

PlantEd

Genome editing in plants

Cost Action CA18111

4th PlantEd Conference

18-20 September 2023

Porto, Portugal

4th PlantEd Conference

18-20 September 2023

Porto, Portugal

Book of Abstracts

Book of Abstracts of the 4th PlantEd Conference

EDITORS:

Dr. Isabel Mafra

Dr. Joana Costa

Dr. Caterina Villa

Dr. Carla Teixeira

Prof. Isabel Ferreira

EDITION:

COST Action CA18111, PlantEd – Genome editing in plants - a technology with transformative potential

Website: <https://plantgenomeediting.eu/>

DATE:

September 2023

Table of contents

Committees	7
Scope	8
Venue	8
Supporters	9
Programme	10
Useful information	13
Oral Presentations	14
Session I – GE applications and molecular mechanisms	15
Genome editing to improve health benefits of root chicory	16
CRISPR/Cas9-directed editing of RelA/SpoT homologs in tomato (<i>Solanum lycopersicum</i> L.)	17
Tomato breeding by design using non-transgenic genome editing.....	18
CRISPR/Cas9-mediated miRNA editing in tetraploid potato	20
Efficient targeted gene insertions in diploid potatoes.....	21
CRISPR-based visualisation of centromere sequences in chicory	22
Session II - Improving resistance to abiotic stress	23
CRISPR - Bridging fundamental knowledge and novel technology to increase rice heat tolerance	24
Optimized <i>Lolium perenne</i> L. protoplasts isolation and transformation for CRISPR-Cas9 downstream applications	25
Establishment of highly efficient and reproducible <i>Agrobacterium</i> -mediated transformation system for tomato (<i>Solanum lycopersicum</i> L.)	26
Improving grape resilience to climate change exploiting the CRISPR/Cas technology: different approaches to face drought	27
Session III – Improved technologies	29
Increase the production of industrially valuable compounds in the microalgae <i>Chlorella</i> – the GeneBEcon approach	30
Optimizing protoplast isolation and transformation efficiency for enhanced plant genome editing in grapevine.....	31
Exploring alternative approaches for efficient gene targeting in plants: high fidelity nonhomologous end-joining with CRISPR-Cas12a in potato protoplasts	32
New Genomic Techniques in citrus, step-by-step solutions for more efficient and successful procedures	33
Session IV - Nutritional improvement and characterisation	35
Developing gene editing in sweetpotato to increase its nutritional status	36

In-depth characterization of Cas9 specificity in tomato using high-throughput amplicon sequencing, GUIDE-seq and whole genome resequencing.....	37
A dual single-guide RNA approach used to edit the <i>β-cyclase 2</i> gene in anthocyanin-rich sweet orange varieties.....	38
HQT gene editing to study chlorogenic acid metabolism and its physiological role in tomato	39
Session V – Improving resistance to biotic factors.....	41
Gene editing in potato to enhance PVY resistance	42
Studying potato resistance and susceptibility factors against pathogens with the use of genome editing.....	43
Gene editing in triploid banana cultivars	44
<i>De novo</i> domestication of wild tuber-bearing <i>Solanum</i> species.....	45
Session VI – Regulation and public perception	46
Preliminary analysis of the European Commission Proposal for a Regulation on the production and marketing of plant reproductive material.....	47
The new NGT legislation proposal of the European Union. Analysis of selected EU and national regulatory obstacles for the introduction and market viability of NGT plant products	48
The awareness of the Polish society on new genomic techniques	49
Session VII – Short Term Scientific Missions - STSM	50
Leveraging system biology and new breeding technologies for water stress tolerance in grapevines	51
Application of multiplexed CRISPR-ACT3.0 gene activation system in tomato roots for enhancing resistance against plant-parasitic nematodes.....	52
Development of resistant sunflower lines to broomrape using CRISPR-CAS9.....	53
Exploring the role of <i>SnRK2</i> genes in salinity stress response of <i>Petunia axillaris</i> through CRISPR-based genome editing	54
Poster presentations	55
Optimization of gene editing in <i>Lactuca sativa</i>	56
Improvement of drought stress tolerance in poplars (<i>Populus</i>) by modification of candidate genes	57
CRISPR/Cas-mediated efficient knock down of vacuolar invertase gene to address cold sweetening in potatoes	58
Use of prime editing to mutate the thiol reductase (TR) activity of AtYUCCA 6 in <i>Arabidopsis Thaliana</i>	59
Multiplex genome editing and trait improvement for complex multicrop systems.....	60
Genome editing technologies for engineering the phytonutrient content in tomato	61
Identification of the Pectate Lyase gene family in <i>Vitis vinifera</i> and its role in the berry development	62

CRISPR/Cas9 mediated resistance to Becurtovirus in sugar beet	63
Generation of the barley why2 knock-out mutant	64
The “SMART-BREED” project: innovative molecular technologies for the adaptation of horticultural species to climate change through precision breeding	65
The application of CRISPR-Cas9 for the creation of multiple mutants in the genes coding for the nascent polypeptide associated complex	66
Employing protein-interaction techniques to identify pathogen effectors to better devise targeted genome editing in plants	67
Changes in the public perception of genetically modified organisms (GMOs) in Poland	68
A rocky road to CRISPR - Dealing with in vitro recalcitrance in European beech (<i>Fagus sylvatica</i> L.)	69
Generation of a <i>brc1</i> knock-out mutant using CRISPR-Cas9	70
New Plant Breeding Techniques to mitigate biotic stresses in grapevine	71
CRISPR/Cas9 technology to reduce the levels of allergenic molecules in tomato fruit	72
Field trials in genome edited plants: a bibliometric approach.....	73
CRISPR/Cas targeted inactivation of guaianolide oxalate formation in chicory	74
Efficient gene editing in tomato facilitated by RNA virus vector systems	75
CRISPR/Cas9-mediated genome editing in petunia plants: enhanced plant architecture with compactness and flower abundance	76
Development of drought-resistant tomato plants by knocking out the AHP genes.....	77
Improving resistance to pod shattering in canola.....	78
Genome editing of VvPDS gene by using biolistics on grapevine (<i>Vitis vinifera</i> L.) somatic embryos.....	79
Genome editing in perennial ryegrass: towards improving abiotic stress tolerance for safe and sustainable food systems (EditGrass4food)	80
Targeted knockout of barley endosperm-specific storage proteins as a prerequisite for molecular farming purposes	81
Using wheat transformation and gene editing strategies for wheat improvement.....	82
Mitochondrial genome editing in potato by mitoTALEN and mitoTALECD: induced mutations and phenotype of edited plants and vegetative progenies	83
Genetic editing of CML genes in potato <i>Solanum tuberosum</i>	84
Freedom to use and share regulatory data under the proposal for the EU revision of the New Genomic Techniques (NGT) legislation	85
CRISPR/Cas goes viral – viral replicons for gene targeting in poplar (<i>Populus</i> spp.).....	86
Protoplast applications in CRISPR/Cas9 genome editing to accelerate cabbage breeding	87
Maximising pineapple production by controlling flowering time using CRISPR/Cas9	88
Heavy metal (Cu, Zn) induced gene expression in <i>Brassica juncea</i>	89

Committees

Scientific Committee

Dennis Eriksson, Swedish University of Agricultural Sciences, Sweden

Isabel Mafra, REQUIMTE-LAQV/Faculty of Pharmacy, University of Porto, Portugal

Götz Hensel, Heinrich-Heine-University, Dusseldorf, Germany

Katrijn Van Laere, EV ILVO, Belgium

Dragana Miladinovic, Institute of Field and Vegetable Crops, Serbia

Jeremy Sweet, JT Environmental Consultants, UK

Jale Tosun, Heidelberg University, Germany

Patrick Rüdelsheim, Perseus bvba, Belgium

Tomasz Twardowski, Institute of Bioorganic Chemistry, Poland

Ewa Wozniak, Polish Academy of Sciences, Poland

Geraint Parry, Association of Applied Biologists, UK

Matina Tsalavouta, University of Liverpool, UK

Vladislava Galovic, Institute of Lowland Forestry and Environment- ILFE, Serbia

Anna Coll, National Institute of Biology, Slovenia

Ankica Kondic-Spika, Institute of Field and Vegetable Crops, Serbia

Sebastien Carpentier, KU Leuven , Belgium

Local Organising Committee

Isabel Mafra, REQUIMTE-LAQV/FFUP, Portugal

Joana Costa, REQUIMTE-LAQV/FFUP, Portugal

Caterina Villa, REQUIMTE-LAQV/FFUP, Portugal

Carla Teixeira, REQUIMTE-LAQV/FFUP, Portugal

Isabel Ferreira, REQUIMTE-LAQV/FFUP, Portugal

Scope

The 4th PlantEd Conference (COST Action 18111) will be held over three days, with open scientific sessions on genome editing technology in plants, followed by PlantEd Working Group (WG) sessions and a Management Committee (MC) meeting. The conference will be a hybrid event, with a limited number of participants physically present, combined with live streaming (Zoom). The PlantEd conference, a network for plant genome editing research across Europe and beyond, is an excellent platform for disseminating information, discussion, and connections and updating the latest research and innovation.

The PlantEd Conference, being a network for research on plant genome editing across Europe and beyond, is an excellent platform for dissemination, discussions and connections, and for updating on the latest research and innovation forefront.

Topics to be covered: The conference will host sessions on the application of genome editing in various types of economically important plants (cereals, oil crops, roots and tubers, legumes, fruits and vegetables, trees, algae), as well as the latest technological advancements for genome editing in plants.

The conference will take place towards the end of the action and final grant period, which marks the closing of PlantEd activities, identifying the main achieved outcomes, but most importantly, planning/on-going activities by the prospection of new resources.

Venue

[Faculty of Pharmacy, University of Porto \(FFUP\)](#)

Rua Jorge Viterbo Ferreira, 228

4050-313 Porto

[DIRECTIONS](#)



The conference will have the support of the [Associated Laboratory REQUIMTE](#) and [Faculty of Pharmacy, University of Porto](#).

Supporters

PlantEd

PlantEd – COST Action CA18111 – Genome editing in plants

Financial supporters



Funded by
the European Union

Local supporters



Programme

4th PlantEd Conference

Porto, Portugal – September 18-20, 2023

Monday 18 Sept		Session Chair: Dennis Eriksson
	08:00-09:00	Registration
	09:00-09:30	Welcome Local Organizer – Isabel Mafra ; REQUIMTE- LAQV, Faculty of Pharmacy, University of Porto/Portugal Welcome Executive Board of Faculty – Marcela Segundo ; Faculty of Pharmacy, University of Porto/Portugal Welcome COST Action Chair – Dennis Eriksson ; Swedish University of Agricultural Sciences/Sweden
	09:30-10:00	Keynote: Dirk Bosch and Katarina Cankar ; Wageningen University/The Netherlands <i>Genome editing to improve health benefits of root chicory</i>
	10:00-10:20	Justyna Boniecka , Department of Genetics, Nicolaus Copernicus University in Toruń/Poland <i>CRISPR/Cas9-directed editing of RelA/SpoT Homologs in tomato (Solanum lycopersicum L.)</i>
	10:20-10:40	Zoe Hilioti , Institute of Applied Biosciences/CERTH/Greece <i>Tomato breeding by design using non-transgenic genome editing</i>
Session I – GE applications and molecular mechanisms	10:40-11:20	Coffee break and poster session
	11:20-11:40	Daria Navrotska , Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine/Ukraine <i>Brachypodium distachyon DOF transcription factor gene analysis and genome editing</i>
	11:40-12:00	Tjaša Lukan , Department of Biotechnology and Systems Biology, National Institute of Biology/Slovenia <i>CRISPR/Cas9-mediated miRNA editing in tetraploid potato</i>
	12:00-12:20	Yordan Dolapchiev , The Sainsbury Laboratory/United Kingdom <i>Efficient targeted gene insertions in diploid potatoes</i>
	12:20-12:40	Katrijn Van Laere , ILVO - Plant Sciences Unit/Belgium <i>CRISPR-based visualisation of centromere sequences in chicory</i>
	12:40-13:50	Lunch
Monday 18 Sept		Session Chair: Isabel Mafra
	13:50-14:20	Keynote: Sílvia Coimbra ; Faculty of Sciences, University of Porto/Portugal <i>CRISPR - Bridging fundamental knowledge and novel technology to increase rice heat tolerance</i>
	14:20-14:40	Cecilia Sarmiento , Tallinn University of Technology/Estonia <i>Optimized Lolium perenne L. protoplasts isolation and transformation for CRISPR-Cas9 downstream applications</i>
Session II – Improving resistance to abiotic stress	14:40-15:00	Muneeb Hassan Hashmi , University of Siegen, Siegen/Germany <i>Establishment of highly efficient and reproducible Agrobacterium-mediated transformation system for tomato (Solanum lycopersicum L.)</i>
	15:00-15:20	Luca Nerva , CREA - Research Centre for Viticulture and Enology/Italy <i>Improving grape resilience to climate change exploiting the CRISPR/Cas technology: different approaches to face drought</i>
	15:20-16:00	Coffee break and poster session
	16:00-17:30	PlantEd Working Group meeting (WG1-WG5 together)

Tuesday 19 Sept		Session Chair: Götz Hensel
Session III – Improved technologies	09:30-09:50	Hilal Betul Kaya , Manisa Celal Bayar University/Turkey <i>Optimizing protoplast isolation and transformation efficiency for enhanced plant genome editing in grapevine</i>
	09:50-10:10	William de Martines , Wageningen University and Research/The Netherlands <i>Exploring alternative approaches for efficient gene targeting in plants: high fidelity nonhomologous end-joining with CRISPR-Cas12a in potato protoplasts</i>
	10:10-10:30	Angelo Ciacciulli , CREA OFA Acireale/Italy <i>New genomic techniques in citrus, step-by-step solutions for more efficient and successful procedures</i>
	10:30-11:10	Coffee break and poster session
Session IV – Nutritional improvement and characterisation	11:10-11:40	Keynote: Nélide Leiva Eriksson ; University of Lund/Sweden <i>Nutritional enrichment of sweetpotato with highly bioavailable iron</i>
	11:40-12:00	Ellen Slaman , VIB-Ugent/Belgium <i>In-depth characterization of Cas9 specificity in tomato using high-throughput amplicon sequencing, GUIDE-seq and whole genome resequencing</i>
	12:00-12:20	Concetta Licciardello , CREA/Italy <i>A dual single-guide RNA approach used to edit the b-cyclase 2 gene in anthocyanin-rich sweet orange varieties</i>
	12:20-12:40	Fabio D'Orso , Research Centre for Genomics and Bioinformatics/Italy <i>HQT gene editing to study chlorogenic acid metabolism and its physiological role in tomato</i>
	12:40-13:50	Lunch
Tuesday 19 Sept		Session Chair: Vladislava Galovic
Session V – Improving resistance to biotic factors	13:50-14:20	Keynote: Johan Hunziker ; INRAE/France <i>Gene editing in potato to enhance PVY resistance</i>
	14:20-14:40	Éva Csaba , ELKH Centre for Agricultural Research/Hungary <i>Studying potato resistance and susceptibility factors against pathogens with the use of genome editing</i>
	14:40-15:00	Senne Van den Broeck , KU Leuven/Belgium <i>Gene editing in triploid banana cultivars</i>
	15:00-15:20	Kim Hebelstrup , Department of Agroecology, Aarhus University/Denmark <i>De novo domestication of wild tuber-bearing Solanum species</i>
	15:20-16:00	Coffee break and poster session
	16:00-17:30	Management Committee meeting
	19:30	Social dinner
Wednesday 20 Sept		Session Chair: Katrijn Van Laere
Session VI – Regulation and public perception	09:00-09:30	Keynote: Elke Vereecke ; EV ILVO/Belgium <i>Increase the production of industrially valuable compounds in the microalgae Chlorella – the GeneBEcon approach</i>
	09:30-09:50	Juan Vives-Vallés , University of the Balearic Islands - INAGEA/Spain <i>Preliminary analysis of the European Commission Proposal for a Regulation on the production and marketing of plant reproductive material</i>
	09:50-10:10	Tomasz Zimny , Institute of Law Studies, Polish Academy of Sciences/Poland <i>The new NGT legislation proposal of the European Union. Analysis of selected EU and national regulatory obstacles for the introduction and market viability of NGT plant products</i>
	10:10-10:30	Anna Linkiewicz , Cardinal Wyszyński University in Warsaw/Poland <i>The awareness of the Polish society on new genomic techniques</i>
	10:30-11:00	Coffee break and poster session

	11:00-11:15	Agnés Ricroch ; AgroParisTech and University of Paris Saclay/France <i>“Roadmap for Plant Genome Editing” – a Springer book production from PlantEd</i>
Session VII - STSM	11:15-11:30	Vladislava Galovic ; University of Novi Sad, Institute of Lowland Forestry and Environment/Serbia <i>Overview on the 4-year STSM activities</i>
	11:30-11:45	Alvaro Valenzuela , Fondazione Edmund Mach/Italy <i>Leveraging system biology and new breeding technologies for water stress tolerance in grapevines</i>
	11:45-12:00	Karam Mostafa , Ondukuz Mayıs University/Turkey and Agriculture Research Center/Egypt <i>Application of multiplexed CRISPR-ACT3.0 gene activation system in tomato roots for enhancing resistance against plant-parasitic nematodes</i>
	12:00-12:15	Kubilay Yıldırım , Ondokuz Mayıs University, Department of Molecular Biology and Genetics, Samsun/Turkey <i>Development of resistant sunflower lines to broomrape using crispr-cas9</i>
	12:15-12:30	Sara Yasemin , Siirt University/Turkey <i>Exploring the role of snrk2 genes in salinity stress response of Petunia axillaris through CRISPR-based genome editing</i>
	12:30-12:45	Poster prizes
	12:45-13:00	Closing of conference

Development of resistant sunflower lines to broomrape using CRISPR-CAS9**Kubilay Yildirim^{1,*}, İlkyay Sevgen Küçük¹, Musa Kavas², Dragana Miladinović³**¹Department of Molecular biology and Genetics, Ondokuz Mayıs University, Samsun, Turkey²Department of Agricultural Biotechnology, Ondokuz Mayıs University, Samsun, Turkey³Institute of Field and Vegetable Crops, Novi Sad, Serbia*E-mail: kubilay.yildirim@omu.edu.tr

Sunflower is one of the most important oil crops in the world that become a strategic plant due to the increased demand for its oil in recent years. Sunflower has low climate demand that enables it to grow in many regions of Europe. The biggest problem in sunflower cultivation is the presence of parasitic plants called broomrape (*O. cumana*). The seeds of these non-photosynthetic parasitic plants germinate with the secretion of Sesquiterpene Lactones (STL) from the roots of the sunflower. After attachment to the roots of the sunflower, it absorbs the water and all the nutrients from the host. Just one broomrape plant can produce millions of tiny seeds that can survive more than 15 years in the soil and can contaminate many fields in a region. Classical herbicides and mechanical techniques are not effective on these parasitic plants, since it already causes great damage to the plant when it rises above the ground. Many sunflower lines resistant to broomrape have been developed in last decades. However, the resistance of these lines was broken by emergence of new virulent broomrape strains. In recent years, secretion of Sesquiterpene Lactones (STLs) from sunflower roots has been found to trigger the germination of broomrape seeds. The genes encoding the enzymes (HaGAS, HaGAO, HaG8H, HaCOS) functional in STL biosynthesis in sunflower have been well characterized in recent years. In the light of all these information, genes of the enzymes that catalyze the production STLS was aimed to knockout with CRISPR/Cas9 technique in the project. It has been hypothesized that mutant sunflower lines developed in this way will have full resistance to broomrape. The sequences of four genes were retrieved from the database and processed with CRISPR-P 2.0 software to find out the best guide RNAs (gRNAs) that can target exon parts of the genes. By this way, four best gRNAs (one gRNA for each gene) were selected for simultaneous targeting of the first exon of the genes. All gRNAs were then transferred into a Cas9 containing agrobacterium plasmid (pHSE401) by using golden gate cloning. gRNA/Cas9 containing Agrobacterium (Gv3101) strains was treated to the seed, cotyledon and hypocotyl parts of the sunflower genotype. Tissue culture-based regeneration process has been established and first transgenic candidate seedlings were obtained in the current study. After obtaining fully regenerated mutants (T0) sunflower lines, transgenic ones will be selected with PCR and sequencing test. STL level in the roots of mutant line will be determined broomrape germination test will be applied to select the resistant genotypes. This is the first study developing broomrape resistant sunflower genotypes by using CRISPR genome editing system. Optimization of CRISPR mediated gene transfer and regeneration protocol will fasten and made important contribution to sunflower breeding. Genome editing-based strategies used to enhance crop resistance to parasitic weeds and its prospective applications will be discussed in the congress.

Funding: The project was supported financially by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) with a project number; TOVAG-1220340 and the Cost Action-CA18111 with STSM.