University of Novi Sad

Institute of Lowland Forestry and Environment

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COST Action CA18111
Genome Editing in Plants

PlantED

1st PlantEd Conference Plant Genome Editing - State of the Art

Book of abstracts

Venue: University of Novi Sad, Central Building
Date: 5-7 November 2019

1st PlantEd Conference Plant Genome Editing - State of the Art

Organized by

Institute of Lowland Forestry and Environment, Novi Sad, Serbia

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COST Action CA18111 (PlantEd)
"GENOME EDITING IN PLANTS - A TECHNOLOGY WITH TRANSFORMATIVE POTENTIAL"

1st PlantEd conference
"Plant Genome Editing - State of the Art"

5th – 7th November 2019
Novi Sad, Serbia

Including:
Open Conference; Working Group Meetings; Management Committee Meeting

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Scope of the Conference

The 1st PlantEd conference within COST Action 18111 (PlantEd) will be the extended three days joint meeting of Action Working Groups and Management Committee, together with an open-to-all scientific introduction dedicated to genome editing technology in plants. This will be a suitable platform for dissemination of the Actions ideas through Working Groups: WG1-Technical platforms, WG2-Impact assessment, WG3-Policies and regulations, WG4-Perceptions and opinions – all with a focus on genome editing in plants.

This conference will gather experts from various backgrounds from science to industry and through the scientific part of the conference will introduce all participants to the powerful transformative technology of genome editing and its applications in plants. The outcome of the conference will help shaping the activities of the COST Action PlantEd (and beyond) over the next four years. There will be plenty of opportunities for networking among the various disciplines and sectors that have an interest in plant genome editing.

PlantEd organizing team

Novi Sad, 5th - 7th November

PlantEd COST Action CA18111 Genome Editing in Plants

1st PlantEd conference
Plant Genome Editing - State of the Art

5th – 7th November 2019 Novi Sad, Serbia

FreeWifi _ ID: PlantEd2019
Pass: ILFEPLANTED

Tuesday 5th Nov 2019 - Action Working Group meetings (WG1-WG4)	
9:00-10:00	**REGISTRATION**
10:00-12:00	Parallel Working Group meetings (WG1-WG4)
12:00-13:00	Lunch
13:00-15:00	Parallel Working Group meetings (WG1-WG4)
15:00-15:30	Coffee break
15:30-16:30	Parallel Working Group meetings (WG1-WG4)
16:30-18:00	Plenum discussion on bilateral interactions between the WGs
Wednesday 6th Nov 2019 - Conference	
09:00-10:30	Plenum session 1 - Opening/Setting the scene
10:30-11:00	Coffee break including poster session
11:00-12:30	Plenum session 2 - Plant genome editing around the world
12:30-13:30	Lunch
13:30-17:30	***Individual networking using the GRIP application***
13:30-17:15	Parallel sessions based on Action WG1-WG4
17:15-17:30	Nomination and prize for best poster
19:00-23:00	Social dinner
Thursday 7th Nov 2019 - Conference / Management Committee meeting	
08:30-14:00	***Individual networking using the GRIP application***
08:00-09:00	Plenum session 3 - Genome editing in commercial plant breeding
09:00-09:45	Plenum session 4 - Initiatives on plant genome editing
09:45-10:00	Official closing of the conference
10:00-10:30	Coffee break
10:30-13:15	2nd Action Management Committee meeting
13:15-14:00	Lunch

PlantEd COST Action CA18111 Genome Editing in Plants

1st PlantEd conference
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5th – 7th November 2019 Novi Sad, Serbia

Tuesday 5 Nov - Action Working Group meetings (WG1-WG4)

9:00-10:00 REGISTRATION

	Amphitheatre	**Hall I-16**	**Hall II-13**	**Hall P-14**
Venue:	*(All plenum sessions+ WG1 parallel sessions)	(WG2+WG4 parallel sessions**)**	* (WG3 parallel sessions)	(WG4+Grip app)
	Ground level	**1st Floor**	**2nd Floor**	**Ground level**

10:00-16:30	**Amphitheatre**	**Hall I-16**	**Hall II-13**	**Hall P-14**
10:00-12:00	WG1 meeting	WG2 meeting	WG3 meeting	WG4 meeting
12:00-13:00	**Lunch**			
13:00-15:00	WG1 meeting	WG2 meeting	WG3 meeting	WG4 meeting
15:00-15:30	**Coffee break**			
15:30-16:30	WG1 meeting	WG2 meeting	WG3 meeting	WG4 meeting
Venue:	**Amphitheatre**			
16:30-18:00	Plenum discussion on bilateral interactions between the WGs			

18:00 Free time, but discussions welcome to continue

PlantEd COST Action CA18111 Genome Editing in Plants

1st PlantEd conference
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Wednesday 6 Nov – Conference

Venue:	**Amphitheatre**
09:00-10:30	**Plenum session 1 - Setting the scene** **Moderator: Vladislava Galovic (WG1)**
09:00-09:10	Official opening of the conference by the Organizing Committee and the Chair
09:10-09:35	Welcome address:; Rector of the University of Novi Sad; Provincial Secretary for Higher Education and Scientific-Research Activity; Provincial Secretary for Agriculture, Water management and Forestry; Director of the Institute of Field and Vegetable Crops; Director of the Host Institution
09:35-09:50	Saša Orlović, Director of the Institute of Lowland Forestry and Environment, Novi Sad, Serbia Introduction about the host, Institute of Lowland Forestry and Environment
09:50-10:10	Dennis Eriksson, Action Chair, Swedish University of Agricultural Sciences, Sweden PlantEd and its place in the world of plant genome editing
10:10-10:30	Petra Jorasch, Euroseeds, Belgium The view of the European seed sector on genome editing in plant breeding
10:30-11:00	Coffee break
10:30-11:00	Poster session (during the coffee break)
Venue:	**Amphitheatre**
11:00-12:30	**Plenum session 2 - Plant genome editing across the world** **Moderator: Dennis Eriksson (Chair of the Action)**
11:00-11:30	Thorben Sprink, Julius Kuehn Institute, Germany Genome editing platforms around the world
11:30-12:00	Wendy Harwood, John Innes Centre, UK Development and application of CRISPR / Cas9 based genome editing resources in crops
12:00-12:15	Jan Schaart, Wageningen University and Research, the Netherlands Gene editing at WUR-Plant Breeding
12:15-12:30	Henk Schouten, Wageningen University and Research, the Netherlands The new CRISPR-Cas toolbox
12:30-13:30	Lunch
13:30-17:30	***Individual networking using the GRIP application (Hall P-14)***
13:30-17:15	**Parallel sessions - see separate program**
Venue:	**Amphitheatre**
17:15-17:30	Nomination and prize for best poster
19:00-23:00	**Social dinner**

13:30-17:30 ***Individual networking using the GRIP application (Hall P-14)***	
Venue:	**Amphitheatre**
13:30-17:15	**Parallel session 1 - Technical platforms** **Moderator: Thorben Sprink (WG1)**
13:30-13:45	Introduction by the moderator
13:45-14:00	Luisa Bortesi, Maastricht University, the Netherlands Current technical limitations and future developments in plant genome editing
14:00-14:15	Jana Murovec, University of Ljubljana, Slovenia The use of ribonucleoprotein complexes (RNPs) for plant genome editing
14:15-14:30	Lena Maas, Wageningen University and Research, the Netherlands Transient Induction of Plant Regeneration
14:30-14:45	Kubilay Yildirim, Tokat Gaziosmanpaşa University, Turkey Conferring Multiple Resistance to DNA Viruses in Plants with the CRISPR/Cas9 Genome Editing Technology
14:45-15:00	Goetz Hensel, IPK Gatersleben, Germany Improvement of barley traits by targeted genome modification
15:00-15:30	Coffee break
15:00-15:30	Poster session (during coffee break)
15:30-15:45	Katarina Cankar, Wageningen University and Research, the Netherlands The CHIC project
15:45-16:00	Rufang Wang, Wageningen University and Research, the Netherlands CRISPR/Cas9-mutagenesis for revisiting the role of master regulators in tomato ripening
16:00-16:15	Katrin Van Laere, ILVO, Belgium CRISPR/Cas9 to modify bitterness in Cichorium intybus
16:15-16:30	Tjaša Lukan, National Institute of Biology, Slovenia Establishment of CRISPR/Cas9-mediated microRNA knock-out in potato
16:30-16:45	Allah Bakhsh, Nigde Omer Halisdemir University, Turkey Elucidation of role of potato invertase inhibitor using Crispr-Cas9 Application
16:45-17:15	Q&A session with all the speakers

Wednesday 6th Nov Parallel Session

13:30-17:30 Individual networking using the GRIP application **(Hall P-14)**

Venue: Hall II-13

13:30-17:15	**Parallel session 2 - Policies and regulations** **Moderator: Patrick Rudelsheim (WG3)**
13:30-13:45	Introduction by the moderator
13:45-14:00	Philipp Aerni, University of Zurich, Switzerland Politizising the precautionary principle
14:00-14:15	Nils Rostoks, University of Latvia, Latvia Development of risk assessment of genome-edited organisms
14:15-14:30	Kai Purnhagen, Wageningen University and Research, the Netherlands
14:30 -14:45	Kristine Margaryan, Institute of Molecular Biology, Armenia Prospects of plant genome editing in Armenia
14:45-15:30	Coffee break
14:45-15:30	Poster session (during coffee break)
15:30-15:45	Juan Antonio Vives-Vallés, University of the Balearic Islands, Spain The Judgment of the CJEU of 25 July 2018 on mutagenesis: interpretation and legislative proposal
15:45-16:00	Aleksej Tarasjev, University of Belgrade, Serbia How Serbia is regulating GMOs and how it will regulate new breeding techniques
16:00-16:15	Selim Cetiner, Sabanci University, Turkey The biosafety law in Turkey and its implications
16:15-16:30	Trine Hvoslef-Eide, Norwegian University of Life Sciences, Norway Proposed revision of the Norwegian Gene Technology Act as a result of the challenges from new breeding technologies
16:30-17:15	Q&A session with all the speakers

Wednesday 6th Nov Parallel Session

13:30-17:30 Individual networking using the GRIP application **(Hall P-14)**

Venue: Hall I-16

13:30-15:00	**Parallel session 3 - Impact** **Moderator: Dragana Miladinović (WG2)**
13:30-13:45	Introduction by the moderator
13:45-14:05	Zoe Hilioti, Centre for Research and Technology Hellas, Greece Zinc Finger Nucleases as Genome Editing Tools for Specific and Efficient Plant Breeding
14:05-14:25	Jeremy Sweet, JT Environmental Consultants, UK Novel disease resistance approaches: are they durable?
14:25-14:45	Simon Bull, ETH Zurich, Switzerland Genome editing of cassava – improving a crop from the Global South
14:45-15:00	Gul Ebru Orhun,Canakkale Onsekiz Mart University, Turkey Cereal Genetic Engineering Studies
15:00-15:30	Coffee break
15:00-15:30	Poster session (during coffee break)
Venue:	**Hall I-16**
15:30-17:15	**Parallelsession4–Public andstakeholder perceptions** **Moderator: Tomasz Zimny (WG4)**
15:30-15:45	**Introduction by the moderator**
15:45-16:00	Tomasz Zimny, Swedish University of Agricultural Sciences, Sweden Why public acceptance is important?
16:00-16:15	Aida Dervishi, University of Tirana, Albania GMOs and genome editing - Public perception and attitude in Albania
16:15-16:30	Nebojša Majstorović, University of Novi Sad, Serbia Purchasing products of gene technology: Psychological factors affecting consumer decision making
16:30-17:15	Q&A session with all the speakers (together WG3 & WG4)

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Thursday 7th Nov - Conference /Management Committee meeting	
08:30-14:00	***Individual networking using the GRIP application (Hall P-14)***
Venue:	**Amphitheatre**
08:00-09:00	**Plenum session 3 – Genome editing in commercial plant breeding** **Moderator: Sebastien Carpentier**
08:00-08:20	Julia Wind, KeyGene, the Netherlands High-throughput genome editing in tomato to create variation in gene expression
08:20-08:40	Muath Alsheik, Graminor, Norway Gene Editing to Innovate Norwegian plant breeding
08:40-09:00	Marcel Kuntz, CNRS, France The global CRISPR patent landscape
Venue:	**Amphitheatre**
9:00-09:45	**Plenum session 4 - Initiatives on plant genome editing** **Moderator: Dennis Eriksson**
09:00-09:15	Lavinia Scudiero, Wageningen University, the Netherlands European Citizens´ Initiative
09:15-09:30	Nikita Sajeev and Vera Veltkamp, Wageningen University, the Netherlands The GeneSprout Initiative
09:30-09:45	Nick Vangheluwe, Vlaams Instituut voor Biotechnologie, Belgium European CRISPR campaign
09:45-10:00	Official closing of the conference by the Action Chair and the Conference Organizers
10:00-10:30	Coffee break
Venue:	**Amphiteatre**
10:30-13:15	**2nd PlantEd MC meeting**
13:15-14:00	Lunch

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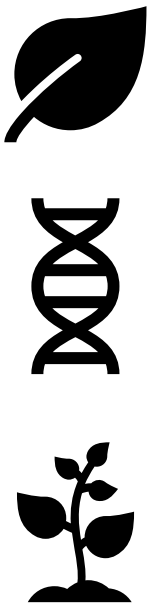
COST Action 1st PlantEd Conference
Oral presentations



https://plantgenomeediting.eu/

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Plenum session 1 - Setting the scene

PlantEd and its place in the world of plant genome editing

Dennis Eriksson, Action Chair

Swedish University of Agricultural Sciences, Alnarp, Sweden

Genome editing has boosted plant research and plant breeding in recent years with its capacity, versatility and ease of use. Despite being still a rather novel technology, it is already having an impact on plant biological systems research and on trait management in breeding. The regulatory status of the resulting products is not fully settled yet though, as the decades-old GMO legislation in the EU may be applicable in many cases despite apparent problems with enforcement of certain provisions such as detection. It is also not clear how society and the general public will perceive this technology once applications become more commonplace. A good communications strategy is therefore of outmost importance.

PlantEd was timely initiated to facilitate the coordination of ongoing research on plant genome editing and to discuss the direction of future research priorities. We connect all the major plant research institutes in Europe and enable the training of early career researchers through targeted activities. We engage not only a large number of academic researchers but also a range of other stakeholders to address technical, legal, perception and communications issues.

This presentation will give you an overview of what PlantEd is about, where it came from and where we are going in the next four years.

European seed sector´s perspectives on genome editing in plant breeding

Petra Jorasch

The United Nation's Sustainable Development Goals commit the world to zero hunger worldwide and call for more productive, reliable harvests to help escape from poverty. Plant breeding innovation is key to advance global food security and contribute to a more sustainable agricultural production. The latest plant breeding methods, like genome editing, can improve crops faster and more efficiently. Governments should enact policies and regulatory frameworks that remove unnecessary barriers and encourage research, promote innovation and enable commercialization. The opportunities must be available for plant breeders who work in small or large organizations, for the public or private sector, and in all crops.

Europe's seed sector, technology developers and public researchers are global leaders in developing improved plant breeding methods. The sector is highly innovative and invests up to 20% of its turnover in research and development, to constantly provide farmers with the best varieties that fit the needs of a highly productive and sustainable agriculture as well as consumer demands. The European Seed Sector considers that the consequences of the European Court of Justice Ruling on mutagenesis breeding (C-528/16) present unacceptable socio-economic risks for European plant breeding, for the wider agri-food chain, for consumers and for our European environment. The prohibitive compliance requirements of the GMO Directive relative to the value of commodity crops effectively cut Europe's breeders off from scientific progress and puts them as well as farmers, processors, traders and consumers at a competitive disadvantage to regions with more enabling regulations.

The EU Seed Sector maintains its position that plant varieties and seeds are subject to a respected and robust regulatory regime. Plant varieties developed using the latest breeding methods should not be subject to any additional regulations if they could have been produced through earlier breeding methods, or might also have been obtained from natural processes without human intervention.

The development of new plant varieties requires up to 15 years before they can be marketed. It is therefore crucial for companies that their investments in the latest plant breeding methods can be based not only on sound science, but also on confidence that the market access of their improved varieties will not be subject to an uncertain outcome of politicized regulatory procedures.

Plenum session 2 – Plant genome editing across the world

Genome editing platforms around the world

Thorben Sprink[1], Dominik Modrzejewski[1], and Ralf Wilhelm[1]

[1]Institute for biosafety in plant biotechnology, Julius Kuehn-Institute, Quedlinburg, 06484, Germany.

Climate change and the rising demand for food driven by the growing world population and changes in nutritional behavior are posing major challenges to food security worldwide. Producers of agricultural goods and breeders have to face these new challenges, as climate change effects become more apparent. This is reflected in the increasing frequency of extreme weathers events such as droughts or floods in recent years accompanied by an increase in the pressure of abiotic- and biotic stresses on crop plants and, as a result, increasing use of pesticides. International agricultural production changed rapidly in the last decades from small field cropping, growing plenty varieties of crops and other agricultural products side by side, to big plains of monocultures. The aims of breeding have always been to genetically alter crops with to achieve superior characteristics of relevant traits. Conventional breeding like simple crossing has been employed since centuries but frequently reached its limits to access sufficient natural variation. Mutation breeding using radiation or chemicals enabled breeders to induce undirected novel but randomly distributed mutations. The backdraw is its need of extended backcrossing. Genetic engineering using transgenesis opened the field for specific changes in the genome conferring traits of interest even between species. But these changes are still undirected integrated into genomes. Moreover, the social acceptance of cultivating transgenic plants is low in many countries. Recent developments in genome editing e.g. especially using clustered regularly interspaced short palindromic repeats (CRISPR) fused with CRISPR associated proteins (e.g. Cas9), enable to change the genome of plants in a directed, trait AND site specific way. The approach is time saving and in some cases allows to avoid creating (intermediate) transgenic lines for breeding.

These new techniques of "Genome Editing" have already been successfully applied to more than 50 different crops and model plants worldwide. Many studies have been basic research, testing and developing the technologies, but there are also plenty of applications to improve agronomical relevant traits of crops e.g. agronomical value, increasing biotic- and abiotic stress tolerance, food and feed quality. Additionally, first varieties created by using genome editing have already been released to national and international markets.

However, regulation of these new genome editing techniques is not harmonized between continents and countries and may hinder international trade in the future. Many American countries established a straight forward regulatory system and application procedure, which defines such genome editing products as non-GMOs if a transgene is not present in the genome. In contrast, the European union has a restrictive regulatory system for GMOs in place which also applies for genome editing as verified by the European court of justice. In this presentation, the newest products of genome editing in agriculture will be presented and a first version of a genome editing atlas will be presented in which developments in the Genome Editing field are projected on a world map to identify the global development of GE.

Development and application of CRISPR / Cas9 based genome editing resources in crops

Tom Lawrenson, Sadiye Hayta, Mark Smedley, Penny Hundleby, Nicola Atkinson, Yvie Morgan, Martha Clarke, Alison Hinchliffe, Wendy Harwood

John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

RNA-guided Cas9 (CRISPR/Cas9) based technologies are providing valuable tools enabling rapid progress in many crop research areas. In addition, they hold great promise for contributing to the development of improved crops for the future. Access to these technologies is required by the research community. This may take the form of training, the opportunity for collaborative projects or simply access to a service platform. The resources offered by the BRACT facility will be described, including the BBSRC funded targeted gene knock-out resource.

Targeted gene knock-outs, using CRISPR/Cas9 are now routine for most crops although with varying efficiencies. Targeted knock-out relies on the non-homologous end joining (NHEJ) repair pathway to repair the double strand break made by Cas9 and in doing so introduce a small error. Examples will be described in a range of crops including wheat, barley and Brassica crops. Opportunities for achieving the desired outcome in crops such as wheat with complex polyploid genomes will be discussed.

CRISPR/Cas9 technologies can now be used for many applications beyond simple targeted gene knock-outs. Alternatives to Cas9 are also available and may offer advantages for specific applications. Gene targeting or targeted knock-in is a key aim of the plant community for many applications. This relies on the homology dependent repair (HDR) pathway rather than NHEJ. HDR is rare in plants meaning that progress achieving gene targeting has been slow. Advances in establishing gene targeting methodology in barley will be described.

Gene editing at WUR-Plant Breeding

Jan Schaart, Henk Schouten, Frans Krens

Wageningen University & Research (WUR) Plant Breeding

At WUR-Plant Breeding we have a strong focus at the development of New Plant Breeding Techniques. We for example designed the concept of cisgenesis, a genetic modification using only native gene sequences of the modified species. Since the arrival of the first gene editing tools we investigate the application of these tools for targeted mutagenesis in several crop species for a number of traits. We started in 2010 with testing Zinc Finger Nucleases in tetraploid potato aiming at changing tuber starch composition, used TALENS for a short period and then switched to gene editing using CRISPR-Cas. We now apply CRISPR-Cas9 for targeted mutagenesis in a number of projects for 1) biochemical pathway engineering (changing fatty acid profile in the oil seed crops Crambe and Camelina; changing flower color in the ornamental species carnation, alstroemeria; 2) removal of undesired genes (antinutritional factors in feed; toxic gluten genes in wheat) and 3) mutagenesis of susceptibility genes to generate disease resistances (potato, tomato, cucumber). In addition we use CRISPR-Cas to facilitate plant crossbreeding. For this we test CRISPR-Cas for targeted recombination, targeted allele-replacement, creating CENH3-based haploid inducers, inducing chromosomal inversions and creating male-sterile plants. In my presentation I will highlight some of our work using CRISPR-Cas for targeted mutagenesis.

CRISPR-Cas toolbox project

Henk J. Schouten, Ruud A. de Maagd, Jan G. Schaart, Gerco C. Angenent

Wageningen University & Research.

In June 2019 Wageningen University & Research (WUR) submitted, together with 10 plant breeding companies, a project proposal on testing and improving components of the CRISPR- Cas toolbox for plant genome editing. The funding (1.4 MEuro) should be provided by the Dutch government (50%) and the participating companies (50%). The decision about granting will be announced in November this year.

The project has 4 Work Packages (WPs). In WP1 different Cas-enzymes (including SpCas9, SaCas9, Mad7, ThermoCas9, Cpf1, Cms1) and promoters driving the guideRNAs and Cas- genes will be compared. Also multiplexing strategies will be evaluated. In WP2, delivery methods will be tested, including Ribonucleoprotein delivery, transient systems, and nanoparticles. WP3 focusses on modulation of expression, by deleting domains of promoters, or modifying the chromatin status. WP3 also studies genome editing via homology-directed repair. In WP1 to WP3 WUR will mainly use tomato as model species, whereas companies use other crop plants too.

In the last WP (WP4), polyploid crops will be mutated, aiming at efficient systems, knocking out all targeted alleles. The project will be flexible, and CRISPR-Cas variants that emerge in the near future, may be incorporated in the studies.

Parallel session 1 – Technical platforms

Current technical limitations and future developments in plant genome editing

Luisa Bortesi

Maastricht University

The recent development of genome-editing tools such as sequence-specific nucleases, base- editing enzymes, and oligonucleotides, has revolutionized basic plant research and crop breeding.
Even though an ever-growing number of publications describes the successful application of these genome-editing tools with high efficiency and precision, there are still several practical aspects which need to be addressed and some major bottlenecks which should be overcome to fully exploit the potential of the technology and expand its application beyond model species and a few selected crops. These include, for example, techniques to deliver the genome editing tools, strategies to regenerate and select edited lines, and approaches to edit multiple genes at a time.
In this talk, I will give an overview of the main current limitations of genome editing technology tools, in general, and specifically in their application for crop improvement. I will also present an outlook on what future developments are expected and needed in the field of plant genome editing.

The use of ribonucleoprotein complexes (RNPs) for plant genome editing

Jana Murovec

University of Ljubljana, Biotechnical Faculty, Jamnikarjeva ulica 101, 1000 Ljubljana, Slovenia
Corresponding author: jana.murovec@bf.uni-lj.si, +386 1 320 3265

Abstract: CRISPR/Cas9 is the most widely used tool for genome editing in human, animal, microbe and plant cells. Besides its efficiency and versatility for numerous applications, it is also the simplest technique to induce small or large changes in genomes. Although the most often used expression method for genome editing of plants remains *Agrobacterium tumefaciens* transformation, the introduction of preassembled ribonucleoprotein (RNP) complexes has already proved its efficiency in several plant species, like *Arabidopsis thaliana*, tobacco, lettuce and rice (Woo et al., 2015), grapevine and apple (Malnoy et al., 2016), maize (Svitashev et al., 2016), Petunia x hybrida (Subburaj et al., 2016), wheat (Liang et al., 2017), potato (Andersson et al., 2018), cabbage and Chinese cabbage (Murovec et al., 2018).
The technique offers several advantages comparing to stable transformation and needs new approaches for delivery of RNPs into plant cells. The presentation will therefore discuss current options for producing RNPs (*in vitro* transcription or chemical synthesis of sgRNAs, in-house purification or use of commercially available proteins Cas9), available methods for introduction of RNPs into plant cells, methods for verification of RNPs activity, mutation and off-target detection methods. Alternative applications of RNPs, like CRISPR/Cas9 targeted capture for NGS, engineered DNA-binding molecule-mediated ChIP system will also be presented.

Transient Induction of Plant Regeneration

Maas L, Angenent GC, Boutilier K*

Wageningen University and Research
Droevendaalsesteeg 1, 6708PB Wageningen, Netherlands. Lena Maas
MSc. PhD candidate, Molecular Biology
**Corresponding author: kim.boutilier@wur.nl*

Recalcitrance for in vitro regeneration in some species or genotypes of common crops limits the use of biotechnology tools such as doubled haploid production and somatic embryogenesis. There is therefore an urgent need to develop a generic tool to improve plant regeneration processes in a germplasm-independent manner.
Embryo-expressed transcription factors like the AP2 domain protein BABY BOOM and the CAAT-box binding factor LEAFY COTYLEDON1 have been used to enhance plant regeneration in a range of crops when stably expressed from a constitutive promoter. Although this transgenic approach has been highly successful, it precludes the routine commercial utilization of these plants. We aim to overcome this problem by transiently activating endogenous BBM/LEC1 gene expression using CRISPR-dCas9 technology. Transcriptional activator or repressor binding sites in the arabidopsis BBM/LEC1 promoters are being identified by producing deletions of various sizes via CRISPR/Cas9. The ability to use different activators fused to a dCas9 protein to regulate LEC1/BBM gene expression will beevaluated.

Establishment of CRISPR/Cas9-mediated microRNA knock-out in potato

Tjaša Lukan*[1], Florian Veillet[2], Laura Chauvin[2], Anna Coll[1], Maja Križnik[1], Jean-Eric Chauvin[2] and Kristina Gruden[1]

[1] *Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia*
[2] *INRA, Agrocampus Ouest, Université Rennes 1, UMR 1349 IGEPP, Domaine de Kéraïber, 29260 Ploudaniel, France*

To date, CRISPR/Cas9-mediated microRNA editing has not been established in potato. However, there is a growing evidence that small noncoding RNAs can be targeted by CRISPR/Cas9 system in plants. The novel gene-editing strategy is a challenge yet worth accepting, due to the compelling robustness, specificity, and stability for the modification of microRNA expression. In potato, CRISPR/Cas9 technology was mostly used in combination with Agrobacterium-mediated stable transformation. This could pose a problem, especially in case of time-consuming stable transformation with sgRNAs not previously confirmed as functional. On the other hand, protoplasts transfection is a faster method, but protoplasts isolation and plant regeneration remain bottlenecks. Therefore, we established a protocol, which consists of the design of CRISPR/Cas9 constructs, transient transfection of protoplast to select functional sgRNAs, followed by stable transformation of potato explants. This was achieved through the optimisation of protoplasts isolation from potato, protoplasts transfection and high-resolution melting analysis (HRM) to confirm functionality of tested constructs already one week after transfection. In the last step, functional constructs were used for stable transformation. Transgenic lines with desired mutations were selected by Sanger sequencing. Thus, we established a fast and efficient protocol for CRISPR/Cas9-mediated microRNA knock-out in potato. In addition, we confirmed our hypothesis that protoplasts transfection followed by HRM is an optimal strategy to test functionality of designed sgRNA constructs in general targeting mRNAs or miRNAs prior to stable transformation.

Improvement of barley traits by targeted genome modification

Goetz Hensel[1], Katarína Holubová[2], Stefan Hiekel[1], Pouneh Pouramini[1], Phillip Reuter[3], Steffen Baier[3], Martina Gastl[4], Véronique Bergougnoux[2] and Jochen Kumlehn[1]

[1]*Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Plant Reproductive Biology, Correnssstr. 3, Seeland, OT Gatersleben, Germany*

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The emergence and implementation of Cas endonuclease technology has undoubtedly taken plant research and biotechnology to a higher level. After the development of transformation vectors harbouring cas9 and gRNA expression units compatible for monocotyledonous plants, transient expression tests relying on biolistic DNA transfer into epidermal cells or PEG- mediated transformation of protoplasts were established to prevalidate candidate target gene- specific constructs to be used for site-directed mutagenesis in stable transgenic plants. Agronomically relevant traits like row-type (VRS1), plant productivity (CKX1) as well as malting quality (LOX1) were considered among the first of our approaches taking advantages of this new technology. Transgenic barley plants of the model cultivar 'Golden Promise' ectopically expressing target gene-specific endonucleases were generated, and by simultaneously targeting different positions within the target region, a wide variety of mutations was obtained. Beside indels in the target motifs, also deletions between pairs of guide-RNA target sites were detected. The typically multiple mutant alleles present in primary transgenics were efficiently resolved and fixed in doubled haploids generated via embryogenic pollen cultures. The ckx1 mutant plants showed reduced cytokinin dehydrogenase activity associated with altered shoot and root dry weight as well as higher grain number and yield per plant, which was also confirmed under field conditions. In the LOX1 approach, knockout experiments were extended to elite malting barley background. Doubled haploid lox1 mutant plants were subsequently used for micro malting experiments and showed differences in some malting parameters. Finally, it was demonstrated that 2-rowed barley can be readily converted to 6-rowed barley by the targeted deletion of just a single nucleotide of the VRS1 gene.

The CHIC project: New Plant Breeding Techniques; Chicory as a multipurpose crop for dietary fibre and medicinal terpenes

Dirk Bosch and Katarina Cankar

The CHIC project (Chicory Innovation Consortium), which is funded by the EU Horizon 2020 programme, consists of 17 partners from 12 different countries. The overall objective is to implement New Plant Breeding Techniques (NPBTs) in root chicory in order to establish it as a multipurpose crop and as a sustainable approach to molecular farming, i.e. the production of health-related products with clear benefits for consumers. CHIC will develop root chicory varieties that on the one hand produce more and healthier inulin food fiber and on the other hand produce sufficient amounts of medicinal terpenes. CHIC is highly interdisciplinary and focussed on interaction with stakeholders. The Consortium will evaluate the technical performance of different NPBTs, as well as the safety, environmental, regulatory, socio- economic and broader societal issues associated with them. At the same time CHIC gives great emphasis to communication about the project and about gene editing in general, also implementing innovative communication methods. For example, artists will make themselves familiar with genome editing techniques and express their feelings and views in artworks to inspire a broader public debate. By involving stakeholders and by raising public awareness at all phases of the project, CHIC strives to ensure responsible and desired innovation.

http://chicproject.eu/

CRISPR/Cas9-mutagenesis for revisiting the role of master regulators in tomato ripening

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Tomato (Solanum lycopersicum) is a model for climacteric fleshy fruit ripening studies. Ripening is regulated by multiple transcription factors (TFs) together with the plant hormone ethylene and their downstream effector genes. TF RIPENING INHIBITOR (MADS-RIN), NON-RIPENING (NAC-NOR) and COLORLESS NON-RIPENING (SPL-CNR) were reported as "master regulators" due to the severe ripening defects in their spontaneous mutants rin, nor and Cnr. The use of CRISPR/Cas9-mutagenesis makes the access to multiple, including knock- out (KO) mutant alleles much easier and more precise. However, we and others found that knockouts of the three underlying genes by CRISPR/Cas9-mutagenesis showed much milder phenotypes than their spontaneous mutants. The nor-KO fruits showed an orange pericarp with other ripening traits also only partially affected, which others also found for a rin-KO mutant. The cnr-KO fruits showed almost normal red pericarp and similar ethylene production compared to that in the wild-type. This suggests that the regulation of ripening is more robust than previously thought, which requires us to revisit our model of regulation of ripening and replace it with one involving a network of partially redundant components. At the same time, the fast rise of CRISPR/Cas-mutagenesis, resulting in unexpectedly weak phenotypes, suggests that compensatory mechanisms may obscure protein functions. This emphasises the need for assessment of these mechanisms in plants and for careful design of mutagenesis experiments.

CRISPR/Cas9 to modify bitterness in Cichorium intybus

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Cichorium intybus var. sativum (chicory) and var. foliosum (witloof) are economically important crops with a high nutritional value attributed to many specialized metabolites with beneficial effects on human health. However, Cichorium also contains sesquiterpene lactones (STL) that impart the bitter taste that limits its use for industrial purposes. Modifying genes in the STL biosynthesis pathway would enable us to alter the bitterness and open new industrial opportunities for Cichorium.

Towards this goal, we successfully implemented CRISPR/Cas9 for Cichorium. After protoplast transfection with transient expression vectors driving guide RNAs targeting the phytoene desaturase gene (CiPDS), mutant phenotypes, including albinism, were observed in 20% of the regenerated calli. DNA sequence analysis of the in vitro albino plantlets by Indel Detection by Amplicon Analysis (IDAA) and the Inference of CRISPR Edits (ICE) tool revealed that mostly frameshift mutations occurred, but also fragment insertions up to 25bp were detected.

Using our optimized CRISPR/Cas9 protocol, we aim to target the Germacrene Acid Synthase (GAS), Germacrene Acid Oxidase (GAO) and Costunolide synthase (COS) genes involved in the three dedicated steps upstream of the STL biosynthesis pathway. Based on genome sequencing data for Cichorium intybus, we constructed a phylogenetic tree for GAS, GAO, and COS, and identified the putative functional paralogs for each gene family, allowing us to design specific gRNAs. Regenerated plants obtained after protoplast transfection are now being analyzed for occurrence of indels in GAS, GAO and COS, using next-generation sequencing of amplicons.

Our successfully implemented CRISPR/Cas9 method for Cichorium is the basis for other gene editing projects at ILVO, e.g. modifying allergenicity in celery and increasing Phytophthora resistance in potato.

Elucidation of role of potato invertase inhibitor using Crispr-Cas9 Application

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Being a prominent crop, potato is world's third-most prominent crop with Turkey ranking 22nd for production area and 15th for quantitative production. Every year potato yield is challenged by pathogen infection causing an immense loss in economy. The use of cell-wall invertase (CWIN) gene has been shown to ameliorate the plant defense-mechanism in several studies in other crops. Here, we make the use of CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeat)-Cas9 (CRISPR-associated nuclease 9) technology to knock-out the potato invertase inhibitor gene (Inv-Inh), which could alter the levels of CWIN enzyme. Two single guided RNAs (sgRNAs) have been used separately in our vector construction and transformed via Agrobacterium-mediated transformation in diploid M6 and tetraploid Desiree potatoes. We have optimized the transformation protocol for recalcitrant diploid potato M6 using pBIN19 binary vector that further contains gusA gene. From the two sgRNAs, we have achieved several regenerated plants and screened them against nptII primers using PCR. The PCR positive plants are being analyzed for possible indels (insertions/deletions) using genotyping for Inv-Inh gene. The Inv-Inh gene of genotype positive plants will then be directly sequenced to determine the mutated sequence.

***Keywords**: invertase inhibitor enzyme, cell wall invertase, apoplastic invertase, CRISPR, potato*

Conferring Multiple Resistance to DNA Viruses in Plants with the CRISPR/Cas9 Genome Editing Technology

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Plant DNA viruses, also known as Geminiviruses, are destructive plant pathogens causing severe crop losses on grain, vegetable and fruit harvests, threatening food security worldwide. Geminiviruses possess circular single-stranded DNA (ssDNA), 2.3 and 3 kb in length. During the Geminivirus replication, ssDNA is converted to double–stranded DNA within the nucleus of plant cells either by rolling-circle amplification mechanism, or by recombination-mediated replication. Based on their genome-wide pairwise organization, host range and insect vectors, Geminivirisus are classified into nine genera including more than 360 species. These viruses can infect more than 300 dicotyledonous plants found in 44 different families. It is not possible to struggle with these viruses chemically. The only way to combat with these viruses is generally based on the killing of vector insects. However, this system is not enough to fight with Geminiviruses due to a number of factors; including the recombination of different Geminiviruses infecting a plant, transportation of infected plant material to new locations, migration of insect vectors that can spread the virus from one plant to another. Global warming in recent years have also been reported to cause increase in Geminiviridae virus epidemics worldwide. Therefore the most effective way is to devolop resistant agricultural plants against these viruses. Newly discovered genome editing technique, Crispr/Cas9, has been recently reported to be used for complete virus resistant in plants. This system depends on targeting of virus DNA with a guide-RNA (gRNA) and cutting of viral DNA with Cas9 enzyme. It has an important potential for development of multiple resistance against all Geminiviruses. In the current study, we investigated utilization of CRISPR/Cas9 system for multiple resistances in sugar beet against two curly top viruses found in two different genera of Geminiviridae. Beet curly top viruses (BCTV) and beet curly top Iranian viruses (BCTIV) are different species found in the family of curtovirus and be-curtovirus, respectively. Both have several different strains creating yield limiting disease in sugar beet with highly similar symptoms. We designed 20 gRNAs for common sequences of replication, capsid and movement protein of BCTV and BCTIVs. We transiently overexpressed the gRNA/cas-9 endonuclease system in sugar beet plants and subsequently challenge these plants with BCTV and BCTIV. Our first data demonstrated that all tested gRNA/cas-9 constructs exhibited interference activity with both viruses at the same time but those targeting the replication and capsid protein was the most effective ones. Sugar beet plants overexpressing the gRNA/cas-9 constructs exhibited delayed and reduced accumulation of both viral DNA. These results demonstrate the utilization of genome editing technologies for viral resistance in plants, thereby extending the efficacy of these technologies for producing plants resistant to multiple viral infections.

Keywords; *genome editing ,CRSPR/Cas-9,disease resistance, sugar beet, geminiviridae, beet curly top virus*

Parallel session 2 – Policies and regulations

Politicizing the Precautionary Principle: Why Disregarding Facts Should Not Pass for Farsightedness

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The EU Communication on the Precautionary Principle (PP), published in 2000, states that the PP should adhere to the general principles of risk management, which include (a) the principle of proportionality between the measures taken and the chosen level of protection;

(b) the principle of nondiscrimination in the application of the measures; (c) consistency of the measures with similar measures already taken in similar situations; (d) the examination of the benefits and costs of action or lack of action; and (e) review of the measures in the light of scientific developments. This interpretation of the PP is scientifically sound and has a long track record in national and international environmental policy. However, when applied to the EU regulation on genetic engineering, and by extension, on gene-editing, these principles of risk management have all been infringed due to the impracticalities of process-based regulation. The paper concludes that a shift from process-based to product-based regulation would resolve the current inconsistency of the European regulatory system on agricultural biotechnology and ensure that the EU and its member will contribute to the global need for sustainable intensification in agriculture, a necessity to achieve the UN Sustainable Development Goals.

Reference: Aerni, P. (2019). Politicizing the Precautionary Principle: why disregarding facts should not pass for farsightedness. Frontiers in Plant Science, 10, 1053.

Development of risk assessment of genome-edited organisms

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Gene or genome editing represents a set of techniques that allow making precise changes in genomes of many species from bacteria to plants and animals. In contrast to early genetic modifications, genome editing does not necessarily result in insertion of foreign DNA in genomes which suggested that this technique may fall outside the GMO legislation. Nevertheless, the recent European Court of Justice ruling indicated that genome-edited organisms fall under the GMO Directive in the European Union. As a consequence of this decision, European Food Safety Authority (EFSA) has been mandated to develop scientific opinion on so called site-directed nucleases 1 and 2 (SDN-1 and -2), as well as on oligonucleotide-directed mutagenesis (ODM) to advice, if existing EFSA methodology is suitable for risk assessment of genome-edited plants under the EU GMO legislation. While scientific rationale for regulating genome-edited organisms as GMOs is questionable, and the risk management related to detection and quantification of genome-edited organisms may prove impractical, the risk assessment of genome-edited organisms appears to be relatively straightforward. SDNs are a collective name for different nucleases capable of producing double stranded DNA breaks in genomes, which then are repaired using endogenous non- homologous end joining repair system (SDN-1) or can facilitate homologous recombination with exogenous DNA (SDN-2). ODM uses oligonucleotides homologous to certain genome regions to introduce nucleotide substitutions. These techniques result in targeted nucleotide substitutions or short deletions. While development of CRISPR/Cas technology has received most of the attention, there are other older SDNs that can be used in genome editing and have resulted in some commercialized products, e.g., Calyxt high oleic acid soybean. Here, I will review suitability of the existing requirements as applied to risk assessment of genome-edited plants. Specific considerations, such as presence or absence of SDN constructs in the genome, type of intended changes and potential off-target effects of genome editing will be discussed. Some genome-edited plants containing only characterized changes in DNA resulting in known phenotypes and no foreign DNA may require substantially less information for risk assessment compared to SDN-3 and conventional GM plants.

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You want it extra CRISPRY? Regulating New Plant Breeding Technologies in the EU

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With the event of new gene editing tools, conventionally termed "new plant breeding technologies" (NPBT), the world faces a major revolution in the way our food is produced. Like technologies such as blockchain, artificial intelligence and robotics, NPBT are part of the fourth industrial revolution, triggering questions about how the EU responds to these as an industrial 'state'. Unlike blockchain, artificial intelligence and robotics, NPBT share essential features with traditional GM techniques and hence face EU regulation from a time when the EU's regulatory state was well pronounced, in particular through a strong application of the precautionary principle. NPBT are hence technologies from the 4th industrial revolution where the clash between the EU as a strong regulatory state and the requirements to enable innovative technologies from the 4th industrial revolution may be best pronounced, making it a superb area to study the EU's dilemma to regulate representing the regulatory and the industrial state.
I will assess how the EU copes with this dilemma. Based on this analyses, I will investigate whether the way the EU balances between these two 'States' is a good way of regulation. The paper will finally develop regulatory options for reform of the regulation of NPBT, which are, in my view, better able to cope with the balancing act between the two "states". In particular, the presentation will proceed in the following way: First, I will analyse how the EU currently regulates products which use NPBT. Second, I will extrapolate the principles for regulating blockchain technology developed by Michéle Finck to NPBT and test against these principles if the current regulatory framework is fit for purpose. Forth, I will make suggestions for a better regulation of NPBT, largely adopting an approach that has been dubbed "incomplete centralisation".

Key words: CRISPR, GMO, regulation, EU Law

The Judgment of the CJEU of 25 July 2018 on mutagenesis: interpretation and legislative proposal

Juan Antonio Vives-Vallés, Cécile Collonnier

The Judgment of 25 July 2018, Confédération paysanne and Others, C-528/16, EU:C:2018:583 of the Court of Justice of the European Union (CJEU) was optimistically awaited by breeders and supporters of agricultural biotechnology, but short after the press release advancing the Judgment, hope turned into frustration. Opinions on how to frame the New Breeding Techniques (NBT) in the context of Directive 2001/18/EC were issued before the Judgment, while proposals to assist the EU legislator to amend the regime driven by the Directive have been also provided afterwards by scholars and institutional bodies around the EU. However, not so much attention seems to have been paid to the Judgment itself. Through legal analysis, this study tries to determine the very meaning of the Judgment. Legal interpretation is also combined with scientific/technical assessment to analyze the impact of the Judgment on the legal status of the existent breeding techniques. It finds out that while some impacts of the Judgment on the NBT might have been slightly overvalued, others have been generally underestimated if not absolutely overlooked. The study also provides some keys to mitigate some of the negative effects of the Judgment on plant breeding and agriculture.

How Serbia is regulating GMOs and how it will regulate new breeding techniques

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Regulatory aspects of genome editing, "new breeding techniques", "new mutagenesis", as well as many current developments in extremely broad and vaguely defined field of "synthetic biology" have come to attention of scientific community and can have significant influence on further scientific development and biotechnology applications in any country. In Serbia, that influence will depend on national legislation that itself should be based on country international obligations, but is also can be significantly affected by public perceptions. Serbia is Party to Convention on Biological Diversity (CBD), Cartagena Protocol on Biosafety to the CBD, Codex Alimentarius of FAO UN and WHO, but also EU candidate country. So, for national legislative regulating new breeding techniques and their uses, it is important whether it will fall under Cartagena Protocol or it will be covered internationally by much broader definition of biotechnology in the CBD. Furthermore, it will depend of guidance documents of Codex, but also on the latest developments in EU, such as decision of European Court of Justice from 2018. As for public perception of GMOs in Serbia, it remains largely negative. Predictability of further regulatory developments in Serbia is additionally complicated in light of recent history of GMO legislation in our country. Serbia (as part of Federal Republic of Yugoslavia that consisted of Serbia and Montenegro) got its first Law on Genetically Modified Organisms (GMOs) in 2001. That Law provided possibilities for issuing all types of permits (field trials, deliberate release and placing on the market) under very strict procedures. From 2001 to 2009, permissions for several field trials were granted, from "traditional" field trials with NK603 corn to interesting attempts to detect landmines by genetically modified Arabidopsis thaliana and tobacco plants. Permit for placing on the market of Roundup Ready soybean meal as a feed was also issued for the period of 10 years. The Law on GMOs was amended in 2009 with the aim to harmonize Serbian legislation with the latest EU regulations (most notably 1829 and 1830 from 2003), as well as implement some provisions of Cartagena Protocol which entered into force in Serbia. Drafted law, as well as proposed Law that was sent to Parliament by the government, were on those lines. However, law proposal was amended in the Parliament and commercial growing and use of GMOs was banned. That amendments made new Law simultaneously restrictive and unsafe. The ban on commercial use and growth of GMOs is seen as an obstacle in harmonization of national legislation with EU legislative, as well as an obstacle to Serbia's accession to WTO. The Law also does not include requirements of unintentionality, technical unavoidableness and existence of positive risk assessment regarding the presence of modification event in concentrations under 0.9% in products and under 0.1% in seed material, thus making it potentially unsafe. No applications for field trials were received since the new Law was adopted. Necessary amendments of existing EU legislation on GMOs in the near future could be coupled with first legal deliberations regarding new breeding techniques. And Serbia's experience shows that while scientific investigation at the levels of contained use and field trials is still allowed, not having perspective of further development and commercialization of new products can affect the scientific research on new breeding techniques, as well as their application for development of new products and varieties.

Key words: *new breeding techniques, Serbia, regulations, public perception*

Biosafety Law and Its Implications on Biotech Research Policies in Turkey

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Turkey's agricultural economy is among the top ten in the world thanks to the size and diversity of its arable land and favourable agroecological conditions. However, biotic and abiotic stresses coupled with climate change cause significant challenges for the farmers. Turkey also has a modern and developed food processing industry which represents 16 percent of all manufacturing in the country. Agricultural research and classical plant breeding employed so far have been short in meeting the raw material demand by the agri-food industry. Therefore, Turkey has to import substantial amounts of grains and oil seeds for food, feed and processing purposes to meet the needs of its processed food industry which is a net exporter.

Priorities for utilizing modern biotechnologies including plant genetic engineering have been determined to address and solve important crop production problems almost 25 years ago. Newer versions of the research policy documents also set agricultural biotechnology as a priority, and government has allocated millions of dollars in infrastructure development. However, antitechnology sentiments supported by the European NGOs resulted in an extremely severe Biosafety Law that prohibits any GMO cultivation and practically renders field trials impossible. The agri-food industry also has to face an estimated burden of one billion USD per year due to the implementation of the said Law.

Therefore, many stakeholders including scientists are in need of immediate revision of the Biosafety Law in order to benefit from modern biotechnologies including plant genome editing.

Prospects of plant genome editing in Armenia

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The Republic of Armenia is a small, mountainous country located at the junction of the biogeographic zones of the Lesser Caucasus and the Iranian and Mediterranean zones. The geographical location and topography of the country have resulted in rich plant biodiversity with high level of endemism. Armenia is considered as one of the five centers of diversity and origin of the world's major crops described by N.I. Vavilov, the creator of the world's largest collections of plant germplasm.

On a territory of 29,740 km^2 Armenian flora comprises about 3 600 species of vascular plants, which makes about half of entire Caucasian flora. Being one of the centers of cultivated plants origin, Armenia is famous for the indigenous diversity of numerous species of cereals, vegetables, oilbearing plants and fruit crops. Plant genetic resources for food and agriculture are represented in the country by different plant species of economic value, crop wild relatives, old local varieties and wild edible plants. Existed plant genetic diversity is an important source of variation in plant breeding, highly contribute to economic growth and food security and is part of our national heritage and sovereignty, having a great role in maintaining environmental balance in Armenia too.

Lack of awareness increased human impact, increased pests and diseases virulence and global climate changes are the main factors seriously threaten plant diversity in country. The effective conservation of Armenian plant biodiversity and its sustainable use are a priority for Armenia as it intends to reinforce economic power through sustainable agricultural development.

Hence, it has critical importance for Armenia to strengthen its capacity to pursue biosafety policies based on well-balanced decisions on the introduction of innovations like plant genome editing. Genome editing is a promising tool with great potential and key role in speeding up crop breeding and in meeting the ever-increasing global demand for food all over the world. The urgency of climate change call for great flexibility and innovation in crop resilience and production systems. There is a strong need also to take into account government regulations and consumer acceptance around the use of these new breeding technologies. The country should be responsible for providing a safe and healthy environment and conserving its genetic diversity for present and future generations. Poorly characterized but rich genetic diversity of Armenian plant resources, formed by millenniums, represents a unique source of genetic variation for the development of sustainable agriculture.

Proposed revision of the Norwegian Gene Technology Act as a result of the challenges from new breeding technologies

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Norway has a long standing international reputation as a country of a firm stand on GMO, with a Gene Technology Act with a purpose is "to ensure that the production and use of genetically modified organisms and the production of cloned animals take place in an ethically justifiable and socially acceptable manner, in accordance with the principle of sustainable development and without adverse effects on health and the environment". With CRISPR and other gene editing tools entering the toolbox, the Norwegian Biotechnology Advisory Board has taken it on to advice the authorities on how a revision of the law could be done; without adverse effects on health and the environment. The propositions were handed over to the Minister for Climate and the Environment last December and is presently being handled by the Competent Authorities (Directorate for the Environment).

The presentation will use our recently co-funded GENEinnovate project to illustrate our aims of using CRISPR as a tool in potato breeding and how the proposed changes in the Norwegian Gene Technology Act may affect how the products from our project may be handled by the Competent Authorities in Norway, if we succeed in providing Norwegian potato cultivars with improved resistance towards late blight (Phytophthora infestans).

In short, the proposition is to divide GMOs into three risk categories, with Class 1 being where no foreign DNA is added, Class 2 where cis-genesis is the method used and Class 3 where transgenes are the result. The proposition from the Norwegian Biotechnology Advisory Board may serve as an inspiration for the countries in the EU when they start drafting revised directives on the production and use of GMOs.

Parallel session 3 – Impact

Zinc Finger Nucleases as Genome Editing Tools for Specific and Efficient Plant Breeding

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Recent developments in genome editing have paved the way for a non-transgenic and high-throughput approach in which targeted mutagenesis in plants occurs efficiently. This approach involves site-specific DNA double-stranded breaks (DSBs) by zinc finger nucleases (ZFNs) transiently expressed in seeds and subsequent activation of Non-Homologous End Joining (NHEJ) mechanism, which creates local genetic modifications. A novel ZFN design with DNA binding sites spaced by an intron edited the tomato genome in an efficient and specific manner offering thus a versatile tool with excellent prospects for applications in plant breeding. The creation of new seed varieties using the efficient ZFN-based technology addresses the challenges posed by climate change and creates products with variable fruit quality. The transition to a sustainable, bio-based society is increasingly dependent on agricultural output and requires tailored products that meet food production and consumer nutritional needs.

Novel disease resistance approaches – are they durable ?

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GE techniques for disease resistance can be used to develop or enhance molecular immunity and resistance against different plant pathogens. Gene sequencing techniques have advanced to the stage where both plant and pathogen genes and transcriptomes have been mapped so that targeting of SDNs can be developed. Also improved understanding of the complex interactions between plants and pathogens and related cross talk at RNA level are better understood.
The genome editing (GE) techniques for disease resistance, include SDNs such as:

- Clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) systems,
- Transcription activator-like effector nucleases (TALENs),
- Zinc-finger nucleases (ZFNs)
- Meganucleases (LAGLIDADG homing endonucleases)

These techniques have been used for engineering disease resistance by targeted mutagenesis of crop plants, including gene knockouts, knockdowns, modifications, suppression and activation of target genes. CRISPR/Cas9 leads other GE techniques including TALENs and ZFNs for editing genes due to its high efficiency, relative simplicity and low risk of off-target effects. Disease resistance can be engineered in crops by GE of either specific host-susceptibility genes (S genes), or cleaving DNA of pathogens to inhibit their development.
This presentation will discuss the range of techniques for developing disease resistance and the efficacy and merits of these techniques. Multiplexing and other management strategies which can improve their efficacy and durability will be discussed. Finally, the likely impact of these techniques on crop development and sustainable agriculture will be considered.

Bio

Jeremy Sweet has been conducting research on Crop improvement and plant diseases. Much of this work was conducted at NIAB Cambridge studying sustainable crop production, integrated disease management, environmental and agronomic impacts of GM crops, and gene flow to crops and wild relatives. He was coordinator of UK, ESF and EU projects studying agronomic and environmental Impacts of GMOs. He was an advisory Board member of several EU and National programmes on GMOs. He was a member of the EFSA GMO Panel and Environmental Working Group for 15 years, providing scientific opinions on the risks associated with GMO applications in the EU and developing guidance documents on risk assessment. He participated in the ALGEBRA project on GM algae and in an EFSA study of RNAi GM plants. He is an author in over 50 scientific papers on GMOs, numerous plant pathology papers and of 2 books.
He is director of JT Environmental Consultants Ltd which provides research, advice and technology transfer on GMOs and Plant Health to agencies and governments around the world. He is vice–chair of the iPLANTA COST action studying RNAi applications in plant production and protection.

The Economics of Regulating New Plant Breeding Technologies - Implications for the Bioeconomy illustrated by a Survey Among Dutch Plant Breeders

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New plant breeding technologies (NPBTs) are increasingly used for developing plants with novel traits. Scientific research suggests that those plants in general are safer than the ones developed using "conventional" plant breeding methods. The NPBT technologies are much more precise than traditional plant breeding technologies and generate more accurate knowledge about the induced changes and properties of the plants. This implies that plants developed using NPBTs should not be face harsher regulations than those developed using "conventional" plant breeding methods.

This contribution discusses the economics of regulating new plant breeding technologies. We first present alternative regulatory approaches being used and conceptually compare their advantages and disadvantages. It provides us with a perspective on EU regulation of mutagenesis-based New Plant Breeding Techniques (NPBT). Further insights on the regulation are obtained from the results of survey measuring the attitude of Dutch breeding companies towards the ruling of the EU Court of Justice that subjected the use of CRISPR-Cas 9 in the development of new plant varieties under the regulations of GMOs.

The results of the survey show that the ruling expand the financial constraints facing plant breeders, resulting in a perceived negative impact on competitiveness and much lower investments in CRISPR-Cas9.

Cereal Genetic Engineering Studies

Gul Ebru Orhun

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Cereal are major meal source for both human and animals due to it is very nutritious.

Crop cultivation and yield should also be increased in order to feed the growing population all over the world. Today the world population is increasing at the most rapid rate ever. It is forecast that by the year 2050, the world's population will double to nearly 12 billion people. In fact, it has been estimated that the world will need to produce more than twice as much food during the next 45 years as was produced since the beginning of agriculture 10000 years ago

Nowadays, genetic engineering has gained importance. So, plant genetic engineering is becoming important increasingly. Because genetic engineering methods reduce both the time and cost for improve of desired feature. Genetic engineering provides a great development for cereal breeding programs, allowing the production of novel and genetically diverse plant materials. This review study examines some advancement for cereal genetic transformation.

Keywords: Cereal, genetic transformation, wheat, genetic engineering

Parallel session 4 – Public and stakeholder perceptions

Why public acceptance is important?

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Public acceptance of new biotechnologies in agriculture is urgently needed for the development of bioeconomy and prevention further restrictions on the production of genetically modified (GM) crops (Tyczewska et al., 2018). Scientific and technological innovations dominate our daily lives. Most European consumers have had little opportunity of experiencing the products of genetic engineering applied to crop breeding. Nowadays a need is being stressed for wider use of agriculture not only for food production and to ensure food security but also for production of biomass, as a renewable source of raw materials for the industry of new types of goods and services, including bioenergy and biopharmaceuticals.

Published opinion polls offer a variable picture of European consumer attitudes. We have to stress the public attitudes have high market value. Some show that many consumers are against GMOs. Other evidence suggests little real interest: when offered products labelled "GM" at a favourable price, consumers tend to buy. Do opinion polls actually provide reliable indications of consumer behaviour when presented with real rather than theoretical choices? How significant is legislation and what is the role of politicians? Do we have the food security question in front of us? We should answer all these questions keeping in mind population of the globe in 2050 and climate changes.

Attitudes to GM food (and its labelling) are linked to moral, existential and epistemological issues about trust and people's sense of agency. Lay scepticism to GM food may be influenced by a lack of trust in the institutions and actors responsible for the new technology. In addition, GM food is sometimes perceived as "unnatural", challenging traditional perceptions of nature and of humanity's place in nature, which may bring about moral objections. To increase the societal acceptance of genetic modification in agriculture, we must ensure that research in biotechnology and bioscience is presented in an understandable and clear manner (Malyska et al., 2018).

Food safety as well as food security issues are important for European consumers. According to report "Food safety in the EU" (2019) around two in five Europeans say that they are personally interested in the topic of food security. Europeans have a high level of awareness of food safety topics. Around 60% of Europeans have heard about genetically modified ingredients in food drinks, whereas 21% have heard about genome editing.

In this presentation the data concerning public acceptance and perception of agribiotechnology innovation techniques in Poland [as the example of European Union countries] will be shown. The GM foods debate is global, impacting all societies and remains largely unresolved till today.

Key words: agribiotechnology, bioeconomy, biotechnology, GMO, public perception

GMOS AND GENOME EDITING: PUBLIC PERCEPTION AND ATTITUDE IN ALBANIA

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In this study we explore the perception and attitude on the use of GMOs and genome editing and their derived products consumption inAlbanian society. The survey included different demographic characteristics as age, education level and the profession in order to evaluate the factors and their effects in given opinions. A total of 300 respondents were interviewed, the age varied from 17 to 68 years with a mean of 32.7. Our results show that 71.7 % has average knowledge about GMOs and interestingly they show great interest to get more information. About 58.6 % of responders were concerned and believed that the use of GM food pose to human health risk, while 46.2% believed that the GMOs are potentially harmful for the environment. Nevertheless, the responders showed higher positive attitude (42%) on the improvement of plants disease resistance and adaptability by applying genome editing techniques, but the majority of them believed that genome editing should be used only under a strict control. Interestingly, more than the half of responders (51%) showed positive opinion and advocated the application of genome editing in medicinal production. The responders coming from big cities had more positive attitude towards the use of GMOs compared to those of rural areas.

Our findings give the first insight on GMOs and genome editing perception and attitude, they suggest the necessity of development of activities and education programs to increase the public awareness on GMOs and highlight benefits and their advantages in the future.

***Keywords**: GMO, Genome Editing, Perception, Attitude, Albania*

Purchasing GMO products: Psychological factors that affect consumers' decision making

Nebojša Majstorović

This work is about controversies in consumers' behavior when buying GMO products. Previous studies indicate that most Europeans are skeptical about GMOs (food) (e.g., France, Germany), but that the public in some European countries is more accepting (e.g., Spain, Portugal). According to Lucht, (2015), 67% of the general public is convinced that scientists are not fully aware of the consequences of GMO food and its hazards to human health, even though over 70% of American scientists from the branch believe GMO food is safe for humans. The hypothetical model offered here is the Theory of planned behavior (Ajzen, 1991) which suggests that an individual will engage in concrete behavior (e.g., purchasing) after establishing an intention to do something, and after he/she forms the belief that the consequences of such behavior are controllable. This theory was tested in organic food consumption with the assumption that the opposite findings hold for GMO food consumption as well (Aertsens et al., 2009). Additionally, to fully understand purchasing behavior it is also proposed to analyze consumers' compensatory/non-compensatory purchasing strategies and product attribute evaluation processes, as they are described in Kotler & Keller (2006). According to their Expectancy-value model, price is one of these attributes; it can be significant but when compared with food benefits, safety for children and other attributes, it might not be a key factor for some consumers in making a purchasing decision in favor of GMO products. Differences in risk and a product's attribute assessment, but also differences in attitudes, knowledge, certain irrational beliefs, emotions or societal and political developments might explain international diversities in purchasing GMO products. In order to create a change in consumers' behavior via advertising tools, it is suggested to scan the public condition, not only for attitudes and opinions but also for other decision-relevant psychological factors, then to create homogenous consumers strata and to define advertising tactics in targeting these groups.

***Key words**: GMO products, purchasing behavior, theory of planned behavior, product attributes.*

Plenum session 3 - Genome editing in commercial breeding

High-throughput genome editing in tomato to create variation in gene expression

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Creating genetic variation in a promoter of a gene can modulate its activity. This can be used for the optimization of traits, such as the colour of tomatoes. Improving colour is attractive to customers, but also involves changes in the metabolic profile, which provides the opportunity for the selection of healthier or tastier tomatoes. In a collaborative project between Wageningen University & Research and KeyGene, we are developing a high-throughput method for the deletion of promoter fragments by genome editing. Following these deletions, we assess the edited plants for changes in gene expression, phenotype and metabolites. Although most guide RNAs bound to CAS9 can cleave DNA in vitro, their performance in living protoplasts is highly variable and hard to predict. Therefore, to make deletions efficiently, guide RNAs need to be selected based on their in vivo activity. A high-throughput 96-well transfection assay has been developed, to quickly and effectively test guide RNA activity. Edited protoplasts have been regenerated into adult plants. Gene expression in the next generation will be measured to link the specific deletions detected to changes in the activity of the edited promoter.

A worldwide CRISPR patent landscape

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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 and by a deliberate political strategy. 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 appears unfortunate for the future of European agriculture, especially since our previous work has shown there are considerable gaps between farmer's needs and current breeding and biotechnology research.

Plenum session 4 - Initiatives on plant genome editing

The European Citizens' Initiative "Grow Scientific Progress: Crops Matter!"

Lavinia Scudiero

One year after the European Court of Justice decision on new plant breeding techniques (NBPTs), a group of students pursuing degrees in Life Sciences, dissatisfied with the outcome, have submitted a legislative proposal asking for an update of Directive 2001/18/EC. The proposal was submitted in the form of the European Citizens' Initiative (ECI), a unique instrument of democracy that allows European citizens to have a voice over European political issues. The objective is to urge the European Commission to act and amend the legal framework governing the deliberate release into the environment of genetically modified organisms (GMOs). The ECI, under the name "Grow Scientific Progress: Crops Matter!" (GSP), acknowledges that the Directive, as present, is not suitable for genome editing advancements and calls for its revision in order to enable the European Union to be more progressive and a more sustainable leading force. In particular, the initiative advocates for a clear distinction between organisms obtained through new mutagenesis techniques and conventional GMOs. It further wants to facilitate the authorization procedure for organisms obtained through NPBTs that carry only natural existing traits and are indistinguishable from crops obtained through traditional breeding. Ultimately, the ECI aims at generating democratic debate around NPBTs and representing citizens who support responsible scientific progress. GSP is currently campaigning throughout the European countries to reach citizens and get their support by gathering no less than one million signatures.

GeneSprout Initiative: A Young Researchers Initiative for Open Dialogue on New Plant Breeding technologies

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As young plant scientists, we were quite disheartened and concerned when the European Court of Justice (ECJ) ruled that any organism obtained through New Plant Breeding Techniques would be classified and subjected to laws as for a genetically modified organism (GMO). We believe that one of the underlying reasons behind the ECJ ruling is the wide knowledge and communication gap between policy, technology and the public perception of NPBTs. A large part of the public is either misinformed or emotionally polarized regarding NPBTs. As the future generation of agricultural plant scientists, breeders and biotechnologists, we feel responsible to bridge this gap and have a voice in the policy-making processes on NPBTs. Furthermore, we feel that we should contribute to a more open and communicative environment to allow for important collaboration of science and society. In this talk we will explain our goals and the approaches we take to achieve them.

European CRISPR campaign

European research institutes jointly call for action: Give CRISPR a Chance!

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Innovative techniques such as genome editing represent a revolution in crop breeding. Many researchers across Europe feel that CRISPR can contribute to the development of solutions for the challenges we are facing today and will face in near future.

To our surprise, the European Court of Justice ruled on July 25, 2017 that organisms obtained through modern tools of mutagenesis such as CRISPR are not exempt from the current GMO legislation. From a scientific point of view, the ruling makes no sense. As citizens and scientists, we are concerned that this will restrict the EU from using this innovation to meet challenges such as climate change, biodiversity loss and degradation of arable land.

"I want Europe to become the first climate-neutral continent in the world by 2050." shared Ursula von der Leyen, president of the European Commission in her opening statement. Sadly, no political guidelines are mentioned to facilitate the potential of innovative crop breeding in her proposed Green Deal for Europe. We have to make policy makers aware of its potential!

More than 129 European plant and life sciences research centers and institutes have endorsed a position paper that urgently calls upon European policy makers. It clearly illustrates that plant scientists all over Europe are united and concerned.

I will highlight on behalf of Dirk Inzé, Science Director at VIB-UGent (Belgium) and one of the initiators of the position paper what the next steps are that we have to undertake to minimize the irresponsible consequences of an outdated regulatory framework in the face of the world's current far-reaching agricultural challenges.

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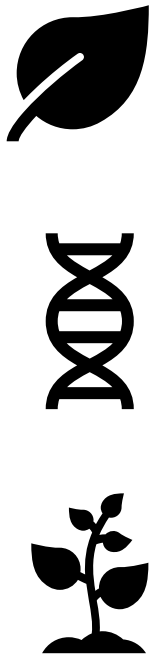

Exploitation of genetic resources in breeding of Brassica species

Ana Marjanović Jeromela, Dragana Miladinović, Igor Balalić, Sandra Cvejić, Nada Grahovac, Milan Jocković, Biljana Kiprovski, Jelena Ovuka, Aleksandra Radanović, Dragana Rajković, Sreten Terzić, Ankica Kondić Špika

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The Brassicaceae family (Cruciferae Juss.) is one of the 10 most economically important plant families. The most prominent members are *Brassica napus* L., with globally widespread cultivars with low-erucic acid content, known as canola, and several types of mustard (*Brassica* spp. and *Sinapis* spp.). IFVCNS is the only research institute in Serbia dealing with the cultivation of oil crops from the Brassicaceae family. The breeding of *Brassica* species from the collection resulted in the introduction and spread of black and white mustards and false flax (*Camelina sativa* (L.) Crantz) cultivation areas. These species are rarely grown and used in Serbia. Rapeseed collection consists of 11 cultivars and 20 lines of spring rapeseed, as well as 56 cultivars and 980 lines of winter rapeseed. All IFVCNS genotypes are phenotypically and cytogenetically characterized, with specific regard to their phenology, morphology of the flowers, pollen characteristics and the number of chromosomes, resistance / sensitivity to diseases and pests, as well as seed quality (oil, protein content, fatty acid and composition tocopherol). Significant genetic variations were found. Because commercial rapeseed breeders are directly engaged in the assessment and selection of the material, the collection is a valuable resource for a more detailed characterization of other traits important for breeding. The constant and systematic use of this collection with the application of conventional breeding methods, combined with various modern molecular techniques, is an effective tool in the development of IFVCNS cultivars and hybrids adapted to changing environmental conditions and market requirements. The novel gene editing instruments can enhance exploitation of genetic resources in the development of productive genotypes as renewable raw materials to produce food and non-food oils, particularly in low-input systems and marginal soils suitable for conventional and organic farming systems.

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Genetic resources and new breeding tools for sunflower improvement

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Sunflower is one of the major oil crops of today, however due to its wide distribution, as well as its drought tolerance it may gain even more on its significance and become the main oil crop of the future, especially in the light of global environmental changes. This imposes a great responsibility on sunflower breeders to create more productive sunflower genotypes for future environmental changes. Exploitation of the available genetic resources in combination with the use of modern molecular and breeding tools could lead to considerable improvements in sunflower, especially with regard to different stresses and better adaptation to the climate change. Utilization of sunflower wild relatives for sunflower improvement is a long-term process which requires a lot of resources and work - from collecting of wild relatives, their maintenance, their testing and mining for desirable genes to the valorization in breeding programs. Genome-wide prediction, also known as genomic selection (GS), is one of the tools that could accelerate this process, through efficient and targeted improvement of populations and identification of parents for rapid genetic gains and improved stress resistant varieties. The application of tissue culture techniques and genetic engineering for improving the existing and introducing new traits from wild relatives into cultivated sunflower did not have much success, mainly due to the difficulty of regenerating plants in a reproducible and efficient way. Development of new breeding techniques, such as genome editing, could provide new perspectives for more efficient sunflower breeding. Generally, traits related to stress resistance are complex phenotypic traits controlled by polygenes, and it is usually necessary to study more than a single gene or single class of genes to understand molecular mechanisms underlying respective tolerance. Genome editing could be very useful to evaluate and validate the strength of the predictive value of a given candidate gene by easily transferring its best alleles into a set of different genetic backgrounds representative of the diversity of the genetic material used in the selection schemes. Institute of Field and Vegetable Crops, Novi Sad, Serbia, handles the largest World collection of sunflower genetic resources, consisting of over 7,000 sunflower inbred lines developed from different genetic sources and 21 perennial and 7 annual species (447 accessions in total). The new breeding tools will be used for further exploitation of this collection for improvement of cultivated sunflower and creation of resilient varieties for the areas and traits where classical breeding reached its limits.

***Key* words**: *Helianthu annuus* L., gene pool exploitation, resilience, breeding

Identification of virus-derived and virus-activated host siRNAs in *Tomato yellow leaf curl virus*-tomato interaction: A screening method for host gene editing leading to resistance

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The current virus control methods are limited in number, efficacy and environmental suitability with current EU decisions restricting crop improvement strategies employing transgenic plants. To protect crops against existing and emerging virus diseases new strategies are urgently needed for global food security. Plant viruses, as subcellular pathogens, exploit the host's protein machinery to complete their life cycles. During a compatible plant-virus interaction the host cellular components are compromised by virus resulting in viral infection establishment; these components are classified as susceptibility factors. Functional intervention to the susceptibility factors could lead to the desired trait of plant resistance.

Plant virus infection induces the production of host-derived small interfering (si) RNAs, designated as virus-activated small interfering RNAs (vasiRNAs), in addition to the virus- derived siRNAs (vsiRNAs). In the present study we identified >27,000 vasiRNAs and ~2,500 vsiRNAs responsive to *Tomato yellow leaf curl virus* (TYLCV)-infection in tomato using next generation sequencing and subsequent bioinformatics analyses. *In silico* prediction led to the identification of vasiRNAs and vsiRNAs (21 nt-long) as well as their putative target tomato genes in tomato transcriptome ITAG4.0 release. Gene Ontology analysis revealed that the group of genes targeted by vasiRNAs and vsiRNAs, belonging to the biological and molecular categories, were found to be quite distinct ($p \leq 0.01$). *Dicer-like 2b* (*DCL-2b*) and *Storekeeper* (*STK*) were computationally predicted to be targeted by a high number of vasiRNAs. *Cell division control protein 2 homolog* (*CDC2*) and *Phosphatase type 2C* (*PP2C*) were computationally predicted to be targeted by vsiRNAs with corresponding reads ≥50. Interestingly, the comparative study performed identified 2,774 tomato genes targeted by both vasiRNAs and vsiRNAs (i.e. *Ubiquitin-like protease 1* [*ULP1*] and *Mini-chromosome- maintenance 3* [*MCM3*]-*associated protein*). The abundance of selected vasiRNAs and vsiRNAs was negatively correlated to the mRNA amounts/expression of the corresponding target gene. Such an approach could rapidly aid in the identification of genes involved in plant- virus interaction. The functional annotation of the identified vsiRNA- and vasiRNA-targeted genes as susceptibility factors would pave the way for their gene editing that could lead to the development of TYLCV resistance in tomato.

Keywords: *RNA silencing; RNAi; small interfering RNAs; siRNAs; TYLCV; next generation sequencing; host susceptibility genes; gene editing*

PLANT X-TENDER: a toolbox to assist plant biotechnology

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Cloning multiple DNA fragments for delivery of several genes of interest into the plant genome is one of the main technological challenges in plant synthetic biology. Despite several modular assembly methods developed in recent years, the plant biotechnology community has not widely adopted them yet, probably due to the lack of appropriate vectors and software tools. Here I will present Plant X-tender, an extension of the highly efficient, scar-free and sequence- independent multigene assembly strategy AssemblX, based on overlap-depended cloning methods and rare-cutting restriction enzymes. Plant X-tender consists of a set of plant expression vectors and the protocols for most efficient cloning into the novel vector set needed for plant expression and thus introduces advantages of AssemblX into plant synthetic biology. The novel vector set covers different backbones and selection markers to allow full design flexibility. We have included ccdB counterselection, thereby allowing the transfer of multigene constructs into the novel vector set in a straightforward and highly efficient way. Vectors are available as empty backbones and are fully flexible regarding the orientation of expression cassettes and addition of linkers between them, if required. We optimised the assembly and subcloning protocol by testing different scar-less assembly approaches: the noncommercial SLiCE and TAR methods and the commercial Gibson assembly and NEBuilder HiFi DNA assembly kits. Plant X-tender was applicable even in combination with low efficient homemade chemically competent or electrocompetent *Escherichia coli*. We have further validated the developed procedure for plant protein expression by cloning two cassettes into the newly developed vectors and subsequently transferred them to *Nicotiana benthamiana* in a transient expression setup. Thereby we show that multigene constructs can be delivered into plant cells in a streamlined and highly efficient way. Our results will support faster introduction of synthetic biology into plant science.

Alteration of Auxin Homeostasis during Gametogenesis using Genome Editing in Tomato

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Auxins are known to be the key players in the majority of processes in plants, including generative organs development. Nevertheless, the role of their overall metabolism, especially inactivation and storage, during plant reproductive process still needs to be elucidated. We applied the combination of hormonal metabolome analysis and transcriptome profiling to reveal the components of auxin hormonal system, which can regulate tomato reproductive organ development. The metabolome and transcriptome data point on several genes and gene families which might have an important role in controlling auxin homeostasis during gametogenesis. Including members of the Grethen-Hagen 3 (*SlGH3*) gene family, IAA- methyltransferase (*SlIAMT*) and several auxin transporter genes. Functional characterization of these genes performed using CRISPR-Cas9-mediated mutagenesis in tomato. *Slgh3-15* knock-out lines exhibited a dramatic decrease in pollen viability and germination coupled with the increased IAA in maturating stamen, leading to the development of predominantly parthenocarpic fruits. The similar aberrant phenotype also observed in *iamt1* mutants. These results demonstrate that CRISPR-Cas9-mediated genome editing can be used in tomato to manipulate important regulation process. We currently continue to produce more knock-out lines of these genes and further phenotype the mutants. Soon we plant to silence also several auxins transporter genes which specifically expressed in stamen late developmental stages and in pollen. The lines genets in this study will serve our long term aims to produce tomato lines with improved fruit set under temperature stress.

CRISPR-mediated genome editing in tomato: off-targets and opportunities

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The ability of CRISPR/Cas9 or Cas12a to specifically induce mutations at virtually every position in the genome has already been shown to have the potential to speed up plant breeding significantly. We have tested the application of Cas12a in tomato protoplasts and found that Cas12a can indeed give rise to targeted mutations, which are mostly 6-10 basepair deletions. Although Cas9 and Cas12a show great promise, concerns about off-target effects exist. In plants, no large screens have been performed yet when it comes to both the mutagenic spectrum and off-target effects of these nucleases. Creating stably transformed plants in large numbers is very time consuming – especially for species, such as tomato, that have to go through a tissue culture phase. To overcome this hurdle, we developed high-throughput tomato protoplast transfection methods. To assess the efficacy and specificity of Cas9, we designed a library of 89 guide RNAs with varying degrees of predicted specificity that target members of a transcription factor family. On- and off-target effects of these gRNAs will be tested by transfecting plasmids encoding these gRNAs and Cas9 into protoplasts, followed by deep sequencing of target- and predicted off-target sites. For a subset of the gRNA library, we will perform DNA-free RNP transfections to determine whether this enhances nuclease specificity. This subset will also be stably transformed to tomato to see how well the results from protoplasts correlate with regenerated plants. Similar experiments will be performed for Cas12a. Additionally, an unbiased off-target detection system will be developed and applied to identify off-target sites that prediction software might overlook. The obtained data will help develop plant-specific rules for guide RNA design in order to minimize off-target effects, give us more insight in the undesired effects of plant genome editing, and make it possible to fine- tune CRISPR mutagenesis experiments even further. We are also exploring epigenetic modifications of the genome. In this research, the utility and specificity of CRISPR-Cas9 based systems for methylation or demethylation of DNA at specific regions of the tomato genome are investigated.

Revealing the Role Of Nascent Polypeptide-Associated Complex During Flower And Male Gametophyte Development Of *Arabidopsis Thaliana*

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The development of plant flowers represents a complex process controlled by numerous mechanisms. The creation of double homozygous knock-down mutant of both □ subunits (also referred to as basic transcription factor 3) of nascent polypeptide associated complex (NAC) in *Arabidopsis thaliana* (further referred to as *nac□1 nac□2*) caused defective phenotype including abnormal number of flower organs, shorter siliques with a reduced seed set, and inferior pollen germination rate together with a lower ovule targeting efficiency. Moreover, a delayed development of plants and lower chlorophyll content was observed. Previously, NAC complex was described as a heterodimer composed of an □- and □-subunit, which binds to the ribosomes and acts as a chaperone in *Saccharomyces cerevisiae*. In plants, NAC□ was connected to stress tolerance and to plant development as a transcription regulator. However, little is known of NAC heterodimer function in plants. Interactions between both NAC□ paralogues and five NAC□ paralogues in *Arabidopsis thaliana* were previously studied in our lab and certain interaction preferences between subunits were discovered. To deepen the understanding about molecular mechanisms behind the *nac□1 nac□2* phenotype, flower bud transcriptome and proteome of the *nac□1 nac2□* double homozygous mutants were analysed resulting in 1965 differential expressed genes (DEG) and 407 proteins (DEPs) when compared to Columbia-0 (Col-0) wild type flower bud transcriptome and proteome, respectively. These data imply NAC□'s involvement in male specific germ-line development, stress tolerance, ribosome assembly and photosynthesis together with starch metabolism. Also, upregulation of other chaperones such as HSP70 was observed in both studies. The study follows with the use of CRISPR-Cas9 genome editing to obtain knock-out NAC□ mutants and multiple NAC□/NAC□ mutant combinations. Lastly, the effect of candidate chaperones knock-out in NAC□ knock-out background will be studied. The results will bring new understanding of NAC heterodimer function in plants.

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Potentials of Gene Editing Application In Wheat Breeding

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Constant increase in human population causes an increased demand for wheat, which production should rise at a rate of 1.6% annually until 2050. To achieve this goal, scientists and plant breeders must have access to all possible breeding tools. The most recent is precision breeding with CRISPR/Cas9 system, which allows creation of desired crop varieties in a fast, simple and much more direct way compared to previous breeding techniques. Many agriculturally important traits of wheat have been targeted by genome editing, resulting in resistance to powdery mildew and *Fusarium graminearum*, improved drought tolerance, water use efficiency and herbicide tolerance, enhanced grain size and yield, as well as reduced amount of alfa-gliadins and immunoreactivity for consumers with coeliac disease. Also, mutant wheat plants which abort pollen development were produced using this technology, resulting in male sterility. Production of male-sterile and doubled haploid plants can facilitate development of hybrid seed production in wheat. However, all these promising results obtained by advanced methods will have only scientific significance if their wide application in wheat breeding is not allowed. In the European Union plants obtained by precision breeding techniques like CRISPR are considered as genetically modified organisms (GMOs) which are not exempt from the GMO legislation. Even crops with the smallest CRISPR-mediated alteration, which can also arise spontaneously in nature, are subjected to these provisions. Some part of the scientific community thinks that there are no scientific reasons to consider genome-edited crops differently than conventionally bred varieties. They stated that plants obtained by simple and targeted genome editing and which do not contain foreign genes are at least as safe as varieties derived from conventional breeding techniques. Recently, many European research institutions have taken the initiative to change the EU legislation by signing an open statement which says that gene editing with CRISPR should be used as a faster and more efficient way of producing food sustainably. If this initiative is accepted, it will allow new methods to be applied more widely in breeding and their results can be translated to the field through the precise introgression of desired traits into conventional wheat varieties.

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Genome editing of *Solanum tuberosum*: A DNA-free, CRISPR Cas9 mediated method for modifying potato.

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As climate change becomes an ever-growing concern coupled with population growth and legislative changes governing the availability of agrichemicals for pest control, particularly in relation to global food security, novel crop production methods which include genome modification must be allowed to be a routine part of the crop production toolkit. With this in mind we are working on a DNA-free method of CRISPR-Cas9 genome editing of potato. *Solanum tuberosum* is the fourth-largest food crop globally and is a crop with grave historic ties to Ireland. In this project we are editing the genome of *Solanum tuberosum* by downregulating the production of harmful glycoalkaloids which are toxic to humans in high quantities. Specifically, the target here is the *SGT3* gene (Rhamnose:beta-solanine/beta- chaconine rhamnosyltransferase), one of the final genes in the solanine production pathway, a pathway which is key to glycoalkaloid synthesis. By introducing a small deletion in this gene, its function can be disrupted, thus halting the pathway. By using a modified version of CRISPR-Cas9, delivered via gene gun (proteolistics), a 20-nucleotide region of the *SGT3* gene has been targeted. To date a number of donor plants (pre-treated with various LED wavelengths) have been bombarded using the gene gun and callus has been observed on explant tissue. To date, preliminary sequencing has not identified an indel, but a re-design of the guide RNA has been carried out using Synthego® material. This work is part of an ongoing Masters by research in UCC, which will be completed in January 2020.

The importance of establishing healthy donor plants of Solanum tuberosum prior to CRISPR-Cas9 genome editing.

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Genetic engineering of crop plants can be used to enhance, introduce or delete specific desirable traits for crop improvement. There are several different approaches available to edit a crop plants genome, however in recent times genome engineering using CRISPR-Cas9 has seen a large increase in popularity due to its high precision targeting and improved versatility. Prior to any intervention in terms of genetic engineering, it is important to establish healthy donor plant material. To that end we have been investigating the impact of factors such as light (Light Emitting Diodes), heat treatment and exposure to a number of bacterial volatile organic compounds on donor plant material. Using this treated donor material, the goal is to create a deletion in the Rhamnose:beta-solanine/beta-chaconine rhamnosyltransferase (SGT3) gene which codes for an enzyme necessary for the production of solanine, a glycoalkaloid found in potatoes. Glycoalkaloids, in large amounts can be dangerous (recommended safety levels < 20 mg per 100 g of potato). The ability to reduce the level of glycoalkaoids routinely could be of great commercial value in potato breeding. Preliminary experiments using *Agrobacterium* mediated co-cultivation transformation with the CRISPR vector pGNK-LeCas9-AtUbp-gRNA were carried out in order to create a deletion in a section of the SGT3 gene. A portion of the SGT3 was amplified using specific primers. Amplicons were sequenced and checked for a deletion. Preliminary results would indicate that no INDELS were detected at the intitial stage. However, on-going research is continuing with the re-design of the single guide RNAs. This project forms part of a research Masters.

Abce Proteins in Plants

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ATP-binding cassette sub-family E member 1 (ABCE1) is a highly conserved protein among eukaryotes and archaea. ABCE1 is currently recognized as an essential translation factor involved in several stages of eukaryotic translation and ribosome biogenesis, but its role in plants has not been experimentally confirmed.

Analysing the genomes of 90 plant species we have found that over 60% of the species possess either one or two *ABCE* genes, while the rest have 3 to 10 paralogs. In *Arabidopsis thaliana,* there are two genes: *AtABCE1* and *AtABCE2*. *AtABCE1* shows expression in floral organs and *AtABCE2* is expressed ubiquitously in all plant organs. We have found that AtABCE2 functions as a suppressor of RNA silencing in *Nicotiana benthamiana* and so does AtABCE1 though in a weaker manner.

There is no mutant line available for *AtABCE1* and only one T-DNA insertional line for *AtABCE2*, but we have not been able to obtain homozygous plants containing the disrupted gene. Human ABCE1 orthologues are crucial for the viability of several organisms (knockouts in yeast, *Caenorhabditis elegans* and *Trypanosoma brucei* are lethal) and therefore it may be difficult to obtain homozygous plants. Using CRISPR/Cas9 we aimed to knockout *AtABCE1* and/or *AtABCE2*. Our first attempt was to knockout the *AtABCE* genes independently using a vector, where Cas9 is under the control of 35S constitutive promoter and where two sites of the gene can be targeted at the same time. Additionally, we cloned a GFP cassette into the T- DNA sequence for easier selection. In the third generation, 40 T-DNA free (GFP negative) mutant plants were selected and sequenced. All lines had indels in *AtABCE1* gene sequence and 18 lines (45%) were homozygous in both sites. The analysis of the sequences show a clear preference for the addition of one "A" at the first targeted site and a preference for deletions (- 12 bp and -38 bp) at the second site. In 17 out of the 18 lines, an early stop codon was created and these knockout lines are being analysed. We have noticed defects in the reproductive organs of *AtABCE1* mutants. Some lines show reduced growth and malformations, especially of flowers and siliques. In order to try to obtain *AtABCE2* knockout lines with CRISPR/Cas9 technique, we are using now a different construct that includes an egg-cell specific promoter DD45 for Cas9.

Isolation and characterization of the myrosinase genes from the hoary cress (*Lepidium draba*) as a target for a genetic knock out by genome editing

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Myrosinases (thioglucoside glucohydrolases) are hydrolytic enzymes that together with glucosinolates form a two component defense system of the *Brassicacea* family, so called "mustard oil bombs". When plant tissues are damaged, glucosinolates, represented by numerous compounds, are degraded into various organic products by myrosinases. Although many of these products are very reactive and potentially toxic there were defined the compounds with health benefit properties as well. The best-known example is glucoraphanin, or its active form the sulforaphane, with anti-cancer activity confirmed recently in clinic studies. Glucoraphanin is the only one aliphatic glucosinolate in hoary cress plants, that makes this species very interesting because of isolation simplicity. In addition, as shown in the metabolic pathway of *Arabidopsis thaliana*, production of aliphatic glucosinolates can be increased by overexpression of one key gene - the transcription factor (myb28). However, a change expression profile from a spatial to a constitutive and significant increase glucosinolates content can result in growth retardation of plants probably due to the interaction of myrosinases with glucosinolates and the formation of toxic products. Hoary cress with eliminated myrosinase activity and, in a second step, increased glucoraphanin production could be a promising source of this substance for the needs of the pharmaceutical and food industry.
In our strategy, we will design a consensus sequence of myrosinase genes based on the sequences available in the NCBI and phytozome.org biological databases. Subsequently, using universal primers and a genome walking technique we will isolate complete myrosinase genes from hoary cress. Candidate genes will be characterized by expression analysis and their expression profile will be evaluated considering to the enzymatic activity in individual tissues. Resultant sequences will be used as a basis for the preparation of hoary cress plants lacking myrosinase activity by CRISPR/Cas9 technology.

***Key words**: glucosinolates, hoary cress, gene isolation, glucoraphanin*

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MicroProteins - small but mighty regulators

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Small open reading frames (sORFs) encoding small proteins are emerging as important regulators of protein activity. We are focusing on microProteins, a subgroup of sORFs that share an evolutionary history with larger proteins. I will present how we study microProteins in the laboratory, how genome engineering impacted our ability to obtain loss of function mutants and how we are envisioning to impact future crop breeding.

Assessment of potato genetic resources

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Potato is one of the most important food crops. Potato is vegetatively propagated and its tetraploid genome is highly heterozygous. The genetic variability of potatoes is decreasing because potato breeding usually involves crossing new varieties with mostly good traits and even then approximately one breeding line in 100 000 becomes a new variety. Introducing old varieties in breeding schemes can have adverse effects due to the high number of recessive alleles. Preserving older potato varieties as genetic resources is important in order to avoid losing the potentially beneficial recessive alleles which have been bred out of new varieties. Estonian Crop Research Institute preserves potato genetic resources as an *in vitro* collection.

To find the potential benefits of genetic resources, the material needs to be assessed both phenotypically and genetically. Our potato collection was phenotyped using the key access and utilization descriptors for cultivated potato genetic resources, composed by the potato working group of the European Cooperative Programme for Plant Genetic Resources (ECPGR). Genetic assessment was done using eight SSR markers: STG0001, STM1052, STM1104, STM5127, STI0004, STG0016, STI0012, and STM5114. SSR markers have been widely used for fingerprinting potato cultivars and there are many lists of markers to choose from. SSR markers have identified some of Estonian landraces to be genetically indistinguishable from known cultivars and some landraces to be of unknown origin.

Currently old varieties and landraces are rarely used for plant breeding. One future use of genome editing could be to "repair" adverse traits of old varieties to make improved versions for practical use in plant breeding.

Knowledge of the Polish society about the genetically modified organisms (GMOs)

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In recent years, the share of genetically modified organisms (GMOs) in the natural environment has been increasing. GMOs are currently used in plant and animal production as well as in the agri-food, pharmaceutical and cosmetics industries. However, the practical use of genetically improved organisms through genetic engineering is still controversial in society. The level of knowledge on genetically modified organisms (GMOs) in Poland is constantly changing. The survey was performed among 361 anonymous respondents about genetically modified organisms and their products used in everyday life. Most of the respondents (70%) were women. The respondents lived mainly in the city (72%) and were overwhelmingly 20 to 60 years old. Most also had higher education (46%) or secondary education (37%). It was found that 96% of the respondents met with the term GMO. However, 50% of people do not agree to the cultivation of agricultural GMO crops and 70% of responders are afraid of the negative effects of using GMO products in the production of food, forage and pharmacology. But 59% of responders agree to the application of the GMO products to pharmaceutical purposes.

Rape (*Brassica napus* L.) transformation and shoot organogenesis – important steps towards successful genome editing

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Seeds *of B. napus* are used in a large scale for the production of one of the most health promoting plant oils in the food industry as well as for biofuel production. Thus, any successful attemp towards increasing crop yield is of crucial economic and ecological importance. However, conventional breeding programs are slow, laborious and time consuming. Genome editing is a very promising option that can be used to significantly accelerate the breeding progress due to effective gene targeting, especially in polyploid species such as *B. napus*.

One of the milestones of rape molecular breeding is an efficient *in vitro* regeneration as the transformation rate of *B. napus* plants using the floral-dip method is very low. Moreover, the long generation time of the rape plant enables floral-dip transformation only in a short time period during a year.

The rate of regeneration depends mainly on the genotype of the used species. Thus, we analyzed twenty varieties of *B. napus* for their ability of shoot organogenesis. Fragments of cotyledons and hypocotyls were isolated from 7-days-old seedlings cultured *in vitro* and transferred onto MS medium supplemented with B5 vitamins and cytokines, with or without $AgNO_3$. Both explant series showed shoot induction on proliferation media, but with significant differences in morphogenetic response between the varieties. Plants with the highest shoot regeneration potential were selected for further transformation experiments.

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Increased homeologous crossovers in an interspecific tomato hybrid by CRISPR/Cas9-inactivation of *RECQ4*

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During meiosis in plants, crossover formation is required for proper chromosome segregation, and crossovers are essential for crop breeding as they allow the formation of new combinations of traits by mixing parental alleles on each chromosome. Crossover formation starts with the production of a large number of DNA double-strand breaks, of which only a few, in the end, will produce crossovers. Earlier work in *Arabidopisis thaliana* has identified a number of genes that drive the resolution of DNA crossover intermediates towards non-crossovers. We are exploring the potential of modification of these genes in interspecific hybrids between crops and their wild relatives in order to increase the production of crossovers. Here, we have used CRISPR/Cas9-mutagenesis in an interspecific tomato hybrid to knock out *RecQ4*. A biallelic *recq4* mutant was obtained in the F1 hybrid of *Solanum lycopersicum* and *S. pimpinellifolium*. Compared to the wild type F1 hybrid, the F1 *recq4* mutant had a significant increase in crossovers: a 1.53-fold increase when directly observing ring bivalents in male meiocytes microscopically and a 1.8-fold extension of the genetic map when measured by analysing SNP markers in the progeny (F2) plants. This result is one of the first demonstrations of increased crossover frequency in interspecific hybrids by manipulation of genes in crossover intermediate resolution pathways and the first to do so by directed mutagenesis.

Genome editing for imrovement sunflower oil quality – possibilities and problems

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Modern sunflower breeding dedicate a great attention in altering oil quality. Sunflower oil gain importance due to the frequent transition to the Mediterranean diet (using oils rich in oleic acid), and the requirements of biodiesel industry, preferring the use of high-oleic sunflower oil for biodiesel production compared to the standard sunflower oil. Although sunflower oil is one of the finest plant oils, sunflower breeders have reacted to market demands and managed to make certain changes in sunflower oil quality, concerning fatty acid composition and tocopherol content. Besides mid and high oleic acid content, "new" traits such as, both low saturated and high saturated fatty acid content and different combinations of increased levels of beta-, gamma-, and delta-tocopherol have been developed. Combination of "new" and "old" traits for oil quality enables their accumulation in one genotype and use for various purposes. Recent breakthrough in sunflower genome sequencing is expected to facilitate the use of genomics and other new breeding techniques, including genome editing, and work on understanding the molecular mechanisms. Genome editing could provide new perspectives for more efficient breeding, especially complex phenotypic traits, such as oil quality traits.

***Key words**: sunflower, oil quality, genome editing, oleic acid, tocopherols*

Implementation of genome editing for apple breeding

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Apple (*Malus x domestica*) is the most important fruit in Switzerland and the second most important fruit worldwide, with approximately 90 million metric tonnes being produced annually. The highly heterozygous genome, long juvenility period and outcrossing nature of this species means that breeding is relatively unpredictable and lengthy (more than 15 years to breed and select a new variety). Apple cultivation is subsequently hampered by diseases, including fire blight, apple scab and powdery mildew, necessitating extensive use of chemicals to minimise crop damage and financial losses. With genome editing technology, we are currently implementing methods to improve the popular apple variety, 'Gala Galaxy'. We are using Cas-based ribonucleoprotein complexes for targeted gene modification that should lead to the production of disease resistant, transgene-free varieties. Ultimately, these varieties should contribute to the global action towards reducing chemical use in crop cultivation.

Use of geminiviral vectors in gene editing

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For long time plant viruses were used for efficient delivery of genetic sequences for the overexpression of heterologous genes or silencing of plant genes. Some properties of these intracellular parasites make them interesting candidates also for the delivery of reagents for plant genome editing.

Here we present results from experiments where a modular system based on BeYDV mastrevirus was used to deliver gRNA targeting reporter Timer fluorescent protein to N. benthamiana plants constitutively expressing Cas9 nuclease. Our modular system simplifies the construction of replicating viruses with either up to 3 individual gRNAs or up to 9 in concatenated form. It also allows the regulation of the replication by the titration of the Rep protein levels using chemically inducible expression. The efficiency of gene editing can be estimated from the ratio of red to green fluorescence.

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The arrival of the plant age: A plant platform to manufacture therapeutic proteins with a human-like-glycosylation using CRISPR/Cas9 technology to mediate knockout of six glycosyltransferase genes in order to eliminate plant specific β 1,2-xylose and core α 1,3-fucose residues.

Zohar Katz, Ofri Launer, Zvi Zvirin, Amit Yaari and Oded Shoseyov.

In recent years, plants have become a viable alternative for the production of recombinant proteins [1-6]. However, their inability to perform authentic mammalian N -glycosylation may cause limitations for the production of therapeutics. A major concern is the presence of β 1,2- xylose and core α 1,3-fucose residues on complex N -linked glycans, as these N -glycan epitopes are immunogenic in mammals and can also affect the activity and potency of plant-derived proteins[3,6-7]. In order to address this challenge, we demonstrate CRISPR/Cas9-mediated knockout of six glycosyltransferase genes in *Nicotiana tabacum* plants. Stable knockout (KO) lines in all six genes will be confirmed through screening of *N.tabacum* plants using NGS sequencing, western blot and mass spectrometry-based N-glycan analysis of endogenous proteins and the recombinant monoclonal antibody adalimumab (Humira®). These lines will serve as a platform for the generation of therapeutic proteins.

Previous groups have shown that antibodies produced in plants have the same qualities of those produced by the mammalian cell-based expression platforms [2,8-10]. Once a plant's immunogenic-to-human residues are cut-off, these transgenic lines will serve as a much safer method of production, since plants do not harbor mammalian pathogens.[11] Moreover, generation of therapeutic proteins in plants will allow a significant reduction in production costs, therefore increasing the economic viability of these medicines[5].

To summarize, by using CRISPR/cas9 genome editing system, we eliminate plant-specific a- 1,3-fucosyltransferase and b-1,2-xylosyltransferase activity and allow the production of human-like-glycosylation of therapeutic proteins in *Nicotiana tabacum* plants. This plant platform will provide a cost effective, highly scalable, safer and more sustainable way to manufacture biological drugs.

Applications of genome editing in plant: benefit, risk and safety considerations

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Over the past three decades, agricultural biotechnology research has extended beyond GM products and the development of several emerging new breeding techniques, such as genome editing. Universally, GM crops have provided farmers in accepting countries arrange of economic, environmental, and health benefits. GM crops have donated considerably to the reduction of environmental impacts from pesticide use Genome-edited crops promise a host of benefits for consumers. Such as the production of soybeans with improved oil profiles, tomatoes with enhanced flavor qualities, non-browning apples, potatoes. In our paper will discuss the benefit, risk and safety considerations of using of genome-edited crops.

***Key words**: Genome editing, GM crop*

Inducing mutations in potato *via* genome editing in demand of climate change

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Potato is one of the most important crop plant in the world; it is ranked as third in the term of human consumption after rice and wheat. Increasing world population and the alarming fast climate change represent a big issue for food security. Potato is a very sensitive crop for high temperatures because the genes, which are responsible for tuber induction, are temperature sensitive and their expression changes with increasing temperatures. Tuber formation of potato is guided by a homolog of the flowering locus T (FT), namely SP6A. Under favourable environmental conditions, a mobile tuberization signal is produced in the leaves, which is encoded by SP6A and transferred to the roots where it leads to tuber formation. However, rising temperatures change these signals and under elevated temperatures the expression of SP6A is repressed by a small RNA, named suppressing expression of SP6A (SES) and, consequently, tuber formation is blocked and yield reduction even down to a total loss of the harvest can be the consequence. A fact that could already be observed in the last years (2018 and 2019) in central Europe as the temperatures where increasing during spring and summer leading to reduction in tuber production already.

Genome editing using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 is a powerful method to induce deletions, insertions or substitutions in different genes. As the Cas9 protein induces double stranded breaks (DSBs) in the target DNA, which are repaired by the cell e.g. *via* the error-prone non-homologous end-joining (NHEJ) pathway or by precise homologous recombination (HR). We have induced mutations in potato protoplasts using isolated Cas9 and guide RNA as proof of concept of DNA-free genome editing in the PDS (*phytoene desaturase*) gene with 0,7% editing rate. Furthermore, we were also able to induce mutations in the SES locus at protoplast level. After regeneration of these protoplasts will result in a transgene free heat tolerant potato.

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Development of Male Sterile Lines of Tomato Using CRISPR-Cas9 System

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Tomato is an important vegetable and economic crop worldwide, and it is a model plant for fruit development research works. Today, hybrid varieties are widely used in vegetable production. Therefore, the development and adoption of hybrid seed technology has led to dramatic increases in agricultural productivity. Male sterility research has been directed toward two goals: identifying genes required for the pollen development pathway and, more practically, identifying genetically stable lines that can be used in hybrid seed-breeding programs. Pollen development in flowering plants is critical for male reproductive success. The pollen cell wall that protects the pollen from various environment stresses and pathogens plays an essential role in pollen cell wall development. The formation of pollen cell wall is associated with the biosynthesis and transport of sporopollenin components. *ACOS5* gene in Arabidopsis encodes an *acyl-CoA synthetase 5* required for sporopollenin biosynthesis. In present study, we identified the tomato homolog of *ACOS5* as *4CL-like ACOS* by bioinformatic analysis tools. The CRISPR/Cas9 system was used to knockout *ACOS5* gene in tomato. We designed two guide RNAs to target distinct sites of tomato *ACOS5* gene. Subsequently, the PKI1.1R vector was transformed into tomato cotyledons through *Agrobacterium-mediated* transformation, resulting in efficient target gene editing. Evaluation by using sequence analysis was confirmed the mutations in tomato *ACOS5* gene. ACOS plays a critical and conserved role in pollen cell wall formation and pollen development and has implications in tomato breeding. Our results suggest that CRISPR/Cas9 system was an efficient and specific tool for target mutagenesis of tomato genome.

Keywords: *Tomato, male sterility, CRISPR/Cas9, knock-out, gene silencing*

Increasing the range of base editing by using different deaminases in plants

Thomas Jacobs

Base editing with CRISPR/Cas systems has emerged as a powerful technique to make specific nucleotide substitutions in genomes. Base editors most often contain a Cas nickase fused to an enzyme that deaminates either cytidine or adenine. The use of several cytidine deaminases has been reported in plants with the most widely used being the rat APOBEC1, but the sea lamprey PmCDA1 and APOBEC3A have also been used. Our experience with rat APOBEC1 base editors has been that if the vector works, it is very efficient, making it easy to identify edited events and recover edited lines. However, only ~25% of our targets have resulted in observable editing. This greatly limits the types of edits we can make and the utility of the system overall. To overcome this limitation we compared four deaminases, APOBEC1, APOBEC3A, APOBEC3B, and PmCDA1 for activity in cell suspension cultures and stable Arabidopsis and tobacco plants. To facilitate the screen, we adapted the BE-FLARE base- editing reporter that utilizes a BFP-to-GFP conversion by making CAC->TAT mutations at amino acid 66. While cloning these deaminases, we observed that the vector containing APOBEC3A was lethal in *Escherichia coli*. We also observed premature editing of the BE- FLARE reporter in the APOBEC3B plasmid, indicating that a low level of Cas9 and gRNA expression is occurring in *E. coli*. To overcome these limitation, introns were added to APOBEC3A and APOBEC3B. Using the BE-FLARE system, we clearly observed BFP-to-GFP conversion and the corresponding DNA edits in Arabidopsis cell suspension cultures using all deaminases. We also targeted endogenous genes with APOBEC1, APOBEC3B and PmCDA1. All three deaminases induce mutations, but the activity of a given deaminase appears to be dependent on the target site. APOBEC3B was active at four out of five targets while the others were only active at three of the targets. In general, APOBEC1 has the lowest activity. Together, our data shows that the editing range of base editors can be improved by using different deaminases in plants and the context-dependent activity we observe suggests that researchers should use a few different deaminases to obtain the edits they want.

Crispr Induced Resistance to Wheat Dwarf Virus (Wdv) In Barley

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Wheat dwarf virus (WDV) is an economically important cereal pathogen.WDV strains infect exclusively barley and wheat negatively affecting yield up to 90 %. Currently, there is not a natural source of resistance against WDV infection. WDV was first detected in former Czechoslovakia, it has been extensively studied since that. As a result, a collection of viral isolates was characterized. Biotechnological progress provided alternative approaches to deal with viral infection via various ways of induction of resistance. CRISPR/Cas9 technology was originally described as an adaptive bacterial immune system against viral infection, in plants. The optimal design of one or multiple sgRNA enables targeting key viral genes in their conserved sequences to efficiently restrict virus replication in plant cells to a broad range of currently existing WDV isolates. WDV genome consists of genes for replication (Rep/RepA), systemic spread in plant tissues (CP, MP), and intergenic regions LIR and SIR that are required for replication. Three guide RNAs were designed to target distinct parts of the WDV genome. Particularly, sequences for Rep, CP, and MP proteins. Plants carrying Cas9 transgene and guide RNA are in development and will be tested for resistance against WDV.

***Keywords**: Wheat dwarf virus, CRISPR/Cas9, resistance, barley*

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Biosafety Capacity Enhancement Activities: Key Practices of the ICGEB

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The International Centre for Genetic Engineering and Biotechnology (ICGEB) represents an advisory, scientific, and technical point of reference for Parties of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity regarding the safe use of genetically modified organisms (GMOs). Based upon a collaborative approach, ICGEB Biosafety is increasing the capacity of governments in Project Countries, principally in sub-Saharan Africa and Latin America and the Caribbean, to effectively regulate the products of modern biotechnology.

Collaborations range from the development of regulatory processes and tools tailored to national biosafety legislative frameworks, to the provision of training in their practical implementation. These are carried out through a number of different activities including: bilateral exchanges between novice regulators in Project Countries with experienced current and former regulators in/from Argentina, Australia, Canada, Uganda and the United States of America; in-country mentored fora in which novice regulators and their technical experts are trained in, and assisted in tailoring to national circumstances, model approaches and tools in regulatory practices. Further, a range of public awareness materials, such as scripts for radio spots, factsheets, Q&As and brochures, have been created to facilitate regulatory authorities to include public participation in decision-making when required.

ICGEB Biosafety has also developed a portfolio of online eLearning biosafety modules which has recently been adopted and used by African regulatory offices, thereby providing them with a tool for autonomous in-house staff training. The portfolio is also available for adoption by product developers, universities and additional government offices and can be viewed at https://showcase-icgeb.elearning.it/. In order to maintain the relevance of the eLearning portfolio, we are keen to extend the geographical coverage and the content of the portfolio, especially with regard to issues raised by genome editing and other emerging technologies.

Challenges of genome editing tools in forestry

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One of the main research tasks from the perspective of climate smart forestry concept in the future will be to mitigate detrimental effects of climate change through selection and generation of woody plant species that are more resilient to abiotic and biotic stress factors that is the focus of our investigation in the future. This area of research is where genome editing using site-directed nucleases emphasising CRISPR-cas9 technology can revolutionize the breeding processes.
The objectives of future investigation of our group will be to employ this technology in testing the response of poplar genome regarding genes encoding for stress oxidative enzymes diamine oxigenase (DAO, EC1.4.3.6) and polyamine oxigenase (PAO, EC 1.5.3.3.) involved in polyamine metabolism. Another utility of this novel technology in forestry can be in objective that microbiome and mycorrhizal fungi within can improve plant health and tolerance to environmental stress in return for carbon. *Rhizopogon irregularis* (formerly *Glomus intraradices*) is the first mycorrhizal fungus which genome is fully sequenced. The extensive knowledge about this very common generalist fungus could find application in creation of synthetically modified mycorrhizae. Using techniques of genome editing in mycorrhizal fungus or/and in host plant, especially CRISP/Cas 9 techology, could increase plant tolerance to drought and heavy metals, nitrogen and phosphorus uptake, plant defence mechanisms and formation of symbiosis could be facilitated as well.
The interest of lignocellulosic biomass has received growing attention as raw material for the production of second-generation biofuels. Not only the favorable as mechanical support, lignin can also have a defense role being the recalcitrant barrier upon various biotic and abiotic stress conditions. Conversely, the presence of lignin in secondary cell wall (SCW) is a major, unfavorable, factor preventing hydrolytic enzymes from gaining access to cellulose. Hence, research efforts are currently aimed at designing plants that either deposit less lignin or produce lignin that are more amenable to chemical degradation. Due to highly regulated genetic control of wood formation mainly at the transcriptomic level on which we focused in our recent study, we choose secondary cell wall-associated NAC domain protein 1 (SND1) that acts as a first-level master switch in SCW biosynthesis. Exploring the novel possibilities of application of these cutting-edge techniques (particularly CRISPR-cas9) and expanding knowledge about technical principles of this methodologies (that is aim of WG1 group within COST PlantEd action) will greatly contribute to our research interests.

Key words: forestry, polyamine metabolism, mycorrhizae, lignin, genome editing

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Stacked genetically modified maize events are present in Algerian marketed foods

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Since the first genetically modified organism (GMO) approval in 1996, the number of genetically modified (GM) crops introduced to the market has been dramatically increasing, as well as the number of countries involved in their production/commercialization, the diversity of novel traits and the global trade. So far, 31 different GM crops, accounting 519 transgenic events, have been authorized for food and feed production in 44 countries [1]. The cultivation area of GM crops reached 189.8 million hectares by 2017, from which, soybean and maize accounted for 50% and 30% of the total area, respectively [2]. Maize, one of the most used staple food and feed ingredients, is the second most cultivated GM crop with the highest number of approved events (238) (ISAAA, 2019). Despite the prohibition of the importation, production, distribution, marketing and use of GM plants in Algeria, no legislation regarding their use in food and feed production has been established.

The present work describes, for the first time, a full-stage study to monitor GMO in Algeria, based on a comprehensive survey of maize-derived foods, providing screening, event-specific identification and quantitative data targeting 11 maize events (Bt176, Bt11, MON810, GA21, NK603, MON863, TC1507, MIR604, DAS59122, 3272 and DAS40278). The results show that,

out of 91 maize-derived samples positive for an endogenous maize gene, 20% contained at least one screening GM element (35S promoter and NOS terminator). Six events were identified in 16 samples, being MON810, NK603 and TC1507 the most frequent (16%, 15% and 14% of the samples, respectively), followed by GA21, Bt11 and DAS59122 (7.6%, 6.6% and 2.2%, respectively). Interestingly, out of those samples, 14 had 3 to 5 GM events, while only 2 had one or 2 events. The quantitative real-time PCR results show very high levels of GM maize events in all samples resultant from the multiple-event accounting (34.9-222.7%), suggesting the presence of stacked events, together with single-trait ones. These findings highlight the need for specific labelling legislation regarding the GMO presence in food and the verification of its compliance.

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Spatial genetics of plant communities

Meredith C. Schuman

Spatial genetics is the spatially resolved analysis of heritable traits, their frequency and distribution in communities, and how this affects adaptation and community interactions. Spatial genetics research includes approaches from ecology, remote sensing, chemistry, genetics, and bioinformatics. Our focus is the spatial genetics of plant-based communities. This is because plants are the trophic basis of terrestrial ecosystems, and their diversity structures ecological communities. Specifically, plant genetic and species-level diversity have large effects, but the mechanisms underlying these effects are still poorly understood. To understand these mechanisms and employ them to achieve e.g. conservation or sustainability goals, and to improve our projections of future habitats under climate change, we require a spatially explicit understanding of genetics and chemical signaling in plant communities. We furthermore require methods for large-scale and long-term monitoring. Remote sensing technologies can provide spatiotemporally resolved, large-scale, long-term data.

A Balanced ABS System: Stakeholder Insights on Provider Country Legislation

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The over-arching aim of the access and benefit-sharing (ABS) of genetic resources is to enable fair distribution of benefits between the users (such as universities and biotech companies) and providers (such as biodiversity rich countries) so as to both open the doors for innovation and create incentives for biodiversity conservation.

Access to genetic resources is crucial for research related to conservation of plant genetic resources as well as R&D for agricultural products and evolved crops that can attain to the new weather conditions climate change brings. Therefore, access to genetic resources in general as well as benefit-sharing from that access is a key element for sustainable development in order to secure research as well as environmental sustainability and resource availability.

ABS is currently a rapidly developing and evolving field that is shaped by the implementation of the Parties. This means that the national implementation of the Parties determine how ABS goals are realized and how ABS principles find form within regulatory mechanisms. These principles are found in international legal documents such as the Convention on Biological Diversity (CBD) as well as Nagoya Protocol. Additionally, decisions and guidelines drafted by the Conference of the Parties to the Convention on Biological Diversity shape these principles that are then to be fulfilled by the Parties when drafting their ABS laws by means of implementing regulatory mechanisms that comply with the international law. This presentation focuses on a study that reviews 20 nationals as well as one regional framework on ABS with the aim of describing the different types of regulatory mechanisms provider countries use. This descriptive approach is then followed by an empirical comparative analysis through semi-structured stakeholder interviews in order to identify the most favored regulatory mechanisms according to ABS experts that belong in four different stakeholder groups (provider countries, academic users, industrial users and collections).

CRISPR/Cas9-mediated genome editing to improve the nutritional quality of tomato fruit

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Metabolic engineering in tomato has been successfully applied for its nutritional improvement, since tomato is an excellent candidate for the genetic engineering/manipulation, by using either the conventional transgenesis, or the novel approach of genome editing (GE).

CRISPR/Cas9-mediated GE is emerging as a modern technology in plant research field, especially for the possible introduction of genetic traits, important for agronomy and nutritional quality of fruits, vegetables and staple crops. Site-specific, precise modifications can be addressed on the target gene, using the CRISPR/Cas9 system associated with RNA guide/s (sgRNA). The double strand breaks introduced by Cas9, can be repaired through the endogenous systems of repair (NHEJ and HR), creating insertions/deletions (indels). The NHEJ (non-homologous end joining) system re-join imprecisely the separated ends, while the HR (homologous recombination) system uses a donor template to repair the break. To date, few studies have been carried out using the HR system of repair in tomato. In some cases, it is necessary to use molecular strategies to increase the copy number of the donor template in the cell, in order to increase the probability of the targeting events. Among them, the use of viral sequences (i.e. LIR and RepLIR from BeYV), inducing a rolling circle replication, can be a valid approach. We aimed to introduce the whole sequence of the fruit-specific promoter E8 upstream the MYB12 gene, involved in the enhancement of polyphenol biosynthesis in tomato fruit. We identified one correct gene targeting event and 3 partial insertions out of 60 hairy roots samples, in a transient expression assay mediated by *Agrobacterium rhizogenes*. Furthermore, in our hands the presence of the viral sequences LIR and RepLIR, also induced molecular re-arrangements in the plasmid backbone, during the *A.tumefaciens*-mediated transformations. Such re-arrangements affected plasmid stability and were bacterial strain-dependent. In this context, the HR remains challenging, although it would be a novel way to engineer the metabolic pathways for tomato nutritional improvement.

Comparison of toxicity of GM and non-GM plants under stress conditions.

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We offer to potential collaborators toxicity characterisation and comparison of prepared engineered plants. For the purposes of the Action is important the assessment of cytotoxicity and haemolytic activity of crop extracts. We are very well equipped to perform various tests, e.g. antimicrobial, immunomodulatory, antioxidant, enzyme inhibition, anti-cancer etc., using also new and unique automated robotic station for high-throughput toxicity/bioactivity screening.

We investigate whether crop plants grown under influence of different stress factors will reveal higher toxicity than the non-stressed ones, and whether transgenic plants tailored for increased stress resistance (of the same species, cultivar) under the same conditions and same stressor, will reveal higher or lower toxicity compared with the non-transgenic ones. The result that some crops with increased stress resistance are better prepared for climatic changes with additional benefits for consumers represented by toxicity decrease can have a positive impact on GMO acceptance.

Toxicity assay according to OECD Guidelines is done in dose-dependent action of plant extracts, using several lines of mammalian cells kept at our department, HEK293T (epithelial cells derived from kidney of human foetus), HaCat (human primary epidermal keratinocytes), HDF (human dermal fibroblast), MDBK (bovine kidney), RAW (mouse macrophages) and others. Further haemolytic activity, inflammation markers, genotoxicity, plasma membrane permeability, mitochondrial membrane potential or mitochondrial mass will be estimated due to the wide spectrum of specific probes forming a fluorescent complex. The ability of extracts to damage homeostasis of endocrine system or testing of antimicrobial activity on beneficial bacteria of digestive tract can be performed as well.

1st PlantEd conference

"Plant Genome Editing - State of the Art"

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