

Sunday Evening News No. 93

Week 36 (2018-09-03 / 09-09)

Selected and edited by **BGF** Jany

Some press releases and media reports on the JCEU ruling:

Purnhagen K.P, Kok E., Kleter G., Schebesta H., Visser R.G.F. and Wesseler J. (2018) **EU court casts new plant breeding techniques into regulatory limbo**. Nature Biotechnology 36, 799–800

We note that the CJEU is legally not allowed to go on a fact-finding mission. The judges interpret the materials presented to them by the referring court and the (public) parties consulted.

<https://www.nature.com/articles/nbt.4251> pdf-file available

Urnov F.D., Ronold P.C. and Carrol D. (2018): **A call for science-based review of the European court's decision on gene-edited crops**. Nature Biotechnology 36, 800-802

<https://www.nature.com/articles/nbt.4252> text available

Overview in German: Übersicht über Nutz- und Zierpflanzen, die mittels Gentechnik und neuer molekularbiologischer Techniken für die Bereiche Ernährung, Landwirtschaft, Gartenbau, Arzneimittelherstellung und -forschung entwickelt wurden.

https://www.bvl.bund.de/DE/06_Gentechnik/02_Verbraucher/09_Monitoring_Molekulare_Techniken/gentechnik_molekulare_techniken_node.html

Discussion: Standing Committee on Plants, Animals, Food and Feed Section *Genetically Modified Food and Feed*, September, 11 2018

Prof. Dr. Ralf Meyer: Mathematisch-Naturwissenschaftlicher Fakultätentag Deutschlands **Gleiches Recht für alle biologischen Stoffe - Konsequenzen aus dem EuGH-Urteil**

<https://idw-online.de/de/news701584>

transkript: **Grüne Biotechnologie: Sargnagel oder Neubeginn?**

Der Europäische Gerichtshof hat entschieden: Pflanzen, die mit neuen Verfahren zur zielgerichteten Erbgutveränderung erzeugt wurden, unterliegen künftig der Gentechnik-Richtlinie. Damit schlägt Europa global gesehen einen Sonderweg ein. Pflanzenzüchter, Landwirte und Wissenschaftler sind alarmiert.

Transkript 9, 8-13 (2018) pdf-file available

Bioökonomierat: **Genome Editing: Bioökonomierat fordert neues EU-Gentechnikrecht**

<http://biooekonomierat.de/aktuelles/stellungnahme-zu-genome-editing/>

Genome Editing: **Europa benötigt ein neues Gentechnikrecht**

http://biooekonomierat.de/fileadmin/Publikationen/berichte/BOER-Memo_Genome-Editing.pdf

Gesellschaft für Pflanzenzüchtung e.V. **EuGH-Urteil: Moderne Züchtungsverfahren gelten als Gentechnik**

<https://gpz-online.de/eugh-urteil-moderne-zuechtungsverfahren-gelten-als-gentechnik/>

Posthof C. (2018): **EuGH stärkt Vorsorge**

Der Europäische Gerichtshof (EuGH) hat sich in seinem Urteil vom 25.Juli zu der Regulierung neuer Gentechnik-Verfahren geäußert. Die RichterInnen vertreten die Ansicht, dass das derzeit gültige EU-Recht auch auf neue Gentechnik-Verfahren anwendbar ist – eine gute Nachricht für die Gentechnik-kritische Bewegung.

Genethischer Informationsdienst 246, 26-27 pdf-file available

Petition gegen Gentechnik durch die Hintertür eingereicht

Um die neusten Methoden zur Veränderungen von Organismen dem Gentechnik-Gesetz zu unterstellen, wurde eine Petition mit zirka 30'000 Unterschriften eingereicht.

<https://www.nau.ch/nachrichten/schweiz/2018/08/31/petition-gegen-gentechnik-durch-die-hintertur-eingereicht-65408646>

GMWatch: **GMO law change mooted as way to get round court ruling**

The NBT Platform [believes](#), contrary to the legal ruling from the ECJ, that "most products derived through the use of NBTs are not considered GMOs".

This is [scientifically](#) and [technically](#), as well as [legally](#), incorrect. The vast majority of organisms produced by new GM techniques are clearly [GMOs](#) under existing definitions relevant to the EU.

But since when did the GMO lobby let science and democratically established law stand in the way of their monetary ambitions?

<https://www.gmwatch.org/en/news/latest-news/18433>

Callaway E. (2018): **CRISPR plants now subject to tough GM laws in European Union**
Top court's ruling threatens research on gene-edited crops in the bloc. Nature

<https://www.nature.com/articles/d41586-018-05814-6>

Katrina Pirner: **ECJ Surprises Observers By Ruling Against New Plant Breeding Technique**

Last month the European Court of Justice diverged from the Advocate-General's opinion by ruling that mutagenesis, a new plant breeding technique, should be subject to the bloc's GMO directive. While celebrated by environmental groups, scientists and agricultural organisations believe the decision is a significant setback for the agricultural industry.

<https://www.southeusummit.com/europe/ecj-surprises-observers-by-ruling-against-new-plant-breeding-technique/>

Jean-Paul Oury: **Mutagenesis: does the ECJ understand the concept of Nature?**

<https://www.europeanscientist.com/en/editors-corner/mutagenesis-does-the-ecj-understand-the-concept-of-nature/>

Peter Teffer: **Juncker's rules on GMOs going nowhere soon**

'It is not right that when EU countries cannot decide among themselves whether or not to ban the use of glyphosate in herbicides, the Commission is forced ... to take a decision,' said Juncker

<https://euobserver.com/institutional/142720>

Patrick Clinton

Europe is going to regulate gene-edited organisms as if they were alien plants from space

That's too bad, because gene editing, for better or worse, is probably going to be one of the techniques that bring us what we need to feed our battered planet.

<https://newfoodeconomy.org/european-union-court-gene-editing-debate-france/>

Petition · IPMB2018: Immediate Review of the ECJ Ruling on Plant Genome Editing.

https://www.change.org/p/ipmb2018-immediate-review-of-the-ecj-ruling-on-plant-genome-editing-9ff3df10-9f7d-44de-b379-8a01a1d71ba2?recruiter=29491788&utm_source=share_petition&utm_medium=twitter&utm_campaign=psf_combo_share_abi.pacific_abi_gmail_send.variation.pacific_abi_select_all_contacts.fake_control.pacific_email_copy_en_us_3.control.pacific_email_copy_en_gb_3.control.lightning_share_by_medium.share_by_medium.lightning_2primary_share_options.variant

European Biotechnology

Germany to pump €1bn into breakthrough innovation

Germany's government announced that it will create an agency for breakthrough ("jump") innovation to foster technology transfer of risky research projects with high market potential into products.

<https://european-biotechnology.com/up-to-date/latest-news/news/germany-to-pump-eur1bn-into-breakthrough-innovation.html>

Giovanni Tagliabue, Henry I. Miller: **"Fakebook Science" and Democracy**

<https://www.europeanscientist.com/en/features/fakebook-science-and-democracy/>

Andrew Porterfield: **How genetics could help agroecology—the science, not the political movement**

Agroecology isn't rocket science

<https://geneticliteracyproject.org/2018/09/04/genetics-could-help-agroecology-the-science-not-the-political-movement/>

Publications:

The Australian Council of Learned Academies '**Synthetic Biology in Australia: an outlook to 2030**'

<https://acola.org.au/wp/sbio/>

Christiaens O, Dzhambazova T, Kostov K, Arpaia S, Joga MR, Urru I, Sweet J, Smagghe G. (2018): **Literature review of baseline information on RNAi to support the environmental risk assessment of RNAi-based GM plants**. EFSA supporting publication 2018:EN-1424. 173; doi:10.2903/sp.efsa.2018.EN-1424

<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2018.EN-1424>

EEA Report No 8/2018: **The circular economy and the bioeconomy**. Partners in sustainability

https://www.eea.europa.eu/publications/circular-economy-and-bioeconomy?mc_cid=d946509efd&mc_eid=b89825d9e5

Deutsch C. et al. (2018): **Increase in crop losses to insect pests in a wartime climate**. *Science*; 36: 916-919. DOI: 10.1126/science.aat3466.

Insect pests substantially reduce yields of three staple grains—rice, maize, and wheat—but models assessing the agricultural impacts of global warming rarely consider crop losses to insects. We use established relationships between temperature and the population growth and metabolic rates of insects to estimate how and where climate warming will augment losses of rice, maize, and wheat to insects. Global yield losses of these grains are projected to increase by 10 to 25% per degree of global mean surface warming. Crop losses will be most acute in areas where warming increases both population growth and metabolic rates of insects. These conditions are centered primarily in temperate regions, where most grain is produced.

<http://science.sciencemag.org/content/361/6405/916>

Markus Riegler: **Insect threats to food security**

Pest damage to crops will increase substantially in many regions as the planet continues to warm. *Science* **361** (6405), 846. DOI: 10.1126/science.aau7311

Muzammil, S. et al. (2018): **An ancestral allele of Pyrroline-5-carboxylate synthase1 promotes proline accumulation and drought adaptation in cultivated barley**. *Plant Physiol.*, doi: 10.1104/pp.18.00169.

Water scarcity is a critical threat to global crop production. Here, we used the natural diversity of barley (*Hordeum vulgare*) to dissect the genetic control of proline-mediated drought stress adaptation. Genetic mapping and positional cloning of a major drought-inducible quantitative trait locus (QTL; QPro.S42-1H) revealed unique allelic variation in pyrroline-5-carboxylate synthase (P5cs1) between the cultivated cultivar Scarlett (*ssp. vulgare*) and the wild barley accession ISR42-8 (*ssp. spontaneum*). The putative causative mutations were located in the promoter of P5cs1 across the DNA binding motifs for abscisic acid-responsive element binding transcription factors (ABFs). Introgression line (IL) S42IL-143 carrying the wild allele of P5cs1 showed significant up-regulation of P5cs1 expression compared to Scarlett, which was consistent with variation in proline accumulation under drought. Next, we transiently expressed promoter:reporter constructs of ISR42-8 and Scarlett alleles in *Arabidopsis* (*Arabidopsis thaliana*) mesophyll protoplasts. β -glucuronidase (GUS) expression analysis showed a significantly higher activation of the ISR42-8 promoter compared to Scarlett upon abscisic acid treatment. Notably, the ISR42-8 promoter activity was impaired in protoplasts isolated from the loss-of-function *abf1abf2abf3abf4* quadruple mutant. A series of phenotypic evaluations demonstrated that S42IL-143 maintained leaf water content and photosynthetic activity longer than Scarlett under drought. These findings suggest that the ancestral variant of P5cs1 has the potential for drought tolerance and understanding drought physiology of barley and related crops.

<http://www.plantphysiol.org/content/early/2018/08/21/pp.18.00169.long>

Wang, W., Simmonds, J., Pan, Q. et al. (2018): **Gene editing and mutagenesis reveal inter-cultivar differences and additivity in the contribution of TaGW2 homoeologues to grain size and weight in wheat**. *Theor Appl Genet* (2018). <https://doi.org/10.1007/s00122-018-3166-7>

The TaGW2 gene homoeologues have been reported to be negative regulators of grain size (GS) and thousand grain weight (TGW) in wheat. However, the contribution of each homoeologue to trait variation among different wheat cultivars is not well documented. We used the CRISPR-Cas9 system and TILLING to mutagenize each homoeologous gene copy in cultivars Bobwhite and Paragon, respectively. Plants carrying single-copy nonsense mutations in different genomes showed different levels of GS/TGW increase, with TGW increasing by an average of 5.5% (edited lines) and 5.3% (TILLING mutants). In any combination, the double homoeologue

mutants showed higher phenotypic effects than the respective single-genome mutants. The double mutants had on average 12.1% (edited) and 10.5% (TILLING) higher TGW with respect to wild-type lines. The highest increase in GS and TGW was shown for triple mutants of both cultivars, with increases in 16.3% (edited) and 20.7% (TILLING) in TGW. The additive effects of the TaGW2 homoeologues were also demonstrated by the negative correlation between the functional gene copy number and GS/TGW in Bobwhite mutants and an F2 population. The highest single-genome increases in GS and TGW in Paragon and Bobwhite were obtained by mutations in the B and D genomes, respectively. These inter-cultivar differences in the phenotypic effects between the TaGW2 gene homoeologues coincide with inter-cultivar differences in the homoeologue expression levels. These results indicate that GS/TGW variation in wheat can be modulated by the dosage of homoeologous genes with inter-cultivar differences in the magnitude of the individual homoeologue effects. <https://link.springer.com/content/pdf/10.1007%2Fs00122-018-3166-7.pdf>

Knott G.J. and Doudna J.A. (2018): **CRISPR-Cas guides the future of genetic engineering.** *Science* 361, Issue 6405, pp. 866-869 DOI: 10.1126/science.aat5011

The diversity, modularity, and efficacy of CRISPR-Cas systems are driving a biotechnological revolution. RNA-guided Cas enzymes have been adopted as tools to manipulate the genomes of cultured cells, animals, and plants, accelerating the pace of fundamental research and enabling clinical and agricultural breakthroughs. We describe the basic mechanisms that set the CRISPR-Cas toolkit apart from other programmable gene-editing technologies, highlighting the diverse and naturally evolved systems now functionalized as biotechnologies. We discuss the rapidly evolving landscape of CRISPR-Cas applications, from gene editing to transcriptional regulation, imaging, and diagnostics. Continuing functional dissection and an expanding landscape of applications position CRISPR-Cas tools at the cutting edge of nucleic acid manipulation that is rewriting biology. <http://science.sciencemag.org/content/361/6405/866> pdf-file available

Otoupal P.B., Cordell W.T., Bachu V., Sittonj.M and Chatterjee A. (2018): **Multiplexed deactivated CRISPR-Cas9 gene expression perturbations deter bacterial adaptation by inducing negative epistasis.** *Communications Biology* 1, article number:129 (2018)

The ever-increasing threat of multi-drug resistant bacteria, a shrinking antibiotic pipeline, and the innate ability of microorganisms to adapt necessitates long-term strategies to slow the evolution of antibiotic resistance. Here we develop an approach, dubbed Controlled Hindrance of Adaptation of OrganismS or CHAOS, involving induction of epistasis between gene perturbations to deter adaptation. We construct a combinatorial library of multiplexed, deactivated CRISPR-Cas9 devices to systematically perturb gene expression in *Escherichia coli*. While individual perturbations improved fitness during antibiotic exposure, multiplexed perturbations caused large fitness loss in a significant epistatic fashion. Strains exhibiting epistasis adapted significantly more slowly over three to fourteen days, and loss in adaptive potential was shown to be sustainable. Finally, we show that multiplexed peptide nucleic acids increase the antibiotic susceptibility of clinically isolated Carbapenem-resistant *E. coli* in an epistatic fashion. Together, these results suggest a new therapeutic strategy for restricting the evolution of antibiotic resistance. <https://www.nature.com/articles/s42003-018-0135-2>

Simon E. Bull et al. (2018): **Accelerated ex situ breeding of GBSS- and PTST1-edited cassava for modified starch.** *Science Advances* 4 (9), eaat6086; DOI: 10.1126/sciadv.aat6086

Crop diversification required to meet demands for food security and industrial use is often challenged by breeding time and amenability of varieties to genome modification. Cassava is one such crop. Grown for its large starch-rich storage roots, it serves as a staple food and a commodity in the multibillion-dollar starch industry. Starch is composed of the glucose polymers amylopectin and amylose, with the latter strongly influencing the physicochemical properties of starch during cooking and processing. We demonstrate that CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9)-mediated targeted mutagenesis of two genes involved in amylose biosynthesis, *PROTEIN TARGETING TO STARCH (PTST1)* or *GRANULE BOUND STARCH SYNTHASE (GBSS)*, can reduce or eliminate amylose content in root starch. Integration of the *Arabidopsis FLOWERING LOCUS T* gene in the genome-editing cassette allowed us to accelerate flowering—an event seldom seen under glasshouse conditions. Germinated seeds yielded S1, a transgene-free progeny that inherited edited genes. This attractive new plant breeding technique for modified cassava could be extended to other crops to provide a suite of novel varieties with useful traits for food and industrial applications. <http://advances.sciencemag.org/content/4/9/eaat6086>

Chinnadurai P. et al. (2018): **Variability of CP4 EPSPS expression in genetically engineered soybean (*Glycine max* L. Merrill).** *Transgenic Res.* <https://doi.org/10.1007/s11248-018-0092-z>

The expression of the CP4 EPSPS protein in genetically engineered (GE) soybean confers tolerance to the *Roundup*® family of agricultural herbicides. This study evaluated the variability of CP4 EPSPS expression using an enzyme-linked immunosorbent assay in soybean tissues collected across diverse germplasm and 74 different

environments in Argentina, Brazil and the USA. Evaluated material included single and combined (stacked) trait products with other GE traits in entries with *cp4 epsps* gene at one or two loci. The highest level of CP4 EPSPS was observed in leaf tissues, intermediate in forage and seed, and lowest in root tissues. Varieties with two loci had approximately twice the level of CP4 EPSPS expression compared to one locus entries. Variable and non-directional level of CP4 EPSPS was observed with other factors like genetic background, trait stacking, growing region or season. The maximum and average CP4 EPSPS expression levels in seed provided large margins of exposure (MOE of approximately 4000 and 11,000, respectively), mitigating concerns over exposure to this protein in food and feed from soybean varieties tolerant to *Roundup*[®] herbicides.

<https://link.springer.com/content/pdf/10.1007%2Fs11248-018-0092-z.pdf>

Campos R. C., Holderbaum D.F., Nodari R.O. and Hernandez M. I.M. (2018): **Indirect exposure to Bt maize through pig faeces causes behavioural changes in dung beetles.**

J Appl Entomol., DOI: 10.1111/jen.12532

Genetically modified (GM) Bt plants currently represent a highly adopted alternative for pest control in agricultural crops. However, their safety to non-target organisms has been an unsolved issue. Non-target organisms associated to nutrient cycling in natural and agricultural systems, such as dung beetles, use faeces of mammals as a food resource and could be exposed to Bt-plant material through faeces of livestock fed with Bt-crops. The aim of this study was to assess whether indirect exposure to transgenic Bt maize MON810 can reduce fitness in dung beetles. Four dung beetles species, *Canthon angularis*, *Canthon rutilans cyanescens*, *Coprophaneus saphirinus* and *Phanaeus splendidulus*, were reared under laboratory conditions and fed with pig faeces using two treatments/diets: faeces of pigs fed transgenic maize and of pigs fed conventional maize. The behaviour of insects was tested by experiments on the incorporation of organic matter in the soil and nesting, and experiments of foraging behaviour with olfactometry measures. Organic matter incorporation in the soil per male–female pairs of *C. rutilans* was similar between GM and conventional treatments, as was their reproductive success, but beetles fed with faeces from transgenic maize produced more brood balls. In another trial regarding the incorporation of organic matter in the soil, *C. saphirinus* fed with faeces derived from conventional maize showed greater ability to bury food resource in comparison with GM fed ones. In an olfactometer test, the time to reach the food source was longer for individuals of *C. rutilans*, previously fed with transgenic faeces during 1 month, than individuals fed with conventional faeces. Our results suggest that differences found in dung beetles' ability represent potential indirect effects of transgenic maize through the food chain and may also affect ecological functions of these organisms in natural habitats, by means of reduced beetle efficiency in removal and burial of faecal masses.

<https://onlinelibrary.wiley.com/doi/pdf/10.1111/jen.12532>

Kahlon J.G., Jacobsen H.-J., Chatterton S., Hassan F., Bowness R. and Hall L. M. (2018): **Lack of efficacy of transgenic pea (*Pisum sativum* L.) stably expressing antifungal genes against *Fusarium* spp. in three years of confined field trials.** *GM Crops & Food* 9,90--108, DOI: 10.1080/21645698.2018.1445471

Fusarium root rot is a major pea disease in Canada and only partial tolerance exists in germplasm. Transgenic technologies may hold promise but the economic benefits of genetically modified (GM) pea will need to surpass the regulatory costs, time and labor involved in bringing a GM crop to market. European pea (*Pisum sativum* L.) cultivars expressing four antifungal genes, 1-3 b glucanase (G), endochitinase (C) (belonging to PR proteins family), polygalacturonase inhibiting proteins (PGIPs) (P) and stilbene synthase (V) have been transformed for disease tolerance and showed disease tolerance under laboratory conditions. Transgenic lines with four antifungal genes inserted either individually or stacked through crossing were tested for their efficacy against *Fusarium* root rot (*Fusarium avenaceum*) in confined trials over three years (2013 to 2015) in comparison with two parental German lines and three Canadian lines. Superior emergence, higher fresh weight or lower disease ratings above and below ground, of transgenic lines in presence of disease inoculum were not observed consistently in the three years of field experiments when compared to the parental and Canadian lines in the presence of disease inoculum. No indication of an advantage of stacked genes over single genes was observed. Most transgenic lines had lower relative gene expression in the roots than in the leaves in greenhouse trials suggesting a possible explanation for poor tolerance to *Fusarium* root rot. Field trials are necessary to verify the agronomic performance and ecological relevance of the promising effects detected under laboratory conditions.

<https://www.tandfonline.com/doi/abs/10.1080/21645698.2018.1445471>

Eriksson E., Ammann K., Chassy B. and Chawad C. (2018): **Comments on two recent publications on GM maize and Roundup.** *Scientific Reports* 8:13338, DOI:10.1038/s41598-018-30440-7

Two -omics studies on genetically modified maize and Roundup-fed rats, recently published in the journal *Scientific Reports*, contain serious flaws in the experimental design, methodology and interpretation of results, which we point out here. The use of -omics technologies are of increasing importance in research, however we argue for a cautious approach to the potential application in food safety assessments as these exceptionally sensitive and complex methods require a thorough and detailed evaluation of the biological significance of obtained results.

Arising from: Mesnage et al. Sci Rep 7:39328 (2017), Mesnage et al. Sci Rep 6:37855 (2016)
<https://rdcu.be/5l6T>

Gabriel Popkin G. (2018): **To save iconic American chestnut, researchers plan introduction of genetically engineered tree into the wild.** doi:10.1126/science.aav2573
<http://www.sciencemag.org/news/2018/08/save-iconic-american-chestnut-researchers-plan-introduction-genetically-engineered-tree>

AgbiInvestor:

The challenges facing agriculture and the plant science industry in the EU

https://croplife-r9qnrxt3qygjra4.netdna-ssl.com/wp-content/uploads/2018/09/Challenges-Facing-Farmers-and-the-Plant-Science-Industry-in-the-EU-report.pdf?mc_cid=2c20c95ca3&mc_eid=b89825d9e5&mc_cid=2c20c95ca3&mc_eid=b89825d9e5

Konferenzen:

Einladung: Genome Editing nach dem EuGH – Konferenz, Workshops, Posterausstellung:

10. & 11. Oktober 2018 Martin-Luther-Universität in Halle (S.)

"Prämissen und Anspruchsgruppen der roten und grünen Genomeditierung

Weitere Informationen zur Veranstaltung, das vollständige Programm unter:

http://kluth.jura.uni-halle.de/bmbf_genomelection/konferenz/

GDCh-Wissenschaftsforum Chemie 2019 (WiFo 2019)

https://www.chemistryviews.org/details/event/9300331/GDCh-Wissenschaftsforum_Chemie_2019_WiFo_2019.html
