_JFbOwBOBS9yxrFGzSjVgpA

Gene Editing Regulation – Acknowledging Uncertainty
When it comes to the regulation of new genetic technologies in the environment, what we don’t know can be as significant as what we do.
Tue, 13 Jun 2023 15:00 - 16:30 CEST – online
https://www.eventbrite.co.uk/e/gene-editing-regulation-acknowledging-uncertainty-tickets-628428796447

Press Releases -Media / Presse- und Medienberichte

Savage S., Brzeziński B.: **EU Commission delays flagship Green Deal package**
https://www.politico.eu/article/commission-delays-green-deal-package/

arc2000: **A Summer off – Commission to avoid NGTs, Pesticides & Nature Restoration?**
https://www.arc2020.eu/a-summer-off-commission-to-avoid-ngts-pesticides-nature-restoration/

Foote N.: **No to pesticide cuts? No gene editing proposal, Commission official warns**
https://www.euractiv.com/section/agriculture-food/news/no-to-pesticide-cuts-no-gene-editing-proposal-commission-official-warns/

EU-Kommission bindet Gentechnik-Lockerung an Pestizidreduktion
https://www.euractiv.de/section/energie-und-umwelt/news/eu-kommission-bindet-gentechnik-lockerung-an-pestizidreduktion/

BMEL: **Tagung des Rates (Landwirtschaft und Fischerei) am 25. April 2023 in Luxemburg**
Ergebnisbericht
https://www.bmel.de/DE/themen/landwirtschaft/eu-agrarpolitik-und-foerderung/gap/agrarrat-03-2023.html

Informationsdienst Gentechnik: **Neue Gentechnik: Biopatente bedrohen Züchtungsfreiheit**
https://www.keine-gentechnik.de/nachricht/34764?cHash=957d0225f7e4b3f8970a07f759f28b7b

Deter A.: **Umweltminister und Bauernverband lehnen Patente auf Pflanzen strikt ab**
https://www.topagrar.com/acker/news/umweltminister-bekraeftigen-absage-an-biopatente-13381148.html

Testbiotech: **Freisetzungsversuche mit Pflanzen aus Neuer Gentechnik**
https://www.testbiotech.org/aktuelles/freisetzungsversuche-mit-pflanzen-aus-neuer-gentechnik
https://www.testbiotech.org/freilandversuche-genomeditierte-pflanzen

Field trials of plants derived from new genetic engineering
https://www.testbiotech.org/en/news/field-trials-plants-derived-new-genetic-engineering
https://www.testbiotech.org/en/field-trials-new-ge-plants

GM-Watch: **EU finally funds detection methods research for new GM plants**
https://gmwatch.org/en/106-news/latest-news/20227

The European Commission will present and discuss its proposal for deregulating new GMOs on 5 July at a meeting of the College of Commissioners, according to a European Commission document published on 17 May, transmitted to the European Parliament and seen by GMWatch.

► Bericht der WGG-Veranstaltung „Potentiale neuer genomischer Techniken (NGT) für eine nachhaltige Landwirtschaft und Lebensmittelproduktion auf der Labvolution-2023

Only some selected press releases or media reports are listed here. The daily up-date of the press releases and media reports are ►here: May week 20

Publications – Publikationen

Tena, G.(2023): **New Green Revolution genes**. Nat. Plants | https://doi.org/10.1038/s41477-023-01426-9
https://www.nature.com/articles/s41477-023-01426-9

Cortés A.J., Castillejo M.A.,Yockteng R. (2023): **'Omics' Approaches for Crop Improvement.** Agronomy 13 (5), 1401; https://doi.org/10.3390/agronomy13051401
https://www.mdpi.com/2073-4395/13/5/1401/htm

Cummings, C., Selfa, T., Lindberg, S. et al. (2023): **Identifying public trust building priorities of gene editing in agriculture and food.** *Agric Hum Values* | https://doi.org/10.1007/s10460-023-10465-z
Gene editing in agriculture and food (GEAF) is a nascent development with few products and is unfamiliar among the wider US public. GEAF has garnered significant praise for its potential to solve for a variety of agronomic problems but has also evoked controversy regarding safety and ethical standards of development and application. Given the wake of other agribiotechnology debates including GMOs (genetically modified organisms), this study made use of 36 in-depth key interviews to build the first U.S. based typology of proponent and critic priorities for shaping public trust in GEAF actors and objects. Key organizational actors provide early and foundational messaging, which is likely to contribute heavily to public salience, comprehension, and decision-making as potential consumers reflect upon their experiences, envision future outcomes, and consider the reputation of those trying to influence them. As is documented in our results, the trust-building priorities of these groups often stand in opposition to one another and are influenced by distinct motivations for how the public will come to trust or distrust GEAF actors and objects as more products are developed and enter the market.
https://link.springer.com/article/10.1007/s10460-023-10465-z

Devi A.M., Devi K.K. Devi, P-P. Devi M.L. and Das S. (2023): **Metabolic engineering of plant secondary metabolites: prospects and its technological challenges.** Front. Plant Sci., Sec. Plant Metabolism and Chemodiversity, Volume 14 - 2023 | https://doi.org/10.3389/fpls.2023.1171154
Plants produce a wide range of secondary metabolites that play vital roles for their primary functions such as growth, defence, adaptations or reproduction. Some of the plant secondary metabolites are beneficial to mankind as nutraceuticals and pharmaceuticals. Metabolic pathways and their regulatory mechanism are crucial for targeting metabolite engineering. The clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated system has been widely applied in genome editing with high accuracy, efficiency, and multiplex targeting ability. Besides its vast application in genetic improvement, the technique also facilitates a comprehensive profiling approach to functional genomics related to gene discovery involved in various plant secondary metabolic pathways. Despite these wide applications, several challenges limit CRISPR/Cas system applicability in genome editing in plants. This review highlights updated applications of CRISPR/Cas system-mediated metabolic engineering of plants and its challenges.
https://www.frontiersin.org/articles/10.3389/fpls.2023.1171154/full

Nakandala U., Masouleh A.K., Smith M.W., Furtado A. et al. (2023):, **Haplotype resolved chromosome level genome assembly of Citrus australis reveals disease resistance and other citrus specific genes**, Horticulture Research | DOI: 10.1093/hr/uhad058
Recent advances in genome sequencing and assembly techniques have made it possible to achieve chromosome level reference genomes for citrus. Relatively few genomes have been anchored at the chromosome level and/or are haplotype phased, with the available genomes of varying accuracy and completeness. We now report a phased high-quality chromosome level genome assembly for an Australian native citrus species; *Citrus australis* (round lime) using highly accurate PacBio HiFi long reads, complemented with Hi-C scaffolding. Hifiasm with Hi-C integrated assembly resulted in a 331 Mb genome of *C. australis* with two haplotypes of nine pseudochromosomes with an N50 of 36.3 Mb and 98.8% genome assembly

completeness (BUSCO). Repeat analysis showed that more than 50% of the genome contained interspersed repeats. Among them, LTR elements were the predominant type (21.0%), of which LTR Gypsy (9.8 %) and LTR copia (7.7 %) elements were the most abundant repeats. A total of 29,464 genes and 32,009 transcripts were identified in the genome. Of these, 28,222 CDS (25,753 genes) had BLAST hits and 21,401 CDS (75.8%) were annotated with at least one GO term. Citrus specific genes for antimicrobial peptides, defense, volatile compounds and acidity regulation were identified. The synteny analysis showed conserved regions between the two haplotypes with some structural variations in Chromosomes 2, 4, 7 and 8. This chromosome scale, and haplotype resolved *C. australis* genome will facilitate the study of important genes for citrus breeding and will also allow the enhanced definition of the evolutionary relationships between wild and domesticated citrus species.
https://academic.oup.com/hr/advance-article/doi/10.1093/hr/uhad058/7100333

Perk, E.A., Arruebarrena Di Palma, A., Colman, S. et al. (2023): **CRISPR/Cas9-mediated phospholipase C 2 knock-out tomato plants are more resistant to *Botrytis cinerea***. Planta 257, 117 | https://doi.org/10.1007/s00425-023-04147-7
Genome-editing technologies allow non-transgenic site-specific mutagenesis of crops, offering a viable alternative to traditional breeding methods. In this study we used CRISPR/Cas9 to inactivate the tomato Phospholipase C2 gene (*SlPLC2*). Plant PLC activation is one of the earliest responses triggered by different pathogens regulating plant responses that, depending on the plant–pathogen interaction, result in plant resistance or susceptibility. The tomato (*Solanum lycopersicum*) *PLC* gene family has six members, named from *SlPLC1* to *SlPLC6*. We previously showed that *SlPLC2* transcript levels increased upon xylanase infiltration (fungal elicitor) and that SlPLC2 participates in plant susceptibility to *Botrytis cinerea*. An efficient strategy to control diseases caused by pathogens is to disable susceptibility genes that facilitate infection. We obtained tomato *SlPLC2*-knock-out lines with decreased ROS production upon *B. cinerea* challenge. Since this fungus requires ROS-induced cell death to proliferate, *SlPLC2*-knock-out plants showed an enhanced resistance with smaller necrotic areas and reduced pathogen proliferation. Thus, we obtained *SlPLC2* loss-of-function tomato lines more resistant to *B. cinerea* by means of CRISPR/Cas9 genome editing technology.
https://link.springer.com/article/10.1007/s00425-023-04147-7

Hu, Y., Patra, P., Pisanty, O. et al. (2023): **Multi-Knock—a multi-targeted genome-scale CRISPR toolbox to overcome functional redundancy in plants**. Nat. Plants 9, 572–587 | https://doi.org/10.1038/s41477-023-01374-4
Plant genomes are characterized by large and complex gene families that often result in similar and partially overlapping functions. This genetic redundancy severely hampers current efforts to uncover novel phenotypes, delaying basic genetic research and breeding programmes. Here we describe the development and validation of Multi-Knock, a genome-scale clustered regularly interspaced short palindromic repeat toolbox that overcomes functional redundancy in *Arabidopsis* by simultaneously targeting multiple gene-family members, thus identifying genetically hidden components. We computationally designed 59,129 optimal single-guide RNAs that each target two to ten genes within a family at once. Furthermore, partitioning the library into ten sublibraries directed towards a different functional group allows flexible and targeted genetic screens. From the 5,635 single-guide RNAs targeting the plant transportome, we generated over 3,500 independent *Arabidopsis* lines that allowed us to identify and characterize the first known cytokinin tonoplast-localized transporters in plants. With the ability to overcome functional redundancy in plants at the genome-scale level, the developed strategy can be readily deployed by scientists and breeders for basic research and to expedite breeding efforts.
https://www.nature.com/articles/s41477-023-01374-4

Guo, H., Cao, P., Wang, C. et al. (2023**): Population analysis reveals the roles of DNA methylation in tomato domestication and metabolic diversity.** Sci. China Life Sci. | https://doi.org/10.1007/s11427-022-2299-5
DNA methylation is an important epigenetic marker, yet its diversity and consequences in tomato breeding at the population level are largely unknown. We performed whole-genome bisulfite sequencing (WGBS), RNA sequencing, and metabolic profiling on a population comprising wild tomatoes, landraces, and cultivars. A total of 8,375 differentially methylated regions (DMRs) were identified, with methylation levels progressively decreasing from domestication to improvement. We found that over 20% of DMRs overlapped with selective sweeps. Moreover, more than 80% of DMRs in tomato were not significantly associated with single-nucleotide polymorphisms (SNPs), and DMRs had strong linkages with adjacent SNPs. We additionally profiled 339 metabolites from 364 diverse accessions and further performed a metabolic association study based on SNPs and DMRs. We detected 971 and 711 large-effect loci via SNP and DMR markers, respectively. Combined with multi-omics, we identified 13 candidate genes and updated the polyphenol biosynthetic pathway. Our results showed that DNA methylation variants could complement SNP profiling of metabolite diversity. Our study thus provides a DNA methylome map across diverse accessions and suggests that DNA methylation variation can be the genetic basis of metabolic diversity in plants.
https://link.springer.com/article/10.1007/s11427-022-2299-5

Keagy J, Drummond C.P.., Gilbert, K.J., Grozinger C.M. et al. (2023): **Landscape transcriptomics as a tool for addressing global change effects across diverse species,** Molecular Ecology Resources | DOI: 10.1111/1755-0998.13796

Landscape transcriptomics is an emerging field studying how genome-wide expression patterns reflect dynamic landscape-scale environmental drivers, including habitat, weather, climate, and contaminants, and the subsequent effects on organismal function. This field is benefitting from advancing and increasingly accessible molecular technologies, which in turn are allowing the necessary characterization of transcriptomes from wild individuals distributed across natural landscapes. This research is especially important given the rapid pace of anthropogenic environmental change and potential impacts that span levels of biological organization. We discuss three major themes in landscape transcriptomic research: connecting transcriptome variation across landscapes to environmental variation, generating and testing hypotheses about the mechanisms and evolution of transcriptomic responses to the environment, and applying this knowledge to species conservation and management. We discuss challenges associated with this approach and suggest potential solutions. We conclude that landscape transcriptomics has great promise for addressing fundamental questions in organismal biology, ecology, and evolution, while providing tools needed for conservation and management of species.
https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13796

Li, C., Wood, J.C., Vu, A.H. et al. (2023): **Single-cell multi-omics in the medicinal plant *Catharanthus roseus*.** Nat Chem Biol | https://doi.org/10.1038/s41589-023-01327-0
Advances in omics technologies now permit the generation of highly contiguous genome assemblies, detection of transcripts and metabolites at the level of single cells and high-resolution determination of gene regulatory features. Here, using a complementary, multi-omics approach, we interrogated the monoterpene indole alkaloid (MIA) biosynthetic pathway in *Catharanthus roseus*, a source of leading anticancer drugs. We identified clusters of genes involved in MIA biosynthesis on the eight *C. roseus* chromosomes and extensive gene duplication of MIA pathway genes. Clustering was not limited to the linear genome, and through chromatin interaction data, MIA pathway genes were present within the same topologically associated domain, permitting the identification of a secologanin transporter. Single-cell RNA-sequencing revealed sequential cell-type-specific partitioning of the leaf MIA biosynthetic pathway that, when coupled with a single-cell metabolomics approach, permitted the identification of a reductase that yields the bis-indole alkaloid anhydrovinblastine. We also revealed cell-type-specific expression in the root MIA pathway.
https://www.nature.com/articles/s41589-023-01327-0

Frandsen P.B., Hotaling S., Powell A., Heckenhauer J. et al. (2023): "**Allelic resolution of insect and spider silk genes reveals hidden genetic diversity"** PNAS 120 (18) e2221528120 | https://doi.org/10.1073/pnas.2221528120
Arthropod silk is vital to the evolutionary success of hundreds of thousands of species. The primary proteins in silks are often encoded by long, repetitive gene sequences. Until recently, sequencing and assembling these complex gene sequences has proven intractable given their repetitive structure. Here, using high-quality long-read sequencing, we show that there is extensive variation—both in terms of length and repeat motif order—between alleles of silk genes within individual arthropods. Further, this variation exists across two deep, independent origins of silk which diverged more than 500 Mya: the insect clade containing caddisflies and butterflies and spiders. This remarkable convergence in previously overlooked patterns of allelic variation across multiple origins of silk suggests common mechanisms for the generation and maintenance of structural protein-coding genes. Future genomic efforts to connect genotypes to phenotypes should account for such allelic variation.
https://www.pnas.org/doi/10.1073/pnas.2221528120

Benevenuto, R.F., Zanatta, C.B., Waßmann, F. et al. (2023): **Integration of omics analyses into GMO risk assessment in Europe: a case study from soybean field trials**. Environ Sci Eur 35, 14 | https://doi.org/10.1186/s12302-023-00715-6
In Europe, genetically modified organisms (GMOs) are subject to an authorization process including a mandatory risk assessment. According to the respective guidance by the European Food Safety Authority (EFSA), one of the pillars of this GMO risk assessment is a comparative analysis of the compositional and agronomic characteristics. This targeted approach has been criticized for its limitations, as it only considers pre-determined compounds, being insufficient to assess a comprehensive range of relevant compounds, including toxins and anti-nutrients, on a case-specific basis. Strategies based on advanced untargeted omics technologies have been proposed as a potential broader approach to be implemented into the initial step of the risk assessment framework. Here, we provide an example of a step-by-step omics analysis based on systems biology approach to fit into the context of European GMO regulation. We have performed field trial experiments with genetically modified (GM) Intacta™ Roundup Ready™ 2 Pro soybean containing both cry1Ac and cp4epsps transgenic inserts and analyzed its proteomic profile against the non-GM counterpart and reference varieties. Based on EFSA's comparative endpoint-by-endpoint approach, the proteomics analysis revealed six proteins from the GMO outside the 99% tolerance intervals of reference varieties (RVs) in the equivalence test. Interestingly, from the near-isogenic (non-GM) comparator we found as many as ten proteins to be outside of the said RVs' equivalence limits. According to EFSA's statistical guidelines, differences found in metabolite abundance between a GMO and its non-GM comparator would not be considered biologically relevant as all compounds of concern remained within the equivalence limits of commercial RVs. By assessing the proteomic and metabolomic data through our proposed systems biology approach, we found 70 proteins, and the metabolite xylobiose as differentially expressed between the GMO and its non-GM comparator. Biological relevance of such results was revealed through a functional biological network analysis, where we found alterations in several metabolic pathways related to protein synthesis and protein processing. Moreover, the allergenicity analysis identified 43 proteins with allergenic potential being differentially expressed in the GM

soybean variety. Our results demonstrate that implementation of advanced untargeted omics technologies in the risk assessment of GMOs will enable early and holistic assessment of possible adverse effects. The proposed approach can provide a better understanding of the specific unintended effects of the genetic modification on the plant's metabolism, the involved biological networks, and their interactions, and allows to formulate and investigate dedicated risk hypotheses in the first place. We draw conclusions on a detailed comparison with the comparative assessment according to EFSA and provide scientific arguments and examples on how the current comparative approach is not fit for purpose.
https://enveurope.springeropen.com/articles/10.1186/s12302-023-00715-6

Feng Z., Du Y., Chen J., Chen X. et al. (2023): **Phenotypic and Genomic Mutations Induced by a Carbon-Ion Beam and Gamma-ray Irradiation in Soybean (Glycine max (L.) Merr.)** Int. J. Mol. Sci. *24* (10), 8825; https://doi.org/10.3390/ijms24108825
Soybean (Glycine max (L.) Merr.) is a nutritious crop that can provide both oil and protein. A variety of mutagenesis methods have been proposed to obtain better soybean germplasm resources. Among the different types of physical mutagens, carbon-ion beams are considered to be highly efficient with high linear energy transfer (LET), and gamma rays have also been widely used for mutation breeding. However, systematic knowledge of the mutagenic effects of these two mutagens during development and on phenotypic and genomic mutations has not yet been elucidated in soybean. To this end, dry seeds of Williams 82 soybean were irradiated with a carbon-ion beam and gamma rays. The biological effects of the M_1 generation included changes in survival rate, yield and fertility. Compared with gamma rays, the relative biological effectiveness (RBE) of the carbon-ion beams was between 2.5 and 3.0. Furthermore, the optimal dose for soybean was determined to be 101 Gy to 115 Gy when using the carbon-ion beam, and it was 263 Gy to 343 Gy when using gamma rays. A total of 325 screened mutant families were detected from out of 2000 M_2 families using the carbon-ion beam, and 336 screened mutant families were found using gamma rays. Regarding the screened phenotypic M_2 mutations, the proportion of low-frequency phenotypic mutations was 23.4% when using a carbon ion beam, and the proportion was 9.8% when using gamma rays. Low-frequency phenotypic mutations were easily obtained with the carbon-ion beam. After screening the mutations from the M_2 generation, their stability was verified, and the genome mutation spectrum of M_3 was systemically profiled. A variety of mutations, including single-base substitutions (SBSs), insertion–deletion mutations (INDELs), multinucleotide variants (MNVs) and structural variants (SVs) were detected with both carbon-ion beam irradiation and gamma-ray irradiation. Overall, 1988 homozygous mutations and 9695 homozygous + heterozygous genotype mutations were detected when using the carbon-ion beam. Additionally, 5279 homozygous mutations and 14,243 homozygous + heterozygous genotype mutations were detected when using gamma rays. The carbon-ion beam, which resulted in low levels of background mutations, has the potential to alleviate the problems caused by linkage drag in soybean mutation breeding. Regarding the genomic mutations, when using the carbon-ion beam, the proportion of homozygous-genotype SVs was 0.45%, and that of homozygous + heterozygous-genotype SVs was 6.27%; meanwhile, the proportions were 0.04% and 4.04% when using gamma rays. A higher proportion of SVs were detected when using the carbon ion beam. The gene effects of missense mutations were greater under carbon-ion beam irradiation, and the gene effects of nonsense mutations were greater under gamma-ray irradiation, which meant that the changes in the amino acid sequences were different between the carbon-ion beam and gamma rays. Taken together, our results demonstrate that both carbon-ion beam and gamma rays are effective techniques for rapid mutation breeding in soybean. If one would like to obtain mutations with a low-frequency phenotype, low levels of background genomic mutations and mutations with a higher proportion of SVs, carbon-ion beams are the best choice.
https://www.mdpi.com/1422-0067/24/10/8825

Ezaki R., Sakuma T., Kodama D., Sasahara R. et al. (2023) : **Transcription activator-like effector nuclease-mediated deletion safely eliminates the major egg allergen ovomucoid in chickens.** Food and Chemical Toxicology 175, 113703 | 10.1016/j.fct.2023.113703
Among the major egg allergens, ovomucoid (OVM) is very stable against heat and digestive enzymes, making it difficult to remove physiochemically and inactivate allergens. However, recent genome editing technology has made it possible to generate OVM-knockout chicken eggs. To use this OVM-knockout chicken egg as food, it is important to evaluate its safety as food. Therefore, in this study, we examined the presence or absence of mutant protein expression, vector sequence insertion, and off-target effects in chickens knocked out with OVM by platinum TALENs. The eggs laid by homozygous OVM-knockout hens showed no evident abnormalities, and immunoblotting showed that the albumen contained neither the mature OVM nor the OVM truncated variant. Whole genome sequencing (WGS) revealed that the potential TALEN-induced off-target effects in *OVM*-knockout chickens were localized in the intergenic and intron regions. The WGS information confirmed that plasmid vectors used for genome editing were only transiently present and did not integrate into the genome of edited chickens. These results indicate the importance of safety evaluation and reveal that the eggs laid by this OVM knockout chicken solve the allergy problem in food and vaccines.
https://www.sciencedirect.com/science/article/pii/S0278691523001059?via%3Dihub

Zadabbas Shahabadi H, Akbarzadeh A, Ofoghi H and Kadkhodaei S (2023): **Sitespecific gene knock-in and bacterial phytase gene expression in Chlamydomonas reinhardtii via Cas9 RNP-mediated HDR.** Front. Plant Sci. 14:1150436. doi: 10.3389/fpls.2023.1150436
In the present study, we applied the HDR (homology-directed DNA repair)CRISPR-Cas9-mediated knock-in system to accurately insert an optimizedforeign bacterial phytase gene at a specific site of the nitrate reductase (NR)gene (exon 2) to achieve homologous recombination with the stability of thetransgene and reduce

insertion site effects or gene silencing. To this end, wesuccessfully knocked-in the targeted NR gene of Chlamydomonas reinhardtiiusing the bacterial phytase gene cassette through direct delivery of the CRISPR/Cas9 system as the ribonucleoprotein (RNP) complex consisting of Cas9 proteinand the specific single guide RNAs (sgRNAs). The NR insertion site editing wasconfirmed by PCR and sequencing of the transgene positive clones. Moreover,24 clones with correct editing were obtained, where the phytase gene cassettewas located in exon 2 of the NR gene, and the editing efficiency was determinedto be 14.81%. Additionally, site-specific gene expression was analyzed andconfirmed using RT-qPCR. Cultivation of the positive knocked-in colonies onthe selective media during 10 generations indicated the stability of the correctedediting without gene silencing or negative insertion site effects. Our resultsdemonstrated that CRISPR-Cas9-mediated knock-in could be applied fornuclear expression of the heterologous gene of interest, and also confirmed itsefficacy as an effective tool for site-specific gene knock-in, avoiding nuclearpositional effects and gene silencing in C. reinhardtii. These findings could also provide a new perspective on the advantageous application of RNP-CRISPR/Cas9 gene-editing to accelerate the commercial production of complexrecombinant proteins in the food-grade organism “C. reinhardtii”.
https://www.readcube.com/articles/10.3389/fpls.2023.1150436

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. M*ost of *the pdf files can be downloaded directly from the links.*

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