PlantEd

Genome editing in plants

Cost Action CA18111

4th PlantEd Conference

18-20 September 2023

Porto, Portugal

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EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

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Book of Abstracts

Book of Abstracts of the 4th PlantEd Conference

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

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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

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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
Session I – GE applications and molecular mechanisms	09:30-10:00	**Keynote: Dirk Bosch and Katarina Cankar**; Wageningen University/The Netherlands *Genome editing to improve health benefits of root chicory*
	10:00-10:20	**Justyna Boniecka**, Department of Genetics, Nicolaus Copernicus University in Toruń/Poland *CRISPR/Cas9-directed editing of RelA/SpoT Homologs in tomato (Solanum lycopersicum L.)*
	10:20-10:40	**Zoe Hilioti**, Institute of Applied Biosciences/CERTH/Greece *Tomato breeding by design using non-transgenic genome editing*
	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 *Brachypodium distachyon DOF transcription factor gene analysis and genome editing*
	11:40-12:00	**Tjaša Lukan**, Department of Biotechnology and Systems Biology, National Institute of Biology/Slovenia *CRISPR/Cas9-mediated miRNA editing in tetraploid potato*
	12:00-12:20	**Yordan Dolaptchiev**, The Sainsbury Laboratory/United Kingdom *Efficient targeted gene insertions in diploid potatoes*
	12:20-12:40	**Katrijn Van Laere**, ILVO - Plant Sciences Unit/Belgium *CRISPR-based visualisation of centromere sequences in chicory*
	12:40-13:50	Lunch
Monday 18 Sept		**Session Chair: Isabel Mafra**
Session II – Improving resistance to abiotic stress	13:50-14:20	**Keynote: Sílvia Coimbra**; Faculty of Sciences, University of Porto/Portugal *CRISPit - Bridging fundamental knowledge and novel technology to increase rice heat tolerance*
	14:20-14:40	**Cecilia Sarmiento**, Tallinn University of Technology/Estonia *Optimized Lolium perenne L. protoplasts isolation and transformation for CRISPR-Cas9 downstream applications*
	14:40-15:00	**Muneeb Hassan Hashmi**, University of Siegen, Siegen/Germany *Establishment of highly efficient and reproducible Agrobacterium-mediated transformation system for tomato (Solanum lycopersicum L.)*
	15:00-15:20	**Luca Nerva**, CREA - Research Centre for Viticulture and Enology/Italy *Improving grape resilience to climate change exploiting the CRISPR/Cas technology: different approaches to face drought*
	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 *Optimizing protoplast isolation and transformation efficiency for enhanced plant genome editing in grapevine*
	09:50-10:10	**William de Martines,** Wageningen University and Research/The Netherlands *Exploring alternative approaches for efficient gene targeting in plants: high fidelity nonhomologous end-joining with CRISPR-Cas12a in potato protoplasts*
	10:10-10:30	**Angelo Ciacciulli,** CREA OFA Acireale/Italy *New genomic techniques in citrus, step-by-step solutions for more efficient and successful procedures*
	10:30-11:10	Coffee break and poster session
Session IV – Nutritional improvement and characterisation	11:10-11:40	**Keynote: Nélida Leiva Eriksson**; University of Lund/Sweden *Nutritional enrichment of sweetpotato with highly bioavailable iron*
	11:40-12:00	**Ellen Slaman**, VIB-Ugent/Belgium *In-depth characterization of Cas9 specificity in tomato using high-throughput amplicon sequencing, GUIDE-seq and whole genome resequencing*
	12:00-12:20	**Concetta Licciardello**, CREA/Italy *A dual single-guide RNA approach used to edit the b-cyclase 2 gene in anthocyanin-rich sweet orange varieties*
	12:20-12:40	**Fabio D'Orso**, Research Centre for Genomics and Bioinformatics/Italy *HQT gene editing to study chlorogenic acid metabolism and its physiological role in tomato*
	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 *Gene editing in potato to enhance PVY resistance*
	14:20-14:40	**Éva Csaba**, ELKH Centre for Agricultural Research/Hungary *Studying potato resistance and susceptibility factors against pathogens with the use of genome editing*
	14:40-15:00	**Senne Van den Broeck,** KU Leuven/Belgium *Gene editing in triploid banana cultivars*
	15:00-15:20	**Kim Hebelstrup**, Department of Agroecology, Aarhus University/Denmark De novo domestication of wild tuber-bearing Solanum species
	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**
	09:00-09:30	**Keynote: Elke Vereecke**; EV ILVO/Belgium *Increase the production of industrially valuable compounds in the microalgae Chlorella – the GeneBEcon approach*
Session VI – Regulation and public perception	09:30-09:50	**Juan Vives-Vallés**, University of the Balearic Islands - INAGEA/Spain *Preliminary analysis of the European Commission Proposal for a Regulation on the production and marketing of plant reproductive material*
	09:50-10:10	**Tomasz Zimny,** Institute of Law Studies, Polish Academy of Sciences/Poland *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*
	10:10-10:30	**Anna Linkiewicz,** Cardinal Wyszyński University in Warsaw/Poland *The awareness of the Polish society on new genomic techniques*
	10:30-11:00	Coffee break and poster session

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

Oral presentation

Development of resistant sunflower lines to broomrape using CRISPR-CAS9

Kubilay Yildirim[1,*], İlkay Sevgen Küçük[1], Musa Kavas[2], Dragana Miladinović[3]

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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.

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