International Conference

Plant Genome Editing & Genome Engineering II

Programme and Abstracts

Vienna, Austria

July 5 – 6, 2019

Organizing Committee

Local Organizing Committee	International Organizing Committee
Alisher Touraev (Austria, VISCEA)	Magdy Mahfouz Kan Wang Hiroaki Saika Sergei Svitashev Shujie Dong Wendy Harwood Erika Toda Panagiotis Moschou Huanbin Zhou Marcel Kuntz David Hamburger

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Welcome to the 2nd International Conference "Plant Genome Editing & Genome Engineering"!

Genome Editing and Genome Engineering are revolutionizing Life Sciences and Plant Biotechnology that seek to develop new technologies for the precise manipulation of genes and genomes *in cellula* and *in vivo*. In addition to its use for advancing our understanding of basic biology, genome engineering has numerous real-world applications, e.g. engineering plants that better provide fuel, food and industrial raw materials. Driven by advances in the CRISPR-toolbox for rapid, cheap, multiplex modification of genomes and breakthroughs in DNA synthesis technologies, the pace of progress enabled by these tools in the last 2 years has been breathtaking.

The **2nd International Conference "Plant Genome Editing & Genome Engineering"** to be held on **July 5-6**, 2019, in Vienna, Austria will discuss the advances in genome editing and engineering, notions, challenges, pros and cons, technologies and methods of applications of the genome editing tools like CRISPR/Cas9, TALENs, ZNFs & AAVs in plants. The case study and sessions will reveal the potential application of Genome editing tools plant biology and crop improvement. Special emphasis on CRISPR system addressing the concept, technology, challenges like off-target effects, efficiency improvement and delivery systems etc.
Vienna is located in the heart of Europe on the banks of the Danube River, and considered as one of the most important economic, cultural and touristic large cities of central Europe. Apart from providing top science, the Conference will capture the spirit of the city thanks to the central location of the venue offering a multitude of cultural events.

This two-days event will provide leading academy and industry scientists a platform to communicate recent advances in **"Plant Genome Editing & Genome Engineering",** and an opportunity to establish multilateral collaboration.
The **2nd International Conference "Plant Genome Editing & Genome Engineering"** will cover the following research topics:

- ***Precision Genome Editing by TALEN, ZFN and others***
- ***CRISPR-CAS9: Revolution in Genome Editing & Engineering***
- ***Current CRISPR-CAS9 Technologies and Design***
- ***Applications of CRISPR-CAS9 in Plants***
- ***Genome Editing & Engineering for Crop Improvement***
- ***Genome Editing & Engineering: Regulatory Aspects***

Approximately 200 participants are expected to attend this exciting scientific forum including almost 40 lectures delivered by worldwide known invited speakers and young, talented speakers selected from submitted abstracts. The program combines plenary lectures, poster sessions, a unique Conference Dinner Party and sightseeing tours of Vienna.

Prof. Alisher Touraev (VISCEA, Austria, Local Organizer)

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SCIENTIFIC PROGRAMME

2nd International Conference "Plant Genome Editing & Genome Engineering"

July 5 (Friday)

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09.20 - 10.30	**Keynote Lecture:** **Magdy Mahfouz (Saudi Arabia):** Plant Genome Engineering for Targeted Improvement of Crop Traits
10.30 - 11.00	***Coffee break***
11.00 - 12.30:	**Session I: Genome Editing Tools & Technology Development**
Chairs	*Hiroaki Saika (Japan) & Magdy Mahfouz (Saudi Arabia)*
11.00 - 11.25 (+5)	**Hiroaki Saika (Japan):** Precise Mutagenesis and its Application in Rice
11.30 - 11.45 (+5)	**Iris Koeppel (Germany):** A Versatile Modular Vector System for RNA-Guided Cas Endonucleases
11.50 - 12.05 (+5)	**Vera Karolina Schoft (Austria):** CRISPR/Cas9 Service for Genome Editing in Plants
12.10 - 12.25 (+5)	**Eszter Alexandra Farkas (Hungary):** Enhancing Powdery Mildew Resistance in Grapevine using the CRISPR/Cas9 System
12.30 - 14.00	***Lunch + Poster Session (all numbers)***
14.00 - 15.30	**Session II: Plant Transformation & Delivery Methods**
Chairs	*Erika Toda (Japan) & Sergei Svitashev (USA)*
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14.25 - 14.45 (+5)	**Sergei Svitashev (USA):** Efficient Genome Editing in Maize Directed by CRISPR-Cas9 Technology
14.50 - 15.05 (+5)	**Radi Aly (Israel):** CRISPR/Cas9-Mediated Mutagenesis of CCD8 Gene in Tomato (Solanum lycopersicum Mill.) Provides Resistance to Parasitic Weeds
15.10 - 15.25 (+5)	**James Chamness (USA):** Developing Monocot Replicon Vectors for Improved Gene Targeting
15.30 - 16.00	***Coffee Break***
16.00 - 17.30	**Session III: Application of Genome Editing in Plant Biology**
Chairs	*Kan Wang (USA) & Shujie Dong (USA)*
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16.25 - 16.45 (+5)	**Shujie Dong (USA):** Accelerating Crop Improvement through Novel Genome Editing Technologies
16.50 - 17.05 (+5)	**Julia Jansing (Netherlands):** CRISPR/Cas9-Mediated Glycoengineering in Nicotiana benthamiana for Plant Molecular Farming
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17.30 - 19.00	***Welcome Reception + Poster Session (all numbers)***
19.00 - 22.00	**Conference Dinner Party** Traditional Austrian food and wine, located in one of Vienna's famous 'Heurigen' **Cost: 50,- EUR**

July 6 (Saturday)

08.00 - 17.00	***Registration***
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09.30 - 09.45 (+5)	**Hrvoje Fulgosi (Croatia):** Thylakoid rhodanase-lile Protein TROL as a Target for CRISPR/Cas9 Editing
09.50 - 10.05 (+5)	**Lucas Lieber (Argentina):** DNA-free Genome Editing in Soybean Explants
10.10 - 10.25 (+5)	**Florian Rösch (Germany):** High-throughput Screening for Genome-Editing Events on Single Cell Level
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11.00 - 12.20	**Session V: Genome Editing for Crop Improvement II**
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11.25 - 11.45 (+5)	**Huanbin Zhou (China):** Development of a toolkit for targeted base editing in rice
11.50 - 12.00 (+5)	**Robert Eric Hoffie (Germany):** PDIL5-1 Knockout induced by Cas9 Endonuclease Confers Resistance of Barley to Bymoviruses
12.05 -12.15 (+5)	**Christian Hertig (Germany):** Targeted Mutagenesis of BRANCHED HEAD Homoeoalleles Causes Alterations of Wheat Spike Architecture
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Chairs: Marcel Kuntz (France), David J.S. Hamburger (Germany), Magdy Mahfouz (Saudi Arabia)

17.10 - 17.30 ***Closing Ceremony & Conference Photo***

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ABSTRACTS OF ORAL PRESENTATIONS

Next-generation plant genome engineering

Magdy Mahfouz

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CRISPR/Cas systems enable high efficiency genome engineering across eukaryotic species and revolutionized biological research and biotechnological applications. CRISPR/Cas systems have been applied in diverse plant species to generate a wide variety of site-specific genome modifications including targeted mutagenesis and editing for various agriculture biotechnology applications. Key technologies are highly needed to unlock the potential of CRISPR applications in crop plants. These technologies include improving the delivery of genome engineering reagents and precise genome editing. Moreover, CRISPR/Cas genome-wide screens make it possible to discover novel traits, expand the range of traits, and accelerate trait development in target crops that are key for food security. Here, I discuss the development and use of various CRISPR/Cas systems for different plant genome¬-engineering applications. I highlight the existing challenges and opportunities to harness these technologies for targeted improvement of traits to enhance crop productivity and resilience to climate change. These cutting-edge genome-editing technologies are thus poised to reshape the future of agriculture and food security.

Precise mutagenesis and its application in rice

Hiroaki Saika

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Precise mutagenesis and its application in rice Hiroaki Saika1, Akiko Mori1, Seiichi Toki1,2 1 Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Japan, 2 Kihara Institute for Biological Research, Yokohama City University, Japan Recently, gene knockout by targeted mutagenesis using sequence-specific nucleases has been applied widely to many plants, including rice. For example, we succeeded in oleic acid fortification of rice grains by knockout of the rice FAD2-1 gene via CRISPR/Cas9-mediated targeted mutagenesis. Gene targeting (GT) enables precise modification of a gene-of-interest by homologous recombination between an endogenous genome and a donor DNA. Since the first report of GT in rice in 2002, more than 10 genes have been modified. GT using positive-negative selection can be used not only to insert gene expression cassettes but also to introduce small mutations such as single base substitutions. We succeeded in achieving site-directed mutagenesis in rice using a combination of positive-negative-selection-mediated GT and subsequent complete marker excision via single-strand annealing (SSA). This system produces plants that are exactly equivalent at the sequence level to mutant plants produced by conventional mutagenesis. We believe our system could be a powerful tool for precise mutagenesis in rice.

A versatile modular vector system for RNA-guided Cas endonucleases

Koeppel1, S. Hiekel1 and J. Kumlehn1.

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Customizable RNA-guided Cas endonucleases are effective tools for plant research and biotechnology. However, under consideration of the ever-growing multitude of functional elements, the experimental throughput of platform establishment, extension and application is limited due to the laborious generation of vectors. Here, we report the establishment of a versatile modular vector system based upon the use of type IIS restriction enzymes that facilitate complex and yet seamless and easy-to-handle cloning steps. One to four guide RNAs driven by monocot- or dicot-compatible promoters can be combined with a Cas endonuclease gene. While individual guide RNA scaffold modules can be loaded with any target-specific fragments, there is the additional opportunity to readily combine various promoters and Cas variants, by which the versatility of the vector set is much extended. Further modularity is provided by the option to integrate a template sequence for homology-directed DNA-repair, which is required for precise genome editing approaches. Alternatively, expression units for any supplementary gene function can be inserted, e.g. to enhance embryogenicity of the primarily transformed cells or to manipulate DNA repair mechanisms.

CRISPR/Cas9 service for genome editing in plants

Vera Karolina Schoft*, **Peggy Stolt-Bergner**

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The VBCF is a publicly funded non-profit research institute, situated at the Vienna Biocenter. The focus of VBCF has been on providing subsidized research services, primarily to the academic community. The CRISPR lab at the VBCF applied the CRISPR/Cas9 system to A. thaliana, Marchantia polymorpha and humulus lupulus (hop) to produce transgenic plants. To this end, we established a flexible molecular cloning system for the generation of customized plant transformation vectors. We developed a robust and efficient system for generation of plant knock-out lines; this includes introducing frame-shift mutations and deletions of up to 12 kb.
We are planning to expand our services to other plant species. Furthermore, we are currently optimizing our gene targeting protocols for Arabidopsis thaliana to be able to offer efficient production of plant lines with precise and heritable changes in the genome.
Standard protocols, new developments and applications of our system will be presented.

Enhancing powdery mildew resistance in grapevine using the CRISPR/Cas9 system

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Grapevine (Vitis vinifera L.) production is challenged by many fungal diseases such as powdery mildew, downy mildew or black rot. Resistance breeding of grapevine incorporates resistance traits from other grape species but these also interfere with wine quality. Promoting plant protection by strategies based on CRISPR/Cas9 can be achieved by targeted modification of susceptibility genes, while wine quality remains unchanged. We have chosen Mildew resistance locus o (Mlo) gene family and Downy mildew resistant 6 (Dmr6) genes as potential sources of resistance to powdery mildew. Therefore, we have designed CRISPR constructs for silencing of these candidate genes in Vitis vinifera L. cv. Furmint. Eighteen members of the Mlo gene family have been described in grapevine and four of them involved in powdery mildew susceptibility. Dmr6, Mlo11 and Mlo13 could be specifically targeted, while Mlo6 and Mlo7 were targeted with a common construct. The designed CRISPR/Cas9 constructs enable fine-tuning in the expression of resistance trait in the grape cultivar Furmint.

An efficient DNA- and selectable-marker-free genome-editing system using zygotes in rice

Erika Toda1,2, N. Koiso1, A. Takebayashi2, M. Ichikawa3, T. Kiba2, K. Osakabe4, Y. Osakabe 2,4, H. Sakakibara2, N. Kato1,2,3, T. Okamoto1,2

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Genome-edited plants from multiple species have been produced successfully using a method in which a Cas9-guide RNA (gRNA) expression cassette and selectable marker are integrated into the genomic DNA by Agrobacterium tumefaciens-mediated transformation or particle bombardment. However, CRISPR-Cas9 integration increases the chance of off-target modifications, and foreign DNA sequences cause legislative concerns about genetically modified organisms. Here, we established a genome-editing system by direct delivery of Cas9-gRNA ribonucleoproteins (RNPs) into plant zygotes via PEG-Ca2+-mediated transfection. Cas9-gRNA RNPs were transfected into rice zygotes produced by in vitro fertilization of isolated gametes and the zygotes were cultured into mature plants in the absence of selection agents, resulting in the regeneration of rice plants with targeted mutations in around 14-64% of plants. This efficient plant-genome-editing system has enormous potential for the improvement of rice as well as other important crop species.

Efficient genome editing in maize directed by CRISPR-Cas9 technology

Sergei Svitashev

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CRISPR-Cas is a powerful technology and has a wide-ranging application in plant breeding programs. However, the efficiency of genome editing depends on various factors including type of genome modification required, species, transformation options, target site etc. At Corteva Agriscience, we combined advances in maize transformation and vector design to improve genome editing efficiency using CRISPR-Cas9 system. Examples of successful utilization of CRISPR-Cas technology as an efficient tool for genome editing in maize will be discussed.

CRISPR/Cas9-mediated mutagenesis of CCD8 gene in tomato (Solanum lycopersicum Mill.) provides resistance to parasitic weeds

Radi Aly

Scientific Manager of Northern R&D, Head Dept. of Weed Science, Newe Yaar Research Center (ARO) P.O.Box 1021, Ramat Yeshai 30095, ISRAEL. Correspondence to: radi@volcani.agri.gov.il

Phelipanche aegyptiaca is an obligate plant parasite that cause extreme damage to crop plants. The parasite seeds have strict requirements for germination, involving exposure to specific chemicals (strigolactones (SLs) exuded by the host roots. SLs are plant hormones derived from carotenoids via a pathway involving the carotenoid cleavage dioxygenase genes CCD7 and CCD8. Having no effective means to control parasitic weeds in most crops, here we demonstrate that CRISPR/Cas9-mediated genome editing of the CCD8 gene can be used to develop host resistance to the parasitic weed P. aegyptiaca. Cas9/single guide RNA construct was targeted to the second exon of CCD8 in tomato (Solanum lycopersicum Mill.) plants. Several mutated tomato lines with variable deletions in CCD8 were obtained with no identified off-targets. Although, mutant lines had morphological changes (excessive shoot branching and adventitious root formation) but some lines showed a significant reduction in parasite infestation compared to non-mutated tomato plants. Considering the impact of parasitic weeds on agriculture, the current study offers insights into the development of a new, efficient method for parasitic weed control.

Developing monocot replicon vectors for improved gene targeting

JAMES CHAMNESS1, **Nathaniel Graham1, Hiroaki Saika2, and Daniel Voytas1**.

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Improved strategies are required for the design and delivery of reagents to perform in planta gene targeting (GT). To enhance the efficiency of GT event generation, deconstructed geminiviruses have been engineered into self-replicating DNA vectors called replicons. Fluorescent protein knock-in studies in rice and wheat callus have shown that delivery of editing reagents mediated by a Wheat Dwarf Virus (WDV)-derived replicon can enhance the efficiency of GT by 10-fold. However, the same studies indicate that replication of WDV may inhibit cell growth, compromising regeneration among transformed callus sectors. In contrast, multiple dicots tolerate transformation with a Bean Yellow Dwarf Virus-derived replicon. We thus hypothesized that the growth arrest in monocots arises from a viral-host interaction specific to WDV, and are currently testing whether a number of different monocot-infecting geminiviruses may be engineered into replicons that both enhance GT and permit normal cell division. We anticipate these studies will identify improved vectors for transformation and gene targeting in monocot systems.

CRISPR/Cas directed gene mutagenesis and site targeted T-DNA integration in crop species

Kan Wang, PhD

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CRISPR-Cas9 and Cas12a nucleases are two of the most frequently used genome editing tools for plants. Although the concerns of off-target activities of these RNA-guided nucleases (RGN) have been continuously raised, it is not fully understood how Cas9 and Cas12a perform in maize. We compared the effectiveness of the two CRISPR/Cas systems and surveyed their specificities in maize plant. *Agrobacterium*-mediated transformation is a critical tool for plant genome engineering and biotechnology, but random T-DNA integration can lead to transgene silencing or insertional mutagenesis. We utilized the CRISPR/Cas9 to direct *Agrobacterium* T-DNA into specific gene sequence and achieved ~5% targeted integration frequency in rice.

Accelerating Crop Improvement through Novel Genome Editing Technologies

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Genome editing technology provides novel solutions to enhance crop productivity by leveraging gene discovery effort for trait improvement across crops. We have developed efficient genome editing platform based on CRISPR systems. Several examples of its application in trait gene discovery and validation will be shown. To overcome the bottleneck of transformation of recalcitrant crops we have also developed a novel technology called HI-Edit which integrates genome editing and the genotype-independent haploid induction process. The Hi-Edit technology enables genome editing in diverse germplasms and significantly reduces the time it takes to develop commercially important crop varieties.

Live imaging of chromatin in plant cells: a challenging task for CRISPR

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The emergence of CRISPR technology has revolutionized the ability of researchers in genome engineering of wide variety of species, though CRISPR's application is not limited to genome editing. Visualizing the spatiotemporal organization of chromosomes which is suggested to have a regulatory role on biological programs like gene expression, transcription and repair, is of a great importance to decipher the basis of these mechanisms. In this regard, a new application for CRISPR was successfully developed to label repetitive and non-repetitive sequences in living mammalian cell cultures. However, the application of this method for plant cells is more challenging. Here we show the establishment of such live cell imaging method by fusion of the catalytically inactive version of Streptococcus pyogenes and Staphylococcus aureus Cas9 (dCas9) to eGFP/mRuby2 for visualization of telomere repeats in live leaf cells of Nicotiana benthamiana. To be able to label other repetitive sequences, improved live imaging vectors containing stem loop motifs of RNA called aptamers were tested. Challenges of the CRISPR-imaging method will be discussed.

CRISPR/Cas9-mediated glycoengineering in Nicotiana benthamiana for plant molecular farming

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Plants are an attractive alternative to conventional microbial and mammalian systems for the production of pharmaceutical proteins. For the transient protein production with agrobacteria, the tobacco variety Nicotiana benthamiana is by far the species of choice.
Transient protein expression in plants is fast and flexible, but differences between plant and mammalian N-glycosylation can affect the activity and immunogenic potential of a plant-produced protein. Consequently, there have been previous efforts to eliminate the plant-specific sugars alpha-1,3-fucose and beta-1,2-xylose from the N-glycans of N. benthamiana. These effort were aimed at silencing or knocking out the corresponding genes with RNAi [1], classical mutagenesis [2] and TALEN [3], but have only been partially successful, as the presence of alpha-1,3-fucose could be reduced but never eliminated.
Here, we have used multiplex CRISPR/Cas9-mediated genome editing to knock out all active alpha-1,3-fucosyltransferase and beta-1,2-xylosyltransferase genes in N. benthamiana.
We generated constructs targeting the xylosyl- (X) or fucosyltransferases (F) separately, and both sets of genes simultaneously. In the T2 generation, we identified F-KO and X-KO lines and confirmed the functional gene knock-out by mass spectrometric analysis of their N-glycans. FX-KO lines were then generated by crossing, and - thanks to the favorable appearance of new germinal mutations by ongoing Cas9 activity – homozygous knock-out lines were quickly identified.

Applications of genome editing in wheat and barley

Tom Lawrenson, Nicola Atkinson, Sadiye Hayta, Mark Smedley, Yvie Morgan, Martha Clarke, Alison Hinchliffe, Wendy Harwood
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RNA-guided Cas9-based editing is revolutionizing gene functional analysis for research purposes as well as providing exciting opportunities for crop improvement. In barley, targeted gene knock-out using CRISPR Cas9 is very efficient, allowing knock out of single and multiple target genes. In wheat, it is possible to knock-out a single gene or to target all homeologues in a single step. Strategies for increasing the efficiency of obtaining the required edits will be described together with consideration of the challenges still limiting applications of the technology. The efficiency of the delivery system for the editing components, which usually involves transformation followed by regeneration in tissue culture, is a step that limits the full potential of new editing technologies. Following the success of targeted gene knock-outs in crops, attention now turns to additional applications of CRISPR Cas9 based genome editing including gene targeting. We will describe recent progress in gene targeting in barley.

PDIL5-1 knockout induced by Cas9 endonuclease confers resistance of barley to bymoviruses

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The bymoviruses Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV) can cause yield losses of up to 50% in winter barley. In autumn, young barley plants are infected by the soil-borne fungal vector Polymyxa graminis. Upon viral establishment and systemic spreading, yellow mosaic symptoms appear on leaves, followed by leaf necrosis and decreased winter hardiness. The rym4 and rym5 allelic variants of the EUKARYOTIC INITIATION FACTOR4E (EIF4E) gene, which are widely used as resistance sources in European winter barley cultivars, have already been overcome by some virus strains. A promising new resistance mechanism for breeding programmes is provided by rym11, a loss-of-function allele of the PROTEIN DISULFIDE ISOMERASE-LIKE5-1 (PDIL5-1) gene. Detected in Asian landraces, it confers resistance to all known strains of BaYMV and BaMMV. By targeted mutagenesis of the coding sequence, PDIL5-1 loss-of-function alleles were generated in the BaMMV/ BaYMV-susceptible cultivars 'Golden Promise' and 'Igri' and resistance was shown in homozygous T1 mutant plants.

High-throughput screening for genome-editing events on single cell level

Florian Roesch1, Mainak Das Gupta1, Lena Hochrein1, Fabian Machens1, Bernd Mueller-Roeber1
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New gene editing tools such as CRISPR or TALEN have made the modification of plant genomes much more precise and faster. However, precise editing techniques, such as those based on the Homology Directed Repair (HDR) pathway occurs at very low frequency in plants. As a result, the selection of precisely edited plants remains a challenge. Furthermore, selecting precisely edited plants using tissue culture methods are laborious and time consuming. Therefore, we aim to develop a DNA-freeCas9-based reporter system to identify edited sequences directly in protoplast at the single cell level. To this purpose, we are creating a split T7-dCas9 system where the two different dCas9s (dead-Cas9) proteins will bring the split T7 enzyme into close proximity of the precisely edited DNA sequence on a sequence dependent manner. If the Locus was successfully edited, the T7 polymerase function can be reconstituted which in turn can drive the expression of a fluorescent protein from an independent vector. The fluorescence signal will then be used to identify successfully edited protoplasts via cell sorting.

Phenotypic Novelty by CRISPR

Panagiotis N. Moschou

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Genome editing by CRISPR is now routinely used in plant biology for unravelling gene functions and improving agronomical traits. CRISPR opens up the possibility of genome manipulations which would have been unthinkable a few years ago. I will discuss and suggest CRISPR-mediated approaches for steering plant development that could be used to overcome potential challenges.

Development of toolkit for targeted base editing in rice

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Genetic variations in plants are important for gene function study and elite gene discovery in plants, as well as essential to economic crop improvement in breeding programs. Here we report a base editing toolkit, developed with Cas9, xCas9, Cas9-NG and its guided effectors (rAPOBEC1, hAID*Δ, TadA), allowing for feasible conversion of C to T, A to G, T to C and G to A at target loci in the rice genome. Pi-d2 involving in the rice blast resistance was successfully corrected at a high frequency and a number of gain-of-function mutants of target gene related to plant defense, such as OsFLS2, OsMPK6, OsBZR1, OsSERK2, OsWRKY45 etc., were generated as anticipated, and these editing events were stably transmitted into the next generation. This base editing toolkit will benefit both biological science study and molecular breeding on rice.

DNA-free genome editing in soybean explants

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Genome editing without the use of DNA vectors has been reported in important crops like maize and wheat. Following a similar approach we have used biolistics to deliver pre-assembled Cas–gRNA ribonucleoproteins into soybean explants. Genomic DNA samples were obtained two days after bombardment and fragments surrounding the targeted sequences were amplified by PCR and analysed by amplicon deep sequencing. Indels, including insertions and deletions, occurring at the Cas–nucleases cleavage sites were considered as mutations. Mutagenesis frequency was calculated as the percentage of reads containing mutations over the total reads sequenced for each sample. We obtained mutagenesis frequencies comparable to what has been obtained using similar protocols in other species. Although we are able to regenerate plants after bombardment, no mutant plants were recovered from our initial experiments. Because no foreign DNA is used in this protocol, by working with larger samples we expect to obtain mutant soybean plants that are completely transgene free as previously demonstrated in other crops.

Targeted mutagenesis of BRANCHED HEAD homoeoalleles causes alterations of wheat spike architecture

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In our project, we aim to modify the spike architecture of the temperate cereal wheat to enhance its yield potential by increasing the number of grains produced per spike. By using specified guide-RNAs (gRNA) and Cas9 endonuclease, suitable motifs have been targeted within the BRANCHED HEAD (BH) coding sequence that is conserved among the three sub-genomes of hexaploid wheat (Triticum aestivum L.). By using biolistic transformation of immature embryos, two conserved regions were targeted simultaneously. Mutagenesis on the target regions was observed in stably transgenic plants, but also in transgene-free ones, where transient expression of gRNA and Cas9 was evidently sufficient to trigger the genetic alterations. Target-specific PCR amplification and Sanger sequencing identified single, double and triple-mutated plants. Haploid technology will be used to fix further new alleles and a variety of combinations thereof. The phenotypic evaluation revealed excessive branching in the spikelets of double and triple mutated KO plants, which was associated with a high rate of infertility.

ARTIFICIAL NUCLEASES-MOLECULAR TOOLS FOR GENE THERAPY

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Nuclease-mediated genome editing offers a powerful tool for gene therapy since it allows the targeted correction/alteration of genomic sequences in cells. Artificial DNA nucleases have provided scientists with the unprecedented ability to probe, regulate, and manipulate the genome [1]. Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and the clustered regularly interspaced short palindromic repeat-Cas9 system (CRISPR/Cas9) represent tools that can bind to and cleave a specified DNA sequence. Recently, CRISPR/Cas9 has become the most popular system for gene modification.

Our research focuses on the development of a new type of ZFNs, which can be regulated in a multiple manners by the help of metal ions. Based on our previous studies the C-terminal nuclease domain of the Colicin E7 protein (NColE7), a member of the HNH nuclease family, can be a good candidate for this purpose. This protein contains the HNH metal binding motif at its C-terminus, which can only become functional in the spatial vicinity of the N-terminal positively charged amino acids. The metal ion binding, which is essential for catalysis, depends on the 3D structure of the protein. The proper folding on NColE7 is also facilitated by the amino acids in the N-terminal sequences [2]. We aim to construct our newly designed ZFNs to target various DNA sequences. To select suitable DNA sequences for efficient targeting, we have designed several guide RNA sequences for CRISPR/Cas9 system as control.

One of the crucial aspects of the artificial nuclease-based therapeutics is their delivery to target cells. This could be achieved by introducing the plasmid DNA carrying the gene of the nuclease into the cell nuclei, or by direct delivery of proteins (and mRNA). The emerging field of nanotechnology provides more options such as the nano-sized carriers, which may have diverse potential in targeted delivery of drug-like molecules to particular sites by designing the physicochemical properties or surface. We encapsulated the plasmids carrying the genes of the nucleases using cationic polymeric delivery systems to increase the targeted delivery efficiency into the cells. The water-soluble lipopolymer (WSLP) used by us consists of polyethylenimine (PEI) as a cationic polymer with modifications for CRISPR/Cas9 and ZFNs. Our goal is the optimization of delivery and function of the constructed artificial nucleases.

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Worldwide CRISPR patent landscape shows strong geographical biases

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Our landscape of CRISPR (CAS 9 and other nucleases) patenting shows that the technology is constantly being improved and a diversity of potential applications (medical, industrial, agriculture) and of actors (both public and private). A novel geopolitical balance of forces has emerged in this crucial new biotechnological field. As is known, laboratories in the USA played a pioneer role in the original invention, and laboratories in this country remain leaders in technical improvements and in the medically applied sector. However, China is now taking the lead in the industrial and agricultural (plants and animals) sectors and in the total number of patents per year since 2016. This can be explained by the massive investment in biotechnology in China. Strikingly, in all sectors, the number of CRISPR patents originating from Europe trails far behind the USA and China. Korea and Japan are next in this ranking. We suggest that the weakening position of Europe is due to the GMO debacle on this continent and also to a "cultural" reluctance to file patents. This trend is also observed for patents in relation to health aspects (which is not a controversial biotechnological field as is the agricultural one).

Normative Criteria and Their Inclusion in a Regulatory Framework for New Plant Varieties Derived From Genome Editing (oral)

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Any legal regulation has to take into account fundamental interests and concerns, whether of private or public nature. This applies in particular to the politically and socially sensitive question of regulating plant biotechnology. With the advent of new breeding techniques, such as genome editing, new challenges are arising for legislators around the world. However, in coping with them not only the technical particularities of the new breeding techniques must be taken into account but also the diverse and sometimes conflicting interests of the various stakeholders. These normative criteria, which can have an impact on regulatory decisions regarding genome edited plants and products derived from them, include inter alia industry interests, farmer interests, public opinion, consumer rights and interests, human health and food safety, food security, environmental protection, consistency and coherence of the regulatory framework, and ethical or religious convictions. Since those interests differ from country to country depending on the respective political, economic, and social circumstances, the respective legislator has the task of identifying these normative criteria and must find a suitable balance between them. As a result, the individual country-specific regulatory outcomes regarding genome edited plants are likely to be as manifold as the interests and regulatory measures at hand.

New breeding techniques in the context of the EU regulation of GM plants

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Two guidance documents produced by the European Food Safety Authority (EFSA) outline the principles for the environmental risk assessment and the risk assessment of food and feed from genetically modified (GM) plants (EFSA GMO Panel 2010 and 2011). In 2011, the European Commission (EC) requested EFSA to assess the adequacy of those guidance documents to perform the risk assessment of plants developed through site-directed-nuclease type 3 (SDN-3). The scientific outcome of the GMO Panel was published in 2012 (EFSA GMO Panel 2012) and concluded that the two guidance are fully applicable. In 2018, the Court of Justice of the European Union has clarified that Directive 2001/18/EC, that regulates the deliberate release of GMOs into the environment, is also applicable to plants obtained by mutagenesis techniques that have emerged since its adoption. Against this background, in April 2019, EFSA was requested by the EC to assess if the methodology described in the scientific opinion of 2012 on SDN-3 is also applicable to plants developed with the new emerging SDN-1and SDN-2 techniques (like CRISPR-Cas9 technique) and with oligonucleotide directed mutagenesis (ODM). The GMO Panel is currently involved in this activity which will be published as scientific opinion output in April 2020.

Analysis on Current Situation and Development Trend of Genome Editing for Crop improvement

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Genome editing is a powerful tool for targeted modification of genomic DNA. It has been applied in various crops to improve agricultural traits and showed great potential in crop improvement. To understand fully the landscape and gather insights into R&D of genome editing in crops, we have conducted a large scale data analysis using advanced analysis and visualization tools. The results shows rapid growth trend in the publications and patents of crop genome editing in recent years. The number of MN-related publications is decreasing, while those of CRIPSR is increasing rapidly. Meanwhile, the hot research subject has changed from biological mechanism to application of editing tool, and research species has also shifted from model plants to key crops. Furthermore, The United States, France, Germany are major countries that have carried out the genome editing-related research earlier. They possess more relevant publications and patents than other countries. In addition, R&D activities between public and private sectors are much different, the former mainly are tend to publish research papers, while the latter are tend to apply for patents. It is our hope that the results of this study can offer valuable insights to a broad class of readers including policy makers, industry practitioners as well as professors and students.

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ABSTRACTS OF POSTER PRESENTATIONS

Poster №1: **CRISPR/Cas9-targeted knock-in of the acetolactate synthase (ALS) gene with a single nucleotide polymorphism conferring imidazolium resistance in Barley.**

Yvie Morgan, Tom Lawrenson, Alison Hinchliffe, Macarena Forner, Wendy Harwood.

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CRISPR/Cas9 has shown to be a useful gene-editing tool in Barley, yet precise knock-in frequencies are low. Previous studies in plants have deployed Geminiviral replicons to extend the mitotic S-phase of somatic cells to enhance homologous recombination rates and to enable high copy number of the repair template. In this study we used Agrobacterium-mediated transformation of immature Golden Promise embryos to introduce CRISPR/Cas9 with the Wheat Dwarf Virus replicon flanking a repair template of ALS harbouring an amino acid substitution (S653N), permitting imazamox resistance on targeted knock-in at the WT locus. qPCR analysis demonstrated a difference in repair template copy number with and without the replicon. ALS amplification by PCR has identified 3/47 T0 transformants as knockin transgenics. Heritable knock-ins are to be assessed in subsequent progeny. Viral-replicons used in combination with tissue-specific expression of Cas9 and recombination proteins may prove to enhance rates further. Ultimately, future knock-in frequencies in plants may reach those that have been achieved in some animal species.

Poster №2: **Verification of Cas12a (Cpf1) ability to edit carrot genome**

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CRISPR/Cas12a system has been recently described as a tool for genome modification, alternative to CRISPR/Cas9 system. Unlike Cas9, Cas12a (Cpf1) protein is guided to the target sequence by a single crRNA, recognizes T-rich PAM motifs and generates staggered ends after DNA double strand break. The Francisella novicida FnCpf1 and Lachnospiraceae bacterium LbCpf1 proteins were chosen by us to edit carrot genomes. DNA sequences of these two Cas12a variants and four crRNA sequences targeting different regions of flavanone-3-hydroxylase gene (F3H), substantial for anthocyanins biosynthesis, were used to create a set of eight vectors. They were delivered to carrot callus cells accumulating anthocyanins and exhibiting purple color, and previously validated by us as a model system using CRISPR/Cas9 vectors. After successful Agrobacterium-mediated transformation, some newly developing calli showed discoloration and molecular analyses confirmed mutations at the target site. The results indicated that the LbCas12a protein was more effective in carrot genome editing.

Poster №3: **Barley ovules as a genotype-independent starting material for CRISPR/Cas9 genome editing**

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Most CRISPR/Cas9 studies performed in barley use immature embryos isolated from the cultivar Golden Promise as the starting material. Golden Promise is, however, genetically very different from elite barley cultivars used in current barley breeding programs and transfer of CRISPR/Cas9 modifications to elite cultivars therefore require large numbers of backcross-generations. A less genotype-dependent transfer system for CRISPR/Cas9 is therefore highly desirable. We have previously established a genotype-independent transformation system in barley using the zygote within the ovule as starting material (Holme et al., 2006, Plant Cell Rep. 25, 1325-1335) and the ovule culture system therefore seems a perfect choice to facilitate barley genotype-independent genome editing. Thus, we have recently started optimizing this system to induce targeted mutations by means of a CRISPR/Cas9 construct previously used for barley immature embryos and which shows a high mutation frequency of 46% at the targeted site (Holme et al., 2017, Plant Mol. Biol., 95, 111-121). The first results indicates the zygote within the ovule may serve as an efficient starting material for CRISPR/Cas9 based modifications in barley.

Poster №4: **Identification and characterization of R2R3-MYB and bHLH transcription factors regulating anthocyanin biosynthesis in carrot (Daucus carota)**

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Anthocyanin, an important plant pigment, is responsible for most of the purple, red and blue colors in plants. Anthocyanins are synthesized via the flavonoid pathway, which also leads to the products including proanthocynidins and flavonols. The biosynthesis of anthocyanin pigments is regulated at the transcriptional level by a MYB-bHLH-WD40 (MBW) transcription factor (TF) complex, composed of TFs from the R2R3-MYB, basic Helix-Loop-Helix (bHLH) and WD40 classes. Carrot (Daucus carota), a globally important root crop, is a rich source of carbohydrates, minerals, vitamins as well as terpene volatile compounds. Furthermore, the black or purple carrot (Daucus carota ssp. sativus var. atrorubens Alef.) is characterized by the significant amount of anthocyanins, which confer to this variety an antioxidant activity greater than in the other colored cultivars. In our study, three carrot genetic stocks that differ in storage root color and anthocyanin accumulation were chosen. RNA-Sequencing analyses (RNA-Seq) were performed in outer and inner tissues of three carrot cultivars, respectively, to identify candidate R2R3-MYB and bHLH TF genes controlling anthocyanin pigmentation. Two MYB transcription factors (namely DcMYB6 and DcMYB7), and one bHLH gene, were involved in anthocyanin accumulation in genotype specific purple cultivars. In addition, one MYB44-like gene and one MYB1R1 gene were found to be the negative regulator of anthocyanin biosynthesis. CRISPR/Cas9 vectors expressing one single-guide RNA (gRNAs) targeting these candidate genes in carrot were tested for changing the anthocyanin biosynthesis in a purple or orange-colored carrot using Agrobacterium-mediated genetic transformation. Knockout of TF activator or repressor might resulted in the coloration or discoloration of callus, validating the functional role of this gene in the anthocyanin biosynthesis in carrot.

Poster №5: **Establishing RNA targeting via the CRISPR/Cas13 system in Vitis vinifera**

Gaelle Robertson, Manuela Campa, Johan Burger

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Recently, genome editing by the CRISPR/Cas system has been expanded beyond DNA targeting. A novel class 2 Cas effector, Cas13, has been revealed as a programmable RNA-targeting effector. The CRISPR/Cas13 system can be re-purposed for applications such as regulation of gene expression and the inhibition of RNA viruses. Grapevine (Vitis vinifera) is one of the world's most important fruit crops and currently more than 65 different viral species have been reported to infect it, the most known for any perennial crop. Using CRISPR/Cas13, we aim to investigate the potential of this system to confer resistance to a grapevine virus. For this, we will first provide a proof-of-concept for CRISPR/Cas13-mediated cleavage of an annotated mRNA transcript in grapevine and the model plant Nicotiana benthamiana. Once the functionality of CRISPR/Cas13-based transcriptome editing is confirmed, a vector targeting the Grapevine virus A (GVA) infectious clone that infects both grapevine and Nicotiana benthamiana will be assembled. Nicotiana benthamiana plants will be transformed with these vectors using Agrobacterium and the CRISPR/Cas13-mediated viral interference of the plants post-infiltration will be analysed on a molecular basis.

Poster №6: **Establishing RNA targeting via the CRISPR/Cas13 system in Vitis vinifera**

Gaelle Robertson, Manuela Campa, Johan Burger
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