

Abstract Book



TRANSGENIC PLANTS & TRANSFORMATION TECHNOLOGIES VI

JULY 6 - 7, 2022, VIENNA, AUSTRIA



International Conference

Transgenic Plants & Transformation Technologies VI

Programme and Abstracts

Vienna, Austria

July 6 - 7, 2022

Organizing Committee

Local Organizing Committee	International Organizing Committee
Alisher Touraev (Local Organizer, Austria) Huw Jones (Conference Co-Chair, UK)	Kirankumar S. Mysore (USA): Yurong Chen (USA) Goetz Hensel (Germany) Matthew Milner (UK) Fredy Altpeter (USA) Huw Jones (UK) Stanton Bruce Gelvin (USA) Lan-Ying Lee (USA) Catherine Navarre (Belgium) Matthew Milner (UK) Linda Avesani (Italy) Goetz Hensel (Germany), Stephan Wenkel (Denmark)

Welcome to the 6th International Conference on "Transgenic Plants & Transformation Technologies"!

Plant Transformation and Transgenic Plants are nowadays core research tools to fight against hunger, to open up the new avenues of science, to evolve the crop varieties, which are multipurpose, producing high harvests under normal and natural stressful conditions to make Earth green and prosperous. The major technical challenge facing plant transformation is the development of methods and constructs to produce a high proportion of plants showing predictable and precise transgene expression without collateral genetic damage.

The 6th International Conference "**Transgenic Plants & Transformation Technologies**" to be held in **Vienna, Austria** on **July 6-7, 2022** will discuss current key objectives of the plant transformation technologies, including understanding and control of precise transgene integration, expression and stability in plants, improved transformation systems, address biosafety considerations and develop GM crops for the benefit of developing countries.

This two-day event will provide leading academy and industry scientists a platform to communicate recent advances in "**Transgenic Plants & Transformation Technologies**", and an opportunity to establish multilateral collaboration.

The **6th International Conference on "Transgenic Plants & Transformation Technologies"** will cover the following research topics:

- **Agrobacterium and Other Methods of Transformation**
- **Novel Plant Transformation Systym**
- **Transformation Elements: Vectors, Promoters, Selection Systyms**
- **Plastid Transformation & Molecular Pharming**
- **Marker Free Technologies**
- **Plant Transformation of Important Crops**
- **Genetic Engineering of Useful Traits**

Approximately 150 participants are expected to attend this exciting scientific forum including almost 30 lectures delivered by worldwide known invited speakers and young, talented speakers selected from submitted abstracts. The program combines plenary lectures, poster sessions, a unique Conference Dinner Party and sightseeing tours of Vienna.

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

Table of Contents

Scientific Programme.....	6
Abstracts of Oral Presentation.....	9
Abstracts of Poster Presentation.....	19
Publications.....	30
List of Poster Presentation.....	47
List of Participants.....	48

6th International Conference on “Transgenic Plants & Transformation Technologies”
(July 6-7)

July 6 (Wednesday)

08.00 - 17.00	<i>Registration</i>
	Opening
09.00 - 09.10	Welcome address by Alisher Touraev (Local Organizer, Austria) Welcome address by Huw Jones (Conference Co-Chair, United Kingdom)
	Keynote Lecture
09.10 - 10.25 (+5)	Huw Jones (UK) Cereal Functional Genomics using Transformation Technologies
10.30 - 11.00	<i>Coffee break</i>
<u>11.00 - 12.30:</u>	<u>Session I: Agrobacterium & Biolistic Plant Transformation</u>
<i>Chairs</i>	<i>Kirankumar S. Mysore (USA) & Yurong Chen (USA)</i>
11.00 - 11.25 (+5)	Kirankumar S. Mysore (USA): Agrobacterium expressing a type III secretion system delivers Pseudomonas effectors into plant cells to enhance transformation
11.30 - 11.55 (+5)	Yurong Chen (USA): Direct Germline Transformation of Cotton Meristem Explants With No Selection
12.00 - 12.20 (+5)	Andrea Saba Mayoral (Spain): Biolistic-mediated transformation of the Spanish elite rice genotype Bomba
12.25 - 14.00	<i>Lunch + Poster Session (all numbers), Conference Photo</i>
<u>14.00 - 15.20</u>	<u>Session II: Transformation of Important Crops</u>
<i>Chairs</i>	<i>Goetz Hensel (Germany) & Matthew Milner (UK)</i>
14.00 - 14.25 (+5)	Goetz Hensel (Germany): Genetic transformation of Triticeae cereals - Summary of almost three-decade's development.
14.30 - 14.55 (+5)	Matthew Milner (UK): From Delivery to Phenotype: Recent Improvements in Transformation and Gene Editing in Cereals
15.00 - 15.25 (+5)	Fang-Ming Lai (USA): Application of highly efficient assays for evaluating gene editing tools in caneberries
15.30 - 16.00	<i>Coffee break</i>
<u>16.00 - 17.35</u>	<u>Session III: Plant Transformation Elements</u>
<i>Chairs</i>	<i>Huw Jones (UK) & Goetz Hensel (Germany)</i>
16.00 - 16.20 (+5)	Stanton Bruce Gelvin (USA): Understanding the mechanism of T-DNA integration into the plant genome.
16.25 - 16.40 (+5)	Lennart Hoengenaert (Belgium): Transgene-free genome engineering in poplar through transient expression of Cas9-coding DNA
16.45 - 17.05 (+5)	Lan-Ying Lee (USA): Improving Agrobacterium-mediated delivery of genome editing reagents: Potential for improving T-DNA delivery and regulating T-DNA integration.

17.10 - 17.25 (+5)	Laura Hernández-Soriano (Mexico): Functional Analysis of Flowering Locus T During the Reproductive Transition in Agave tequilana
17.30 - 19.00	Welcome Reception + Poster Session (all numbers)
19.00 - 22.00	Conference Dinner Party Traditional Austrian food and wine, located in one of Vienna's famous 'Heurigen' Cost: 50,- EUR

July 7 (Thursday)

08.00 - 17.00	Registration
09.00 - 10.30	Session IV: Plastid Transformation & Molecular Pharming
<i>Chairs</i>	<i>Catherine Navarre (Belgium) & Linda Avesani (Italy)</i>
09.00 - 09.20 (+5)	Catherine Navarre (Belgium): Glycoengineering of Nicotiana tabacum BY-2 cells for pharmaceutical proteins production
09.25 - 09.45 (+5)	Linda Avesani (Italy): Plant Molecular Farming as a Strategy Against COVID-19 – The Italian Perspective.
09.50 - 10.05 (+5)	Wenshu He (Spain): A crucial first step in engineering nitrogenase in cereal crops: expression of functional Fe protein (NifH) in rice mitochondria
10.10 - 10.25 (+5)	Inger Holme (Denmark): Horizontal Stacking of PAPhy_a Cisgenes in Barley Is a Potent Strategy for Increasing Mature Grain Phytase Activity
10.30 - 11.00	Coffee break
11.00 - 12.30	Session V: Genetic Engineering of Useful Traits
<i>Chairs</i>	<i>Fredy Altpeter (USA) & Stephan Wenkel (Denmark)</i>
11.00 - 11.25 (+5)	Fredy Altpeter (USA): Towards Oilcane: Metabolic Engineering for Hyperaccumulation of Lipids in Sugarcane
11.30 - 11.55 (+5)	Xin Huang (Spain): Reconstitution of a heterologous crocin biosynthetic pathway in Nicotiana
12.00 - 13.30	Closing Ceremony + Lunch



Abstracts of Oral Presentations

JULY 6 - 7, 2022, VIENNA, AUSTRIA

Cereal Functional Genomics using Transformation Technologies

Huw D Jones

Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University
ABERYSTWYTH, SY23 3EB, UK

Correspondence to: huw.jones@aber.ac.uk

The use of reverse genetic approaches in cereals is now routine. In part due to developments in sequencing, bioinformatics, tissue culture and other molecular genetic tools that has occurred over the last 50 years. The optimisation of transformations protocols involves parallel and iterative changes in explant choice, pre-treatments, DNA delivery, regeneration and selection conditions. My presentation will describe work to develop robust transformation methods for wheat and some of the function genetic research that this enabled.

Engineering Agrobacterium to improve plant transformation and genome editing

Kiran Mysore

Dept. of Biochemistry and Molecular Biology, Institute for Agricultural Biosciences, Oklahoma State University,
3210 Sam Noble Pky, Ardmore, OK 73401 USA

Correspondence to: kmysore@okstate.edu

Agrobacterium-mediated plant transformation (AMT) is the basis of modern-day plant biotechnology. One major drawback of this technology is the recalcitrance of many plant species/varieties to Agrobacterium infection, most likely caused by elicitation of plant defense responses. Here, we develop a strategy to increase AMT by engineering Agrobacterium tumefaciens to express a type III secretion system (T3SS) from Pseudomonas syringae and individually deliver the P. syringae effectors AvrPto, AvrPtoB, or HopAO1 to suppress host defense responses. Using the engineered Agrobacterium, we demonstrate increase in AMT of wheat, alfalfa and switchgrass by ~250% - 400%. We also show that engineered A. tumefaciens expressing a T3SS can deliver a plant protein, histone H2A-1, to enhance AMT. This strategy is of great significance to both basic research and agricultural biotechnology for transient and stable transformation of recalcitrant plant species/varieties and to deliver proteins into plant cells in a non-transgenic manner.

Development of a high throughput and genotype flexible meristem-based transformation system in cotton (*Gossypium hirsutum* L.)

Yurong Chen

Bayer Crop Science, Plant Biotechnology, 700 Chesterfield Parkway West, Chesterfield, United States

Correspondence to: yurong.chen@bayer.com

Cotton is the most important fiber crop and the third largest oilseed field crop worldwide. Development of an efficient transformation and gene editing delivery system is critical to pest management, stress tolerance, yield improvement, and enhancement of quality traits through biotechnology and genome editing. Transformation through somatic embryogenesis has been the method of choice for many academic and industry laboratories since the regeneration of the first transgenic cotton plants. However, this system is genotype-dependent and has a long plant regeneration cycle with tissue culture-induced low fertility of regenerated plants. In contrast, transformation of meristem explants followed by organogenesis could improve this process. In this talk, the development of high throughput and genotype flexible meristem-based transformation system and demonstration of direct germline transformation of meristem explants with no selection will be described. The application of this system in the development of commercial products in the biotech pipeline and genome editing machinery delivery technology will be discussed.

Biolistic-mediated transformation of the Spanish elite rice genotype Bomba

Andrea Saba Mayoral

Universitat De Lleida, Etsea / Departament Pvcf , Lleida, Spain

Correspondence to: andrea.saba@udl.cat

We report the development of an efficient and reproducible genetic transformation system for the recalcitrant Spanish elite rice paella genotype, Bomba. Preconditioned embryos derived from dry seeds were bombarded with gold particles carrying a plasmid containing a screenable and a selectable marker. We confirmed integration and expression of hpt and gusA in the rice genome. Transformation frequency was ca: 10% in several independent experiments. We show Mendelian inheritance of the input transgenes and zygosity determination of the transgenic lines in the T1 generation. A unique and critical step for the regeneration of plants from transformed tissue was shading during the early stages of regeneration, combined with a specific cytokinin:auxin ration at the onset of shifting callus to regeneration media.

From Delivery to Phenotype: Recent Improvements in Transformation and Gene Editing in Cereals

Mathew Milner

Niab Transformation 93 Lawrence Weaver Road, Cambridge, United Kingdom

Correspondence to: matthew.milner@niab.com

Understanding how robust and translatable different technologies are to plant transformation is key in their adoption and efficacy. With the rapid adoption of CRISPR and its related components in both plants and animals a rapid expansion of different components and designed exist for editing genomes. While previous reports have shown that strong expression of the SpCas9 is crucial for efficient editing in cereals other components have been less well studied. This including understanding of the guide RNA and factors that influence their expression and editing. To understand the relative efficiency of editing in different crops rice, wheat and barley we transformed all three species with different guide delivery systems including a tRNA and ribozyme multiplex molecules to understand their role in successful editing of cereal genomes. Results from this comparison show differences in editing at the same target sequences amongst the species, as well as in differences in homeologue editing and overall efficacy for different systems in different species.

Genetic transformation of Triticeae cereals - Summary of almost three-decade development

Goetz Hensel^{1,2}

¹Centre for Plant Genome Engineering, Institute of Plant Biochemistry, Heinrich-Heine-University, Dusseldorf, Germany

²Centre of Region Haná for Biotechnological and Agricultural Research, Czech Advanced Technology and Research Institute, Palacký University Olomouc, Olomouc, Czech Republic

Correspondence to: goetz.hensel@hhu.de

Triticeae cereals are among the most important crops grown worldwide and used for animal feed, food and beverages. Although breeding of today's crops has evolved over the last ten thousand years, biotechnological methods could help speed up the process and introduce traits that are not possible with conventional breeding.

The genetic transformation of cereals was initially tricky because only a few explants were considered to regenerate new plants. Secondly, cereals do not belong to the natural hosts of the soil bacteria *Rhizobium radiobacter*. Nevertheless, several laboratories succeeded in developing efficient transformation protocols. Factors that play a role in this are the type of, for example, transfer of the functional elements and the choice and development of appropriate expression systems. Furthermore, selection markers are needed to favour the growth of transformed cells. Using reporter genes such as the *green fluorescent protein*, the success of the genetic modification can be monitored in a sample-friendly manner.

The transformation protocols developed in this way could be used for various applications. They include approaches to elucidate the molecular basis as well as practical applications. Methods of overexpression, gene silencing and, more recently, targeted gene modification using programmable endonucleases (TALEN, CRISPR/Cas) are used. The main objective of any breeding programme is to increase or at least maintain yields. The number of stems, the number of grains and their size play an important role here. In wheat, the focus is on baking properties. Other approaches try to understand the process of domestication or to make plants resistant to biotic and abiotic stresses. Finally, cereal grains can produce antigens and antibodies (molecular farming).

Application of highly efficient assays for evaluating gene editing tools in caneberries

Fang-Ming Lai, N. Wada, A. Taylor, H. Kim, N. Graham, R.D. Chauhan, M.R. Willmann, and A. Hummel.
Pairwise, 110 TW Alexander Dr., Durham, NC.

Correspondence to: flai@pairwise.com

Pairwise (www.pairwise.com) is actively developing and applying gene editing tools to generate genetic diversity and create better fruits and vegetables. Precision gene editing technology provides a great alternative to traditional breeding approaches but requires genome sequence information, effective gene editing tools, a robust editing tool delivery and transformation system, and a process to recover and validate plants edited for the intended trait. Because of the long timelines involved with transformation and regeneration of edited plants, it is critically important to assess the efficacy of editing tools in the target plant species before beginning full-scale product development. We have successfully developed and deployed rapid screening assays in multiple caneberry genotypes to test the efficacy of editing tools to support our product development pipeline. These rapid assay systems have facilitated optimization of editing tools in market-ready genotypes and significantly enhanced efficiencies of our gene editing platform in caneberries.

Understanding the mechanism of T-DNA integration into the plant genome

Stanton B. Gelvin

Purdue University, Biological Sciences, 201 South University St., West Lafayette, Indiana, United States

Correspondence to: gelvin@purdue.edu

The mechanism of T-DNA integration into the plant genome remains controversial. A non-homologous end-joining (NHEJ) pathway is used for integration. Our laboratory has shown that single or higher order mutants in both the classical (cNHEJ) pathway and the alternative NHEJ pathway maintain stable transformation frequencies similar to that of wild-type plants. These transformation results were confirmed by blotting of high molecular weight DNA from transformed but not selected plant tissues, and by droplet digital PCR. Recently, it has been proposed that DNA polymerase theta (PolQ) is required for T-DNA integration into the Arabidopsis genome. We examined both Arabidopsis *tebichi* (*teb/polQ*) and rice CRISPR-generated *polQ* mutants for transient and stable Agrobacterium-mediated transformation. These mutants show decreased transient transformation but significant levels of stable transformation. Droplet digital PCR analysis confirms T-DNA integration into plant DNA from both selected and non-selected infections. T-DNA/plant DNA junctions isolated from stably transformed rice and Arabidopsis tissues are similar to those from wild-type plants. Both rice and Arabidopsis *polQ* mutants show growth/developmental phenotypes that may be responsible for decreased transformation frequency. Taken together, these results indicate either that these various DNA repair pathways function redundantly, or that some yet unknown pathway is used for T-DNA integration into the plant genome.

Transgene-free genome engineering in poplar through transient expression of Cas9-coding DNA

Lennart Hoengenaert^{1,2,4}, Ruben Vanholme^{1,2}, Jan Van Doorselaere³, Wout Boerjan^{1,2}

¹ Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Gent, Belgium.

² Center for Plant Systems Biology, VIB, 9052 Gent, Belgium

³ VIVES University College, 8800 Roeselare, Belgium

Correspondence to: lennart.hoengenaert@psb.vib-ugent.be

The use of CRISPR/Cas9 is currently the method of choice for precise genome engineering in plants, including the biomass crop poplar. However, the presence of Cas9-expression cassettes within the genome of trees impedes their valorization and the ability to translate experimental findings to the field. Therefore, there is an urgent need to develop genome-engineered trees, without introducing Cas9-coding DNA into their genome. Here we developed a transient transformation strategy that allows regeneration of gene-edited Cas9-free poplars, using the microprojectile-based delivery of gene-editing reagents in callus. Together with a Cas9-coding DNA, an antibiotic resistance gene was delivered allowing a temporary selective treatment to enrich for (stably and transiently) transformed cells. In a second phase, regenerated plantlets were screened for the envisioned gene edits, and for the absence of Cas9 and vector DNA. As such, we obtained up to 4% of plantlets with simple INDELS in the target locus and that are devoid of Cas9-coding DNA. To further benchmark this promising method, we are further characterizing these lines via long read whole genome sequencing to unambiguously investigate the potential incorporation of foreign DNA.

Improving Agrobacterium-mediated delivery of genome editing reagents: Potential for improving T-DNA delivery and regulating T-DNA integration

Lan-Ying Lee

Purdue University, Biological Sciences, 201 South University St., West Lafayette, Indiana, United States

Correspondence to: lee34@purdue.edu

Since first described in 2012, CRISPR/Cas genome editing systems have been used broadly in plant research. Agrobacterium-mediated transformation (AMT) is still the most commonly used method to deliver CRISPR/Cas reagents efficiently into plants. T-DNA integration into the plant genome is frequently a natural consequence of AMT and is a key step in generating transgenic plants. However, because of regulatory concerns, plants containing genome editing transgenes may not be desirable. The Agrobacterium effector protein VirD2 plays an important role in both T-DNA transfer and in T-DNA integration. Our goal is to uncouple these two important functions of VirD2. We therefore generated VirD2 protein variants using PCR mutagenesis and screened Agrobacterium strains encoding mutant VirD2 proteins for their ability to effect transient transformation but with no/low levels of stable transformation. We subsequently tested these mutant Agrobacterium strains for their ability to generate mutations in *N. benthamiana* genes following delivery of T-DNA encoding Cas9 and sgRNAs. We identified VirD2 mutant strains that effected low levels of stable transformation but that could still support genome editing, although at reduced levels from that supported by strains encoding wild-type VirD2. We propose that these VirD2 mutants will be useful for genome editing in the absence of transgenesis, especially of plant species with long generation times or that are vegetatively propagated.

Functional Analysis of Flowering Locus T During the Reproductive Transition in Agave tequilana

Laura Hernández-Soriano

Cinvestav, Genetic engineering department, Libramiento Norte Carretera Irapuato, Irapuato, Mexico

Correspondence to: laura.hernandez@cinvestav.mx

Agave tequilana is exploited commercially in Mexico for the production of tequila. Moreover, this crop has acquired international relevance for inulin production and as a bioenergy source. Despite this, there are few reports on breeding and selection of characteristics of interest. This is mainly due to the morphology, long life cycles, and monocarpic nature of Agave species. Flowering time is a trait that could benefit from selective breeding since the initiation of flowering in commercial fields indicates readiness for harvesting and inflorescences are suppressed in order to conserve high levels of stored fructans. Transcriptome analysis of the reproductive transition in A. tequilana identified members of the Flowering Locus T (FT, a floral integrator) gene family. The purpose of the present study is to characterize the function of the FT homologs (AtqFTs) by overexpression in A. tequilana. To do this, a transformation protocol for A. tequilana has been established. Meristem explants were co-cultivated with A. tumefaciens and are regenerated by direct organogenesis using PPT as the selective agent. In addition to being one of the few reported transformation protocols for Agave species, this protocol will allow us to develop strategies for the improvement of Agave crops.

Glycoengineering of Nicotiana tabacum BY-2 cells for pharmaceutical proteins production

Catherine Navarre¹, Xavier Herman¹, Nicolas Bailly¹, Laurent Bouhon¹, Marie Peeters¹, Johann Far², Loïc Quinton², François Chaumont¹

¹Louvain Institute of Biomolecular Science and Technology, UCLouvain, Louvain-la-Neuve, Belgium

²Mass Spectrometry Laboratory-MoSys, GIGA Proteomics Facility, University of Liège, Liège, Belgium

Correspondence to: catherine.navarre@uclouvain.be

Recombinant glycoproteins can be produced in different heterologous systems such as insect, mammalian or plant cells. Different N-glycosylation profiles can be obtained depending on the cell type and culture conditions. Furthermore, N-glycosylation gives rise to a huge heterogeneity of the final product as each N-site can bear several different N-glycans. This heterogeneity is challenging for pharmaceutical glycoproteins since the presence or absence of N-glycan structures affects their optimal activity. Nicotiana tabacum Bright Yellow-2 (BY-2) suspension cells is a plant platform that allows growth in bioreactors and protein purification from the culture medium. Our research aims at generating a set of BY-2 cell lines with specific and well-defined glycosylation profiles using CRISPR Cas9 genomic edition of various glycosyltransferase genes. A BY-2 cell line producing humanized N-glycans was obtained through the inactivation of beta(1,2)-xylosyltransferase and alpha(1,3)-fucosyltransferase (FucT) genes. To generate a BY-2 cell line producing glycoproteins with oligomannoses, the N-acetylglucosaminyltransferase I (GnTI) genes were inactivated. A second genome editing targeting both GnTI and FucT genes was also achieved. The N-glycan repertoire analysis of glycoproteins secreted in those cell lines confirmed the absence of complex N-glycans. Only high-mannose N-glycans, mainly Man4 and Man5, were detected. In addition, a BY-2 cell line expressing glycoproteins with a single N-linked GlcNAc was also generated through the ectopic expression of a fungal endoglycosidase in the GnTI/FucT-KO line. Glycoproteins, such as antibodies and viral antigens, were successfully produced in these glycoengineered cell lines. This work was supported by grants from the Service Public de Wallonie in Belgium (WALInnov 1810010).

Plant molecular farming of Virus Nano Particles from diagnosis to therapy.

Linda Avesani

University Of Verona, Department Of Biotechnology, Verona, Italy

Correspondence to: linda.avesani@univr.it

Virus Nano Particles are proteinaceous nanomaterials that can be produced in plant systems by exploiting different strategies (stable or transient transformation). The flexibility of plant systems enables so far to produce a wide plethora of these nanomaterials making them useful in diverse applications ranging from the development of new materials to diagnostics and therapeutics. We will review the main uses of plant-made virus nanoparticles highlighting the tools available by plant biotechnology, the challenges associated and the potential applications. In particular, a focus on the use of these nano particles for autoimmune diseases diagnosis and treatment will be described.

A crucial first step in engineering nitrogenase in cereal crops: expression of functional Fe protein (NifH) in rice mitochondria

Wenshu He

Universitat De Lleida, Etsea / Departament Pvcf, Lleida, Spain

Correspondence to: he.wenshu@udl.cat

Biological nitrogen fixation is the conversion of atmospheric nitrogen to ammonia catalyzed by nitrogenase, which is widespread in prokaryotes, but not in eukaryote species. The integration of biological nitrogen fixation into cereal crops is a long-term goal of plant biotechnology that will allow the direct use of atmospheric nitrogen instead of synthetic and natural fertilizers. However, this is frustrated by the inability to express functional nitrogenase complex. The nitrogenase components such as Fe protein show complex metal cluster machinery, oxygen-sensitivity and sparingly solubility. We addressed these challenges by expressing Fe protein NifH from *Hydrogenobacter thermophilus* in rice mitochondria together with the putative peptidyl prolyl cis- trans isomerase NifM from *Azotobacter vinelandii*. Recombinant rice NifH was active at electron transfer to the iron-molybdenum protein and biosynthesis of the iron-molybdenum cofactor. The stable expression and activity of a nitrogenase component in rice represents a critical first step toward the expression of a functional nitrogenase complex, necessary to achieve biological nitrogen fixation in cereal.

Horizontal stacking of PAPhy_a cisgenes in barley is a potent strategy for increasing mature grain phytase activity

Inger Bæksted Holme, Claus Krogh Madsen, Toni Wendt and Henrik Brinch-Pedersen

Section Department of Agro Ecology, Research Center Flakkebjerg, Aarhus University, Slagelse, Denmark

Correspondence to: inger.holme@agro.au.dk

Mature grain phytase activity (MGPA) in the Triticea tribe cereals has evolved through gene duplications and neo-functionalization of the purple acid phosphatase phytase gene (PAPhy) in a common ancestor. Increased gene copy number of the PAPhy_a gene expressed during seed development has augmented the MGPA in cereals like rye and wheat. PAPhy_a phytase is highly stable and a potent enzyme in feed. However, barley only contains one HvPAPhy_a gene and the MGPA levels needs to be increased to substitute for the addition of microbial phytases to the feed. We previously obtained a substantial increase in MGPA for cisgenic barley with one extra homozygous HvPAPhy_a insert. In the present study, the gene dose response of phytase cisgenes from three different cisgenic barley plants were horizontally stacked. Cisgenic barley with 0, 1, 2, 3, 4 and 6 extra HvPAPhy_a inserts demonstrated a perfect positive linear correlation with the level of MGPA. The current study provides new insight into the potential of stacking of cisgenes in crops and suggests cisgene stacking as a versatile strategy for crop improvement.

Reconstitution of a heterologous crocin biosynthetic pathway in Nicotiana

Xin Huang

Universitat De Lleida, Etsea / Departament Pvcf, Lleida, Spain

Correspondence to: xin.huang@udl.cat

Crocins are high-value soluble pigments that are used as colorants and supplements, their presence in nature is extremely limited and, consequently, the high cost of these metabolites hinders their use by other sectors, such as the pharmaceutical and cosmetic industries. The carotenoid cleavage dioxygenase 2L (CsCCD2L) is the key enzyme in the biosynthetic pathway of crocins in *Crocus sativus*. In this study, CsCCD2L was introduced into *Nicotiana tabacum* and *Nicotiana glauca* for the production of crocins. In addition, a chimeric construct containing the *Brevundimonas* sp. β -carotene hydroxylase (BrCrtZ), the *Arabidopsis thaliana* ORANGE mutant gene (AtOrMut), and CsCCD2L was also introduced into *N. tabacum*. Quantitative and qualitative studies on carotenoids and apocarotenoids in the transgenic plants expressing CsCCD2L alone showed higher crocin level accumulation in *N. glauca* transgenic plants, reaching almost 400 $\mu\text{g/g}$ DW in leaves, while in *N. tabacum* 36 $\mu\text{g/g}$ DW was obtained. In contrast, *N. tabacum* plants coexpressing CsCCD2L, BrCrtZ, and AtOrMut accumulated, 3.5- fold compared to *N. tabacum* plants only expressing CsCCD2L. Crocins with three and four sugar molecules were the main molecular species in both host systems. Our results demonstrate that the production of saffron apocarotenoids is feasible in engineered *Nicotiana* species and establishes a basis for the development of strategies that may ultimately lead to the commercial exploitation of these valuable pigments for multiple applications.

Towards Oilcane: Metabolic Engineering for Hyperaccumulation of Lipids in Sugarcane

FREDY ALTPETER¹, Viet Cao, Guangbin Luo¹, Baskaran Kannan¹, Saroj Parajuli¹, Sofia Cano Alfano¹, Ratna Karan¹, Hui Liu², Eva Garcia-Ruiz³, Vijay Singh⁴, Huimin Zhao³, Steve Long⁵ and John Shanklin²

¹Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida, IFAS, Gainesville, FL

Correspondence to: altpeter@ufl.edu

Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like sugarcane has been proposed as a strategy to boost lipid yields per acre for biodiesel production. The energy content of plant oils in the form of triacylglycerols (TAGs) is two-fold greater compared to carbohydrates. However, vegetative plant tissues do not accumulate oil to a significant amount since fatty acid synthesis in these tissues serves primarily membrane construction, in addition TAGs undergo rapid turnover. Therefore, our objectives include metabolic engineering to:

- 1.) increase fatty acid synthesis,
- 2.) increase TAG synthesis from diacyl-glycerol and acyl-CoA
- 3.) optimize TAG storage
- 4.) minimize TAG hydrolysis in vegetative tissues.

Constitutive single or multiple gene expression/suppression cassettes were generated and co-delivered with the selectable nptII expression cassette by biolistic gene transfer into sugarcane callus. Plants were regenerated and analyzed for presence and expression of target constructs by PCR and RT-PCR, respectively. Quantitative real PCR was performed to study the level of gene expression in transgenic TAG plants in different vegetative tissues. Plants were analyzed for TAG content by gas-chromatography and mass spectrometry (GC-MS).

Accumulation of TAG to 8 % of leaf dry weight and total lipids to 13% of leaf dry weight were recorded. This is equivalent to 400-fold increase of TAG compared with non-transgenic sugarcane. Lines with low TAG accumulation co-expressed either fewer transgenes, or expressed the transgenes at lower levels. Results from the first field trial of oilcane will also be discussed. This research outcome will add value to the abundant sugarcane post-harvest residues for production of advanced biofuels.



Abstracts of Poster Presentations

JULY 6 - 7, 2022, VIENNA, AUSTRIA

Poster №1:

Genetic transformation of *Cannabis sativa* L.

Sinja Svetik, Nataša Hren, Špela Mestinšek Mubi, Jana Murovec
Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
Correspondence to: sinja.svetik@bf.uni-lj.si

Cannabis has great potential for use in various fields. Manipulation of its genome using transformation technology could improve the phenotype of plants. Hypocotyls were used to introduce the hptII selection gene and ZsGreen reporter gene into cannabis cells and further regeneration of transformed cells. Gene transformation was performed by coculturing hypocotyls with *Agrobacterium tumefaciens* LBA4404 for three days. Adventitious callus regeneration was achieved by growing explants on Murashige and Skoog medium supplemented with vitamins, 30 g/l sucrose, 8 g/l agar, 0.2 mg/l TDZ, 0.5 mg/l NAA, 200 µM acetosiringone and pH 5.8. The success of the transformation was confirmed with an epifluorescence microscope, as the regenerated hypocotyls glowed green fluorescently, expressed by the gene ZsGreen. In another experiment, we investigated whether the position of the hypocotyl explants affected adventitious regeneration on the same media. It was found that there were no differences in regeneration.

Poster №2:

Comparison of transformation using plasmids or RNP complexes

Špela Mestinšek Mubi, Sinja Svetik, Jana Murovec
Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
Correspondence to: spela.mestinsekmubi@bf.uni-lj.si

The study compared the transformation performance of protoplasts and microspores of different Brassica species with plasmids or ribonucleoprotein complexes (RNPs). Transformation with CRISPR plasmids expressing the Cas9 enzyme and guide RNAs has long been a well-known method, but these plasmids also have some limitations because their expression can be unpredictable. Genome editing with RNP complexes is more effective because it avoids the problems associated with using plasmids. Introduction into protoplasts and microspores was performed using PEG and electroporation, respectively. In the experiments with protoplasts or microspores, the results showed differences between transformation with plasmids or RNPs. It was found that transformation of protoplasts with plasmids or RNPs was more successful than transformation of microspores, and we observed that the success of transformation also depended on the Brassica species.

Poster №3:

CRISPR/Cas9 of GNTI to overcome previous limitations

Heidi Kaulfürst-Soboll

WWU Münster, Institute of Plant Biologie and Biotechnologie, Schlossplatz 7, Münster, Germany

Correspondence to: hfuerst@uni-muenster.de

N-glycosylation is an essential posttranslational modification important for folding, stability and functionality of proteins targeted to the secretory system, as e.g. the plasma membrane, cell wall or the lytic vacuole. Defects of N-glycosylation itself result in severe developmental defects, while the impact of Golgi-localized complex N-glycan modification is still not well understood in plants. Although rice was unable to reproduce when N-acetyl-glucosaminyltransferase I (GNTI, enzyme initiating complex N-glycan maturation in the Golgi) was knocked out, only minor effects were visible in dicots. Effects concerning root growth, salt sensitivity and flowering period were reported in *Arabidopsis gnt1* (*cgl1*) and a fruit ripening phenotype in GNTI-RNAi tomato, revealing changes in hormone cross talk. Occasionally we observed a higher sensitivity to pathogens, which is important for field performance. An RNAi-approach was also conducted in *N. tabacum* another Solanaceae. Although some phenotypes of these RNAi plants matched observations in other species, not all were linked to complex-glycan reduction. This prompted us to conduct a GNTI-CRISPR/Cas9 approach. Notably complete knock-out was obtained directly in the T0 generation. These GNTI-CRISPR lines and also back-crossed plants showed no obvious phenotypes in the greenhouse. First analyses will be presented.

Poster №4:

CRISPRing trees and maize for a climate-friendly economy

Chantal Anders^{1,2*}, Barbara De Meester^{1,2}, Ruben Vanholme^{1,2}, Wout Boerjan^{1,2},

¹Ghent University, Department of Plant Biotechnology and Bioinformatics, 9052 Ghent, Belgium

²VIB Center for Plant Systems Biology, 9052 Ghent, Belgium

Correspondence to: chantal.anders@psb.vib-ugent.be

Lignocellulosic material, such as wood and straw, is an attractive feedstock for the sustainable production of energy and bio-based chemicals. However, deconstruction of lignocellulosic biomass into fermentable sugars is hindered by the presence of lignin. Previously, the flux into the lignin biosynthesis pathway was reduced in *Arabidopsis* by knocking-out TRANSALDOLASE 2 (TRA2). Compared to the wild type, *tra2* mutants had 15% less lignin and enhanced processing efficiencies whilst growing normally. Here, we translated this strategy into the bio-energy crops poplar and maize using CRISPR/Cas9. As poplar is a heterozygous dicot with long generation times, while maize is a homozygous monocot with relatively short generation times, our strategy to generate *tra2* mutants differs significantly. Via *Agrobacterium*-mediated transformation of callus tissue, *tra2* knock-out poplars were generated. Considering the long generation times (~ 6 years), T0 *tra2* regenerant poplars, containing the stably integrated CRISPR/Cas9 construct, were vegetatively propagated and will be analyzed, as such, soon. Via *Agrobacterium*-mediated transformation of immature embryos, *tra2* knock-out maize was generated. Due to relative short generation times (~3 months), backcrossing T0 mutants and subsequent selfing of T1 allowed the selection of Cas9-free *tra2* T2 plants that will be analyzed soon.

Poster №5:

Properties of *Sarracenia purpurea* plants transformed with *Agrobacterium rhizogenes*.

Kinga Maria Pilarska, Magdalena Wróbel- Kwiatkowska

Correspondence to: kinga.pilarska@upwr.edu.pl

Insectivorous plants of the genus *Sarracenia* have been known for centuries for their health-promoting properties. They were used to treat among others bacterial and viral infections, gynecological diseases, glucose and insulin metabolism disorders, because, these plants possess valuable compounds such as betulinic acid, ursolic acid, betulin aldehyde, plumbagin or quercetin. The presented results were obtained from analyses based on *Sarracenia purpurea* plants transformed in in vitro cultures with *Agrobacterium rhizogenes* ATCC 15834 strain. The generated plants produced hairy roots, which show distinct phenotypic differences compared to the roots of unmodified plants. These plants were characterized by accumulation of valuable compounds with bioactive potential. Thus, the significant increase in the content of triterpenes, phenylpropanoids, carotenoids or photosynthetically active pigments was noted. The plants exhibit increased antimicrobial properties as well as improved antioxidant potential. Regeneration of microspores and protoplasts of *Brassica* species with or without PSK-alpha peptide

Poster №6:

Transgenic flax with improved salt tolerance and resistance to fungi

Magdalena Wróbel-Kwiatkowska*, Maja Słupczyńska, Waldemar Rymowicz

Wrocław University of Environmental and Life Sciences, Norwida 25, Wrocław, Polska

Correspondence to: magdalena.wrobel-kwiatkowska@upwr.edu.pl

Genetic engineering of flax (*Linum usitatissimum* L.) with medium-chain-length polyhydroxyalkanoate (mcl-PHA) synthase gene from *Pseudomonas aeruginosa* resulted in higher resistance of generated plants to biotic and abiotic stress. Transgenic lines showed 2-fold higher resistance to main flax pathogen i.e., *Fusarium oxysporum* and improved salt tolerance when it was compared to untransformed, control flax plants. Salt stress (100 mM NaCl) did not cause the chlorophyll and carotenoids degradation in genetically modified flax plants, which also exhibited higher biomass than control plants. The possible reason for this phenomenon might be the presence of mcl-PHA and improved anti-oxidative properties- ROS scavenging ability, measured for transgenic flax. The metabolism of the amino acids was also changed in generated engineered flax, indicating the decrease in glutamate content and slightly increase in proline and arginine amount, especially in a selected line with observed, the highest resistance to both analyzed stress conditions. Generated flax plants could be considered in the future as a source of the new cultivars, resistant to fungal infections and salinity.

Poster №7:

The role of cis-acting elements in the control of gene expression: restoring functionality of the rice psy1 gene promoter to accumulate endosperm carotenoids

Guillermo Sobrino Mengual

UNIVERSITAT DE LLEIDA, Etsea / Departament PVCF, Lleida, Spain

Correspondence to: guillermo.huertana@gmail.com

Carotenoids are tetraterpenoids which in plants are synthesized in plastids. Their biosynthesis involves the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) to generate the C20 intermediate geranylgeranyl diphosphate¹. Genetic engineering is a powerful tool to modulate endogenous biosynthetic pathways or introduce novel metabolic pathways in plants. Modulation of carotenoid biosynthesis is a major target for the improvement of staple crops due to the multiple nutritional and other health benefits of a number of such molecules for humans and animals^{2,3}.

Cereal endosperm-specific promoters contain cis-acting elements such as the P box and O2-box, 300 bp upstream of the transcriptional start site (the -300 core). The P-box is a 7 bp element recognized by the transcription factor PBF (P-box factor) and the O2 box is a 10 bp element recognized by the transcription factor Opaque-2. These transcription factors are only expressed in the endosperm, but they are also present in undifferentiated tissue allowing callus to be used as a test system for endosperm gene expression studies and functional characterization of candidate genes for metabolic engineering studies⁴. Many promoters contain similar sequences with one or two mismatches which do not permit binding of these transcription factors and reduce or abolish transcriptional activity of the corresponding promoters. We therefore hypothesized that these elements could be reactivated if the sequences were corrected. We tested our hypothesis using the reactivation of the rice Opsy1 promoter which controls expression of the phytoene synthase 1 (psy1) gene, in the rice endosperm. Co-transformation of the corrected version of the Opsy1 promoter together with the Ospds (under the control of the endosperm-specific D-hordein promoter) resulted in the accumulation of β -carotene in the endosperm of the resulting transgenic plants

Poster №8:

Engineering rice endosperm with an ectopic cytosolic MEP pathway induces profound metabolite and transcriptome changes

Guillermo Sobrino Mengual

UNIVERSITAT DE LLEIDA, Etsea / Departament PVCF, Lleida, Spain

Correspondence to: guillermo.huertana@gmail.com

In nature, there are 2 main biosynthetic routes of isoprenoid: mevalonate pathway (MVA), mostly present in most eukaryotes and some bacteria and the non-mevalonate pathway (MEP), which is present in bacteria, plants, and apicomplexan protozoa.

We expressed the E. coli MEP pathway in the cytosol of rice endosperm to ascertain whether this might increase the pool of isoprenoid precursors. We have recovered a transgenic rice callus combinatorial population which is now being characterized at the DNA level to determine the precise transgene complement in each line. This population will be subjected to transcriptomic and metabolomic profiling to understand the mechanistic basis of these genetic interventions.

Independent lines will be regenerated to intact plants to determine whether metabolic changes in callus and seed endosperm are congruent.

Poster №9:

Introducing an algal Carbon Concentration Mechanism into rice to enhance photosynthetic efficiency and productivity.

Ashwin Vargheese

UNIVERSITAT DE LLEIDA, Etsea / Departament PVCF, Lleida, Spain

Correspondence to: ashwin.vargheese@udl.cat

Increases in population growth, decreasing agricultural land and depletion of natural resources require substantial increases in crop productivity, in particular in developing countries. Considerable effort has been made towards enhancing the photosynthetic efficiency of staple crops to achieve higher yields through conventional breeding¹. However, gains have been modest, in part due to limited genetic variation in photosynthetic efficiency within breeding germplasm². The photosynthetic efficiency of C3 plants is far from its theoretical maximum in comparison to C4 crops. We describe ongoing experiments aiming to increase CO₂ uptake, by incorporating the *Chlamydomonas reinhardtii* Carbon Concentrating Mechanism (CCM) into rice^{3,4}.

[1] FAO (2017) The future of food and agriculture – Trends and challenges. FAO, Rome

[2] Simkin et al. (2019) Feeding the world: improving photosynthetic efficiency for sustainable crop production. *J Exp Bot* 70, 1119–1140

[3] Wang, Yingjun, and Martin H. Spalding. "Acclimation to very low CO₂: contribution of limiting CO₂ inducible proteins, LCIB and LCIA, to inorganic carbon uptake in *Chlamydomonas reinhardtii*." *Plant physiology* 166.4 (2014): 2040-2050.

[4] Nölke et al. (2019) The integration of algal carbon concentration mechanism components into tobacco chloroplasts increases photosynthetic efficiency and biomass. *Biotechnol J* 1800170

Poster №10:

Introduction of bacterial glycolate catabolic pathway into rice to enhance photosynthetic efficiency and productivity

Ashwin Vargheese

UNIVERSITAT DE LLEIDA, Etsea / Departament PVCF, Lleida, Spain

Correspondence to: ashwin.vargheese@udl.cat

Yield and productivity of many C3 crop plants are directly related to the carbon fixation activity of the photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). This chloroplastic enzyme is far from being efficient because it catalyzes two competing biochemical reactions, carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP). Under high CO₂ concentrations, RuBisCO favors the carboxylation of RuBP which leads to carbon fixation. However, lower CO₂ concentrations push RuBisCO towards oxygenating RuBP producing phosphoglycolate during photorespiration. Even though photorespiration recycles the toxic phosphoglycolate into phosphoglycerate, it is energetically expensive. Hence, reducing photorespiration increases carbohydrate metabolism and photosynthetic efficiency. A bacterial catabolic pathway bypasses the photorespiratory pathway and recycles glycolate into glycerate in an energy efficient manner in *Arabidopsis*¹ and potato². We describe ongoing experiments aimed at enhancing photosynthetic efficiency by engineering an *E.coli* glycolate catabolic pathway into rice. The introduced pathway comprises the *E.coli* glycolate dehydrogenase (three subunits D, E and F) with glyoxylate carboligase (GCL) and tartronic semialdehyde (TSR).

Poster №11:

Towards the engineering of a novel ectopic oxygen scavenging pathway to enhance the photosynthetic efficiency of rice

Wenshu He

Universitat De Lleida, Etsea / Departament Pvcf, Lleida, Spain

Correspondence to: he.wenshu@udl.cat

Increasing crop productivity is essential to address pressing challenges in agriculture due to current global population growth trends, rising carbon dioxide emissions, and loss of agricultural land. Photosynthetic carbon metabolism is a key factor in plant growth and biomass production. However, the efficiency of photosynthesis in C3 plants is far from its theoretical maximum. One of the major limitations of achieving optimum photosynthetic efficiency in a number of crop plants is that ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), which is the central enzyme in photosynthetic carbon assimilation catalyzes two competing reactions, carboxylation and oxygenation. The rates of these competing processes depend upon the relative concentrations of carbon dioxide and oxygen in the proximity of RubisCO. We describe ongoing experiments aiming to introduce a novel oxygen scavenging pathway into rice consisting of lactate oxidase (LOX), lactate dehydrogenase (LDH) and catalase (CAT), to enhance carbon dioxide uptake and thus photosynthetic productivity.

Poster №12:

Glycosylation analysis of SARS-CoV-2 receptor binding domain (RBD) produced in rice

Victoria Armario-Najera

Universitat De Lleida, Etsea / Departament Pvcf, Lleida, Spain

Correspondence to: victoria.armario@udl.cat

The rapid spread of COVID-19 has made necessary the development of diverse technology platforms for the production of vaccines and protein reagents for diagnosis or therapy. In this context, plants serve as a promising platform that offers low production cost, safety as well as easy and rapid scalability. The receptor-binding domain (RBD) of SARS-CoV-2 is involved in host cell recognition and is the first step of viral infection. This domain contains two N-glycosylation sites (N331 and N343) that are important for correct protein folding, stability and binding to the host cells. In this context, we have expressed a short version of RBD (amino acids 331 to 534) of SARS-CoV-2 in rice plants. We are currently investigating the N-glycosylation pattern of purified RBD in different rice tissues. The RBD gene sequence was expressed under the control of the constitutive Zea mays Ubiquitin 1 promoter (including intron 1). The rice alpha-amylase signal peptide was included at the N-terminal to direct the recombinant protein to the secretory pathway, and the His6x-tag at C-terminal for identification and purification. Preliminary results demonstrate that the RBD produced in rice callus is glycosylated and contains complex type N-glycans. Results from glycoproteomic analysis combined to LC-MS analysis will help us to determine specific site-occupancy as well as precise structure and composition of these complex N-glycans in each N-glycosylation site.

Poster №13:

Microbicidal activity of the Scytovirin domain 1 (SD1) produced in rice against HIV-1

Victoria Armario-Najera

Universitat De Lleida, Etsea / Departament Pvcf, Lleida, Spain

Correspondence to: victoria.armario@udl.cat

Lectins are carbohydrate-binding proteins that bind reversibly to specific glycan structures. Many lectins recognize the N-linked glycans present on viral glycoproteins and can therefore prevent viral proteins from interacting with receptors on the target cell surface, blocking the uptake of viral particles and interrupting the infection cycle. Scytovirin (SVN) is a lectin from the cyanobacterium *Scytonema varium*. The 95-amino-acid SVN polypeptide has a molecular weight of 9.7 kDa and contains two structural domains, namely SD1 (residues 1–48) and SD2 (residues 49–95), linked by five intra-chain disulfide bonds. SVN can neutralize HIV-1 by binding to gp120, gp160 and gp41. SD1 has a similar activity to full-length SVN, whereas SD2 is less potent than the full-length SVN and SD1. The domains have different affinities for HIV-1 and bind to carbohydrate ligands independently. We investigated the suitability of the SVN single-domain 4.5-kDa congener SD1 as a microbicide component by expressing the SD1 polypeptide in transgenic rice plants, directing the protein to accumulate in the endosperm. The crude extract containing SD1 was tested for HIV-binding activity. We also evaluated the ability of this crude extract to prevent HIV-1 infection in vitro using the TRO11 pseudovirus, which covers the diversity of most circulating strains of HIV-1 group M subtype B, as well as primary isolates of live HIV-1.

Poster №14:

Development of a platform for ornamental plant transformation and New Breeding Techniques (NBT)

Christina R Ingvarsdén, Inger Holme, Henrik Brinch-Pedersen

Dept. of Agroecology, Aarhus University, Slagelse, Denmark

Correspondence to: christina.ingvarsdén@agro.au.dk

We aim to develop a NBT platform for ornamental plants strengthening competitiveness in Danish ornamental breeding. We already succeeded with CRISPR/Cas9 editing in bellflower (*Campanula portenschlagiana*) inducing ethylene tolerance in flowers, dependent on the allele-dosage of Eil transcription factors. In a current project, we are pursuing to regulate branching and compactness in poinsettia (*Euphorbia pulcherrima*).

In the genus *Euphorbia*, a unique plant-microbe interaction exists between the plant and the bacterium phytoplasma, resulting in desirable branching and compactness. Transferring phytoplasma to each new generation after breeding is not an easy task and lower, bushier plants without phytoplasma is therefore of great interest for breeders.

Candidate genes are obtained from the literature and/or identified by RNAseq. Plant transformation is performed using *Agrobacterium* or protoplast culture. Overexpression and genome editing using CRISPR/Cas9 are used to develop high quality plants with a naturally bushy phenotype.

A compact phenotype produced without plant growth regulators is often advantageous and it might be very useful to transfer new characters to other ornamentals. As a starting point, we will use *C. portenschlagiana* as a demo-plant for Proof-of-Concept.

Poster №15:

Towards CRISPR/Cas9 mediated enhancement of β -carotene in maize endosperm

Xin Huang

Universitat De Lleida, Etsea / Departament Pvcf, Lleida, Spain

Correspondence to: xin.huang@udl.cat

Carotenoids play vital roles in humans and animals by providing essential precursors for vitamin A synthesis and the loss of provitamin A (mainly β -carotene) during storage reduces nutritional value substantially and in turn profoundly impacts the general health of a large number of populations in developing countries. In our previous work we showed that knocking-down of the endogenous ZmBCH2 expression by RNAi increased β -carotene accumulation in the endosperm of maize hybrids. ZmCCD1 transcript abundance negatively correlates with carotenoid content in maize endosperm. We hypothesized that ZmBCH2 and ZmCCD1 may be two important mediators of β -carotene degradation in the endosperm.

We will explore the enhancement of β -carotene accumulation in maize endosperm by knocking out endogenous ZmBCH2 and ZmCCD1 via CRISPR/Cas9. gRNA spacer sequences for the *Streptococcus pyogenes* (SpCas9) and *Staphylococcus aureus* Cas9 (SaCas9) based on the maize B73 reference genome sequence have been designed and driven by the maize U3 promoter. Co-transformation experiments are underway with six separate vectors containing the bar selectable marker gene, pAL76-SaCas9 and pJIT163-2NLS-SpCas9, four sgRNAs targeting two sites for each target gene (ZmBCH2 and ZmCCD1, respectively) to recover mutant plants with knock-outs in ZmBCH2 and/or ZmCCD1. In addition, we will determine endogenous carotenogenic gene and MEP pathway gene expression, as well as carotenoid profiles and non-targeted transcriptomes and metabolites in endosperm at different developmental stages in order to unravel the regulatory mechanisms of carotenoid degradation controlled by these enzymes (BCH2, CCD1).

Poster №16:

Characterisation of PHO1 knock-out lines generated by CRISPR/Cas9

Martin Friberg*, Shrikant Sharma, Niklas Olsson, Helle Turesson, Mariette Andersson, Per Hofvander
Swedish University of Agriculture, Department of Plantbreeding, Växtskyddsvägen, Alnarp, Sweden

Correspondence to: martin.friberg@slu.se

Plastidial starch phosphorylase (PHO1) has been a subject of study in many plant species, but its exact function has proven hard to pin down. It has been implicated in starch degradation, as well as starch chain initiation. It has even been suggested that it could be involved in photosynthesis, as a regulator of the Glc6P pool. In potato (*Solanum tuberosum*), RNAi lines were used to show that knocking out PHO1 reduces starch accumulation, but was not enough to completely suppress it. This suggests that if PHO1 is involved in starch chain initiation, there seem to be redundancies. To complicate things further, it has also been suggested that Pho1 might in-fact be two closely related genes, PHO1a and PHO1b. To gain a better understanding of its importance and complement older RNAi experiments we generated PHO1 knock-out lines using CRISPR/Cas9.

In this poster we present results that suggest that fully knocking out Pho-1 prevents formation of starch from Glucose-1-phosphate (G1P), but that a single wt allele might be enough for wt or near-wt phenotype. We also present DNA fragment analysis further supporting the idea that *S. tuberosum* carries more than one copy of PHO1.

Poster №17:

Molecular, biochemical and physiological characterization of *Medicago truncatula* mutants in sapogenin pathway obtained by different genetic engineering strategies

Maria Carelli, Massimo Confalonieri, Federica Terlizzi, Elisa Biazzi, Aldo Tava, Carla Scotti
CREA Research Centre for Animal Production and Aquaculture (CREA-ZA), Lodi, Italy
Correspondence to: maria.carelli@crea.gov.it

In the *Medicago* genus, triterpene saponins are a group of bioactive compounds extensively studied for their different biological properties; the modulation of saponin biosynthesis is then an interesting target for breeding and biotechnology approaches. In *M. truncatula* two different classes of saponin with specific biological activities are present: hemolytic and non-hemolytic saponins. In this work three *M. truncatula* mutants previously described obtained by different genetic engineering strategies (Iha mutant by Activation Tagging, AsOXA1 by gene overexpression and CYP93E2 by CRISPR/Cas9-mediated mutagenesis) and presenting a modified saponin pattern were tested under controlled conditions during the vegetative and reproductive phases. The traits evaluated were the sapogenin content and the expression levels of sapogenin pathway-genes in root and leaf tissues. Physiological parameters were also evaluated. Our findings provide interesting insights into the application of metabolic engineering of high-value plant secondary metabolites and a better understanding of the physiological functions of saponins in planta.

Poster №18:

SARS-CoV-2 RBD Plant Based Production for Serological Analysis

Mattia Santoni¹⁻², Elisa Gecchele¹, Riccardo Cecchetto³, Noemi Gutierrez-Valdes⁴, Anneli Ritala⁴, Annarita Mazzariol³ and Linda Avesani¹⁻².
1: Department of Biotechnology, University of Verona, Verona, Italy
2: Diamante Srl, Verona, Italy
3: Department of Hygiene and Public Sanitary, University of Verona, Verona Italy
4: VTT Technical Research Centre of Finland, Espoo, Finland
Correspondence to: mattia.santoni@univr.it

The coronavirus disease outbreak, caused by the SARS-CoV-2 is a significant threat to global public health, among its consequences, high-level expression of recombinant protein is essential for the economic production of viral proteins for different applications. For example, serological tests are useful tools to follow the humoral immunity generated by infection or vaccination. Plants have been used to produce antigens to be employed as diagnostic reagents and biopharmaceuticals from more than 30 years, in an approach named Plant Molecular Farming (PMF). This system can be used to manufacture reagents at scale in a timeframe of weeks, compared to months or even years for other heterologous systems. Further, plants can apply posttranslational modifications which are necessary for their biological activity.

In this framework, our aim is the plant production of a SARS-CoV-2 Spike (S) Glycoprotein portion known as the Receptor Binding Domain (RBD), to be used as a diagnostic reagent in serological tests. RBD is highly immunogenic and is the main target of neutralizing antibodies, hence a good candidate to investigate the humoral immunity. The plant made RBD (pRBD) was expressed in *Nicotiana benthamiana* plants by the magnICON® system, the purification from the transformed tissue yielded 19 µg/g LFW and the protein resulted to be stable over six months if stored at -80 degrees.

In a preliminary analysis, based on an indirect ELISA, the pRDB have been recognized from a total of nine COVID-19 patient's sera out of ten, defining the sensitivity of our test at 90%. While this first attempt provided a qualitative result, our goal, and work in progress, is the set-up of a quantitative test based on pRBD and its comparison with a commercial test already in use.

Poster №19:

Transient expression of five key genes of Artemisinin biosynthetic pathway to boost its content in *A. annua* plants

Fabio Pietrolucci (1), Stefano Negri (1), Carolina Ramos (1), Nicolò Giuliani(1), Mauro Commisso (1), Flavia Guzzo (1), Linda Avesani (1)

Dipartimento di Biotecnologie, Università di Verona, Strada le Grazie 15 – 37134 Verona, Italia.

Correspondence to: fabio.pietrolucci@univr.it

Artemisia annua is an herbaceous plant belonging to the *Artemisia* genus, one of the largest and most widely diffused genera of the family Asteraceae (Compositae). In modern times *Artemisia annua* earned great importance due to its strong antimalarial activity. Artemisinin (AN), a sesquiterpene lactone, is the bioactive compound responsible for this action against malarial disease and it has been isolated from *Artemisia annua* leaves. Nowadays artemisinin-based combination therapies have been recommended from WHO as treatments of malarial disease. Unfortunately, artemisinin yield from plants is rather low, ranging from 0,01% to 1,5 %. Artemisinin biosynthetic pathway involves five important genes: Farnesyl phosphate synthase (FPS), Amorphadiene synthase (ADS), Cytochrome P450 (CYP71AV1), Artemisinic aldehyde reductase (DBR2), Aldehyde dehydrogenase (ALDH1). Using the GFP as reporter gene, we previously developed an efficient protocol for transient expression in *A. annua* plants grown in vitro condition. We used this protocol to study the effect of the overexpression of all the five genes belonging to the biosynthetic pathway. We started by infiltrating only the first gene of the biosynthetic pathway (FPS) up to the simultaneous coinfiltration of all 5 genes. Through LC-MS analysis we monitored the levels of artemisinin and of the various intermediate compounds of the biosynthetic pathway in plant. This study will cast a light on artemisinin biosynthesis and its possible bottlenecks and could be useful to design an effective metabolic engineering strategy to boost AN content in plant. Here we show the result obtained up to this point.



Publications

JULY 6 - 7, 2022, VIENNA, AUSTRIA

Genetic Engineering in Potato: Gene Constructs, Vector and Transformation Method

Mukhtabar Tashmatova

Uzbek-Hungarian Scientific Center for Potato Growing under RIVMPC, Keles-Str. 119, Kuksaroy district, Tashkent region, Uzbekistan

Correspondence to: muhtabar.alisherovna@bk.ru

Potato (*Solanum tuberosum* L.) is a worldwide important crop plant that produces high nutritional quality tubers. It is the fourth most important staple crop (after wheat, corn, and rice) in terms of production and demand. Genetic transformation in potato has many benefits and advantages over conventional breeding methods, and potato was one of the first crops to be transformed. Thus, potato is a candidate of special relevance for improvements through conventional breeding and biotechnology. Improvements in methodology and transformation vectors have helped to increase the transformation efficiency and stable expression of transgenes in potato. There are many methods for improving potatoes through genetic transformation. Most of them include the genes of interest in the nuclear genome however, the development of plastid transformation protocols has expanded the available approaches to potato breeding. Although all methods have their advantages and disadvantages, *Agrobacterium* mediated transformation is the most used approach. Alternative methods such as particle bombardment, polyethylene glycol transfection of protoplasts, and microinjection are also effective. Regardless of the DNA delivery approach, a fast and efficient regeneration protocol and selection system are essential steps for successful transformation. Several critical factors affect transformation efficiency: vector type, insert size, *Agrobacterium* strain, explant type, subculture medium composition, selective agent, and others. Also, world attention is paid to examines the potato research carried out focused on producing lines resistant to pathogens and modification of potato physiology with the application of genetically modified crops for agricultural practices and public concerns are also discussed.

Rhaponticum carthamoides (Willd.) Iljin transformed root - influence of sucrose concentration on root biomass and caffeoylquinic acid derivatives and flavonoid production

Ewa Skala¹, Monika A. Olszewska², Joanna Makowczyńska¹, Agnieszka Kicel²

¹ Department of Biology and Pharmaceutical Botany, Medical University of Lodz

² Department of Pharmacognosy, Medical University of Lodz

Muszynskiego 1, 90-151 Lodz, Poland

Correspondence to: ewa.skala@umed.lodz.pl

Rhaponticum carthamoides (Willd.) Iljn. (Asteraceae) is an endemic and endangered species to Siberia. The roots and rhizome of this species have long been used in traditional folk medicine as stimulant, adaptogen, and tonic. The aim of the study was determine optimum sucrose concentration to ensure root growth and production of caffeoylquinic acid derivatives and flavonoid monoglycosides. The roots obtained after transformation with *Rhizobium rhizogenes* A4 strain were cultured in liquid Woody Plant medium supplemented with different sucrose concentration (0-7%), in light conditions (16/8h light/dark photoperiod), in flask (50 mL of medium) on a rotary shaker for 35 days. Among the tested sucrose concentration, the highest value of transformed root dry weight was obtained by using 7% sucrose (31 g/L). The sucrose also modified the phenolic compound metabolic pathway. The most important secondary metabolites in *R. carthamoides* transformed roots were a tentatively identified tricaffeoylquinic acid derivative, chlorogenic acid, 3,5-, 4,5-di-O-caffeoylquinic acids, and 1,4,5-O-tricaffeoylquinic acid. The medium supplemented with 3% sucrose was found to give the best yield of caffeoylquinic acid derivatives (511 mg/L) and flavonoids (39 mg/L).

Evaluation of germination rate of rice collection samples and varieties at low temperature

Qalandarov Bakhtiyor¹, Akhtamov Mirjalol¹

¹Rice Research Institute, Madaniyat-Str., 111506, Urtachirchik district, Tashkent region, Uzbekistan

Correspondence to: bakhtiyor1451@gmail.com

The rice plant is very prone to damage under the influence of low temperatures during the phases of germination, seeding, flowering and grain filling. This crop is mainly planted in April-May of spring. Because during these months, the temperature drops sharply at night or early in the morning. In such cases, the effect of low temperatures on the plants is primarily due to the germination phase of the rice plant. Under the influence of this factor, the field fertility of varieties decreases, plants grow slowly in the vegetative stage, plant mortality increases, the growth period is prolonged, root sterility increases in the reproductive stage, and yields are not as expected. In experiments on the germination of rice seeds at low temperatures in the laboratory, the methods used by scientists who have conducted research on this stress factor, Lee T., Fujina K. and others. In a specially insulated laboratory room brought to 15–16 ° C using an air cooler, 50 pieces of rice seeds were sown in Petri dishes in 2 reps and inspected daily for 8 days. The germination rate was determined and evaluated. According to the results of research, the local varieties of rice "Iskandar", "Mustaqillik", "Lazurniy" and "Tarona", which were initially used as control varieties at low temperatures, were compared. Initially, from 3DAI, the seeds of control varieties "Iskandar" and "Mustaqillik" began to germinate. In particular, 17% of Iskandar and 2% of Mustaqillik varieties had seed germination. Lazurniy and Tarona varieties started to germinate on the 4DAI. Among the control varieties on germination intensity, "Iskandar" variety showed the highest germination rate from 4DAI, germination rate was 98% on the 8DAI. Among the control varieties, the lowest germination rate was observed in the Tarona variety, where the seeds started germination with 3% at 4DAI, and an average germination rate of 68% at 8DAI.

Sub-cellular location of NtZIP5 protein in tobacco plants

Małgorzata Palusińska, Karolina Maślińska-Gromadka, Anna Barabasz And Danuta Maria Antosiewicz

Department of Plant Metal Homeostasis, Faculty of Biology, University of Warsaw, 1 Ilji Miecznikowa Str., 02-096 Warsaw, Poland

Correspondence to: m.palusinska@uw.edu.pl

ZIP proteins (ZRT-, IRT-like Proteins) are involved in the transport of micronutrients but also toxic metals through biological membranes. The ZIP proteins are very poorly characterized in tobacco (*Nicotiana tabacum*). The aim of this research was to understand the role of NtZIP5 in the regulation of metal homeostasis.

One of the basic tasks was to identify a membrane in which the NtZIP5 is located.

First, the open reading frame (ORF) of the NtZIP5 was amplified, cloned into the pENTR vector, and then into the pMDC43 target vector using the Gateway technique. Obtained construct 35S_{prom}: ZIP5: GFP (green fluorescent protein) was used for the transformation of *Agrobacterium tumefaciens*. Bacteria with the construct were used for transient expression in tobacco leaves by the infiltration method. A confocal microscope was used to determine the subcellular localization of NtZIP5-GFP fusion protein. Propidium iodide was used, to visualize the cell walls.

Co-localization of GFP (green signal) and propidium iodide (red signal) has been demonstrated. The results indicate that the NtZIP5 is targeted to the plasma membrane. Funding: project OPUS-8 no. 2014/15/B/NZ9/02303 supported by National Science Centre, Poland.

Multiplication of quality seeds of soybean variety "Sevinch"

Masudjon Sattarov¹, Ibragimov Feliks¹, Saitkhanova Raya¹, Tuygunov Nodir¹

¹Rice Research Institute, Madaniyat-Str., 111506, Urtachirchik district, Tashkent region, Uzbekistan.

Correspondence to: garasha76@gmail.com

Soybean grains contain 50% protein and up to 28% fat and are an important raw material in the food industry. Seed quality is known to be determined by its varietal and crop characteristics. According to the Law of the Republic of Uzbekistan "On Seed Production", the "crop characteristics" of seeds is a set of indicators that indicate the suitability of seeds for sowing. At the Agricultural Research Institute, scientific and practical research is constantly conducted. A new middle-ripening variety of soy Sevinch was created, included in the state register, and patented. Patent NAP №00263 01.03.2018 y. The description of the soybean variety Sevinch was created by individual selection at the Rice Research Institute. It was included in state register in the 2018. Growth period is 115-125 days. Plant height 145-150 cm, lower legume placement height 14-16 cm, number of branches 2-4, number of legumes per plant 120-130, number of grains per legume 2-4. 1000 grain weight 165-175g. The protein content of the grain is 42.6%, the fat content of the grain is 22.5%. Yield is 30-32 c/ha for grain. Blue mass 250-300 c/ha. Resistant to disease, pests and adapted to harvest using mechanization.

Methods of green biotechnology in in vitro cultures of *Salvia tesquicola* Klok. et Pobed

Julia Kowalska¹, Tomasz Kowalczyk², Karol Górski³, **Przemysław Sitarek** ^{1*}

¹Department of Biology and Pharmaceutical Botany, Medical University of Lodz, Poland

²Department of Molecular Biotechnology and Genetics, University of Lodz, Poland

³Department of Clinical Pharmacology, Medical University of Lodz, 90-151 Lodz, Poland

Correspondence to: przemyslaw.sitarek@umed.lodz.pl

Salvia tesquicola Klok. et Pobed. is a perennial herbaceous plant with height 300-600 mm belonging to the genus *Salvia* and Lamiaceae family. Apical inflorescence has 1 – 2 side branch (es). Leaves are folded, oblongly-oval, toothed on the sides, hoary. Stalk is also covered with filaments. The genus *Salvia* is a natural source of bioactive compounds widely used in medicine as an astringent, bactericidal, anti-inflammatory agent, anticancer compound although the species *Salvia tesquicola* has not yet been described in the literature [1-3]. The aim of this study is the first report on the establishment of *Salvia tesquicola* transformed root cultures via *Rhizobium rhizogenes*-mediated transformation and confirming the genetic transformation process by molecular analysis. Our research showed obtaining various in vitro cultures from this species, such as: shoots, cell suspension cultures, callus cultures and transformed roots. In vitro cultures were grown on various media with the addition of growth regulators in addition to the transformed roots. In addition, molecular analysis confirmed the transgenic nature of the transformed roots using PCR with specific primers for the *rolB*, *rolC*, *virG* and *aux* genes.

References

1. Kurkin VA (2004) Pharmacognosy: textbook for students of pharmaceutical universities (faculties). LLC "Etching", VPO "Samara state medical university", Samara, Russian Federation, 1180 p.
2. Murav'eva DA (1983) Tropical and subtropical medicinal plants. 2 th edition. Medicine. Moscow. Russian Federation.
3. Nikitin AA & Pan'kov IA (1982) Anatomical Atlas of some useful and poisonous plants. Nauka. Leningrad. Russian Federation.

Microclonal Reproduction of the Blueberry Plant

Yakubov Mirakbar¹, Vazira Utkirova²

¹Scientific Research Institute of Plant Genetic Resources, University-Str. 2, 100140, Kibray district, Tashkent region, Uzbekistan

²Center of advanced technologies, Talabalar shaharchasi-Str. 3A, 100174, Tashkent, Uzbekistan

Correspondence to: mirakbardan@yahoo.com

Cultivation of medicinal plants is one of the areas of continuous development of Agriculture. Countries that are advanced in the cultivation of blueberry have established a system of microclonal reproduction of blueberry. Due to the fact that our soil conditions are not suitable for vegetative reproduction of the blueberry plant, in vitro conditions are increased in alkaline environment. For the study, the extracts of the blueberry “Patriot” and “Bluecrop” varieties were sterilized and planted in sterile containers for cultivation in vitro conditions. The root formation was observed in the hormonal environment, and the composition of the harvested plants was adapted to alkaline (pH 4.5-4.7) soil, and for the first time in Uzbekistan in the method of biotechnology “Patriot” and “Bluecrop”, varieties of blueberry, were obtained and transferred to the soil. At present, these varieties have begun to bloom.

Activities of the research institute of vegetable, melon crops and potato in Uzbekistan

Nizomov Rustam¹, Rasulov Fakhridin¹

¹Research Institute of vegetable, melon crops and potato, Keles-Str., 111106, Tashkent district, Tashkent region, Uzbekistan

Correspondence to: uzrivmcp@mail.ru

The main purpose of the institute is to conduct research on improving the scientific and practical basis of breeding, seed production, agrotechnology of vegetable, melon and potato crops, as well as the development and implementation of scientific and technical products on this basis. The main directions of scientific research of the Institute:

- carrying out scientific research in the field of vegetable, melon and potato breeding and seed production;
- development and improvement of resource-saving technologies for growing vegetables, melon crops and potatoes;
- development of integrated methods of protection of crops from diseases, pests and weeds, as well as testing and introduction of new types of biological, chemical and other drugs entering the country;
- testing of new equipment and machines;
- participate in the study of storage and primary processing;
- training in cooperation with foreign and national higher education and scientific institutions, advanced training in foreign scientific institutions, universities and centers;
- obtaining and selling licenses in the manner prescribed by applicable law for the registration of NOU-XAU and breeding achievements in the country and abroad, the technology created and the activities required for licensing of high-quality vegetables, melon crops and potato seeds.

Molecular analysis of *Leonotis nepetifolia* (L.) R. Br. roots overexpressing the AtPAP1transcription factor and preliminary determination of their biological properties

Joanna Stelmach¹, Tomasz Kowalczyk², Joanna Wieczfińska³, **Przemysław Sitarek 1***

¹Department of Biology and Pharmaceutical Botany, Medical University of Lodz, Poland

²Department of Molecular Biotechnology and Genetics, University of Lodz, Poland

³ Department of Immunopathology, Medical University of Lodz, Lodz, Poland

Correspondence to: przemyslaw.sitarek@umed.lodz.pl

Leonotis nepetifolia (L.) R. Br. belongs to family Lamiaceae and it's an pantropical shrub, commonly known as Lion's ear. In Brazil, the plant is known also as "cordão de são francisco" or "cordão de frade". The plant has a traditional use in folk medicine for the treatment of some human diseases such as inflammations, bronchial asthma, wounds, gynecological diseases, skin infections, among others. The chemical analysis of this plant showed the presence of steroids, terpenoids, iridoids, phenylethanoids, phenols, flavonoids, phenolic acids, aliphatic esters and coumarins [1,2]. Our previous studies confirmed the presence of these compounds in transformed roots extract from *Leonotis nepetifolia* (L.) R. Br.

Additionally, we demonstrated various biological properties such as: antioxidant activity, as well as protection against DNA damage induced by H₂O₂ in HUVEC cells or cytotoxic activity against selected tumor cell lines [3].

The aim of our study was for the first time to obtain transgenic *Leonotis nepetifolia* roots with overexpression of the AtPAP1transcription factor, their molecular analysis confirming the occurrence of the transformation process and transgene expression in the plant material. In addition, the obtained extracts from the root showed biological activities related to the change of ROS levels in hydrogen peroxide-induced fibroblasts, inhibition of tyrosinase activity and changes in expression levels of pro- and anti-inflammatory genes.

References:

1. Danuta Sobolewska, Paweł Paśko, Agnieszka Galanty, Justyna Makowska-Wąs, Kinga Padło, Wojciech Wasilak, 2012. Preliminary phytochemical and biological screening of methanolic and acetone extracts from *Leonotis nepetifolia* (L.) R. Br.
 2. Phan Thanh Tung, Tran Hong Ngoc Quyen, Ton That Quang, Nguyen Thien Truong Van, Ngo Thi Thuy Duong, Vu Thi Tuyet Ngan, Lai Thi Nga, Nguyen Kim Phi Phung, Nguyen Thi Hoai Thu, 2020. Chemical constituents of the n-hexane extract of *Leonotis nepetifolia* (L.) R. Br (Lamiaceae)
 3. Tomasz Kowalczyk, Anna Merecz-Sadowska, Patricia Rijo, Vera M. S. Isca, Laurent Picot, Marzena Wielanek, Tomasz Śliwiński, Przemysław Sitarek, 2021. Preliminary Phytochemical Analysis and Evaluation of the Biological Activity of *Leonotis nepetifolia* (L.) R. Br Transformed Roots Extracts Obtained through *Rhizobium rhizogenes*-Mediated Transformation
-

Vegetative grafting of melon on vegetable marrow for increasing the yield and quality of fruits

Aminov Shermukhammad¹, Nizomov Rustam¹, Karimov Bakhtiyor¹, Kalantarov Sanat¹

¹Research Institute of vegetable, melon crops and potato, Keles-Str., 111106, Tashkent district, Tashkent region, Uzbekistan

Correspondence to: uzrivmcp@mail.ru

At the Research Institute of Vegetable, Melon Crops and Potato, for the first time, studies were conducted on the vegetative grafting of melon variety Kichkintoy on 10 varieties of vegetable marrow. Grafting in melon combinations on vegetable marrow had an impact on increasing plant growth and development, yield and fruit quality. According to the complex of economically valuable traits (early ripening on 4-5 days, early harvest yield (57-58%), excess of yield control by 22-28% and average fruit weight by 15-20 g, soluble solids up to 11.9%), combinations of melon variety Kichkintoy grafted on varieties of vegetable marrow Grecheskiye 110 and Unumdor from Uzbekistan, as well as varieties of Delicates and Skvorushka from Russia were revealed.

Transgenesis as a tool of biotechnology in in vitro cultures of *Phlomis tuberosa* L.

Piotr Siciński 1, Tomasz Kowalczyk², Anna Merecz-Sadowska³, **Przemysław Sitarek 1***

¹Department of Biology and Pharmaceutical Botany, Medical University of Lodz, Poland

²Department of Molecular Biotechnology and Genetics, University of Lodz, Poland

³ Department of Computer Science in Economics, University of Lodz, 90-214 Lodz, Poland

Correspondence to: przemyslaw.sitarek@umed.lodz.pl

The genus *Phlomis* L. (Lamiaceae) is represented by *Phlomis tuberosa* L. in the Bulgarian flora. In Asian folk medicine this specie is used as a general roborant, for intoxications, tuberculosis, pulmonary and cardio-vascular diseases and rheumatoid arthritis. Phytochemical analyses of *P. tuberosa* demonstrated the presence of flavonoids, polyphenolic compounds, alkaloids or iridoid glucosides [1,2]. One of the goals of biotechnology is to interfere with metabolic pathways that can increase the production of selected secondary metabolites in plants. An interesting example is the construction of a plant expression vector containing the ATPAP1 gene, also known as Arabidopsis Production of Antocyanin Pigment 1. Our earlier studies showed that obtaining transgenic hairy roots with overexpression of the AtPAP1 transcription factor resulted in increased production of phenolic acids [3]. The subject of our research was for the first time obtaining transgenic hairy root cultures of *Phlomis tuberosa* L. with constitutive overexpression of the AtPAP1 transcription factor, their molecular analysis, confirmation of transformation and expression of the transgene. Our research has shown the production of various in vitro cultures of the *Phlomis tuberosa* L. species and transgenic roots with overexpression of the AtPAP1.

1. Calis I, Kirmizibekmez H, Ersoz T, Donmez AA, Gotfredsen CH, et al. (2005) Iridoid glucosides from Turkish *Phlomis tuberosa*. Z Naturforsch B 60: 1295.

2. Stojanov N., Stephanov B. and Kitanov B. (1967), in: Flora Bulgarica. Nauka i Iskustvo, Sofia, p 904-905 (Bui.).

3. Sitarek P, Kowalczyk T, Rijo P, Białas AJ, Wielanek M, Wysokińska H, Garcia C, Toma M, Śliwiński T, Skąła E. Over-Expression of AtPAP1 Transcriptional Factor Enhances Phenolic Acid Production in Transgenic Roots of *Leonurus sibiricus* L. and Their Biological Activities. Mol Biotechnol. 2018 Jan;60(1):74-82. doi: 10.1007/s12033-017-0048-1.

The significance of applying nanoparticles on tomato

Kalantarov Sanat¹, Ibrokhimov Bakhodir¹

¹Research Institute of vegetable, melon crops and potato, Keles-Str., 111106, Tashkent district, Tashkent region, Uzbekistan

Correspondence to: uzrivmcp@mail.ru

Nano and microparticles play an important role in various biochemical processes taking place in plants. The physiological role of nanoparticles and microparticles for plants is that they participate in redox reactions and photosynthesis, increase resistance to adverse environmental factors, provide additional nutrients to the endogenous bacteria for nitrogen fixation in the atmosphere. Today in our country the synthesis of nano and microparticles is carried out on the basis of a simple and cost-effective method. Research results have shown that nano and microparticles; silicon dioxide (SiO₂), manganese dioxide (MnO₂) and iron oxide (Fe₂O₃) influenced the development of the tomato. That is, the use of nano and micro-particles, flowering and fruiting of tomato plants was ahead by 4-6 and 7-8 days, respectively, compared with untreated plants and increased tomato yield by 116,4-133,7%.

A stud of influence of sowing time, seed and mineral fertilizer rates on triticale "Sardor" variety productivity indicators

Rustamov Subxonberdi¹, Kushmatov Bakhtiyor¹

¹Bakhmal Scientific-Experimental Stations of Scientific Research Institute of Rainfed Agriculture, Uzbuloq-Str. 221, Bahmal district, Jizzakh region, Uzbekistan

Correspondence to: uzniizerno@yahoo.com

The study was conducted in 2019-2022 at the central experimental station of Rainfed Agricultural Research Institute of Gallaaral district of Jizzax region. Based on the results of the study, in the conditions of typical gray soils of Jizzakh region, the norm of sowing triticale "Sardor" in the autumn is 3,0 million seeds and mineral fertilizers (Fon) N₃₀, 9 pieces, average weight of 1 spike 0,9 g, number of productive stems per 1 m² 243 pieces, weight of 1000 pieces 37,2 g, grain yield average 20,8 quintals, In the spring, when the sowing rate is 3,5 million seeds and mineral fertilizers (Fon) N₃₀, the average yield is 10,4 cm, the average number of grains is 20,6, the average weight of 1 grain is 0,7 g, 1 m² The number of productive stems was 216, the weight of 1000 grains was 32.6 g, the average grain yield was 14,6 quintals.

Some ways to improve the ecological situation in conditions of water resources deficit

Sadikov Esbosin¹, Sagidullaev Amudulla¹

¹Scientific and Production Association "Grain and rice", Krantau village, 230910, Nukus district, Republic of Karakalpakstan, Uzbekistan

Correspond to: esbosin64@mail.ru

With intensive use of arable land, intermediate and saturating crops in the rice crop rotation contributes to the enrichment of the soil with plant residues in the form of roots and unused aerial mass. The plant mass of crops plowed into the soil is characterized by an increased content of nitrogen, phosphorus, potassium and other nutrients. It is evenly distributed over the soil profile and is easily susceptible to microbiological decomposition, releasing a large number of nutrients. Thus, in our studies, in one rotation after the main crops, more than 27.19 tons/ha of organic matter was accumulated in the arable horizon. This, in turn, contributes to saving expensive mineral fertilizers and pesticides by 35-40%, thereby creating a prerequisite for improving the environmental situation in the Aral Sea region.

Biomass indicators of drought-resistant varieties

Meyliev Akmal¹, Amanov Oybek¹, Kholik Allanov¹, Oybek Sottorov¹

¹Southern Research Institute of Agriculture, Karshi-Beshkent yoli-Str. 3, 180100, Karshi city, Kashkadarya region, Uzbekistan

Correspondence to: uzniizerno@yahoo.com

The dry land in our country is more than 750,000 hectares. In the dry lands of the country, soft wheat is sown in the autumn after the first rains, in the spring in late February and the first ten days of March. Therefore, it is desirable that the new varieties created for arable lands be drought-resistant, disease-resistant, productive, as well as varieties with high grain quality and high biomass in a short period of growth. One of the urgent tasks is to create and introduce new varieties of soft wheat, which are resistant to drought and rust, productive and high grain quality, due to the low rainfall in recent years in the dry lands, rising global temperatures as a result of global climate change. On Southern Research Institute of Agriculture FZ-2020072911 "Creation of new varieties of climate-resistant, disease-resistant, high-yielding, high-quality cereals and legumes, oilseeds and fodder crops for planting in dry lands of the Republic" in project fs part of the research on the selection of high-yielding and high-quality varieties and ridges of winter soft wheat suitable for climate change, 35 varieties and lines in a competitive variety test plot were placed in 3 rows, sowing area was 30 m² and tested.

Selection of spring wheat in Uzbekistan using SSR markers

Juraev Diyor¹, Boysunov Nurzod¹, Amanov Oybek¹

¹Southern Research Institute of Agriculture, Karshi-Beshkent yoli-Str. 3, 180100, Karshi city, Kashkadarya region, Uzbekistan

Correspondence to: boysunov5511@gmail.com

Currently, complex stress factors occurring in the Aral Sea region in Central Asia have a negative impact on people, plants and animals. In addition, in terms of size and main part, the entire Aral region corresponds to the Republic of Karakalpakstan. The total land area of the Republic of Karakalpakstan is 16656.1 hectares, of which 509.5 thousand hectares, or 3.06 % of the total area. Approximately 75.8 % of these irrigated areas are saline at various levels, of which slightly saline lands account for 30.0 %, medium saline lands - 36.9 %, and highly saline lands - 8.9 %. 65.4 % of the total area of irrigated agricultural land in this region belongs to the low-income group with humus, and the yield is on average 18-20 kg/ha. To solve these problems, scientists from the Southern Research Institute of Agriculture of Uzbekistan, using marker-based selection (mas), use SSR markers, new varieties and varieties of spring crops with a short growing season, high photosynthesis productivity, resistance to diseases, drought, soil salinity, high yield and grain quality is selected. Samples of spring wheat from the world collection are imported from the international centers ICA, CIMMYT, SSR markers are used to determine resistance to salinization and high-yielding samples adapted to local conditions are selected.

Research in the creation of cereals, legumes, fodder grass and oilseeds in the southern regions

Amanov Oybek¹, Meyliev Akmal¹

¹Southern Research Institute of Agriculture, Karshi-Beshkent yoli-Str. 3, 180100, Karshi city, Kashkadarya region, Uzbekistan

Correspondence to: mr.amonov1984@mail.ru

On Southern Research Institute of Agriculture cereals crops, legumes, fodder crops and oil crops are resistant to disease and pests, drought, heat tolerant 6805 hybrids, samples, constant lines and varieties are being studied for irrigated lands and 1223 for arable lands to create high-yielding and high-quality varieties. So, 2.816 varieties of winter wheat, 1.380 varieties of spring wheat, 2.529 varieties of durum wheat and 80 varieties of barley were irrigated and 250 varieties of winter wheat, 750 varieties of spring wheat, 93 varieties of durum wheat are being selected in the dry lands, 50 varieties of legumes, 100 varieties of lentils and 80 varieties of lentils in irrigated areas for the creation of varieties of legumes, 143 varieties of beans and 55 varieties of peas, 75 varieties of green beans, 66 varieties of lentils, 143 varieties and ranges of beans are been selected. Based on the results of the last 5 years, Yuksalish, Sarbon, Turan, Rokhat, Ishonch, Oqsaroy, Dovan, Istiqbol, Ziyokor, Shijoat, Sardor, Yangi hayot varieties of winter wheat, Navruz, Parvoz varieties of spring wheat, Nafis, Musaffo, Hilal of durum wheat, Sultan, Kamashi, Chimkurgan, Asia varieties of barley were created. Over the past years, the Institute has created 40 varieties of cereals, 12 of which are included in the State Register, and 8 varieties are being promoted, along with promising varieties. At present, Gozgan, Bunyodkor, Hisorak, Shams, Yaksart, Gallakor, Mingchinor, Langar varieties are cultivated on more than 300.000 hectares of the republic. Created by the Intellectual Property Agency of the Republic of Uzbekistan. Variety of Yaksart NAP-00121, Gozgan NAP-00123, Turkistan NAP-00120, Hisorak NAP-00122, Hazrati Bashir NAP-00124, Elamon NAP-00122, NAP-00184, Farovon NAP-00183, Bunyodkor NAP-00182, Barkhayot NAP-00181, Janub gavkhari of spring soft wheat NAP-00180, Langar variety of durum wheat NAP-00185, Mingchinor variety NAP-00176 and variety of barley Voxa NAP-00195 patents were granted.

Determination of variability of traits of hybrid alfalfa combinations

Turdishev Bekmurad¹, Saypnazarov Gengebay¹, Berdikeev Baymurza¹

¹Karakalpak Scientific Research Institute of Agriculture, Xodja Axmedov-Str. 1, 231407, Jambas-jap district, Shimbay city, Republic of Karakalpakstan, Uzbekistan

Correspondence to: qqditi@iim.uz

Alfalfa is an important agricultural crop in Uzbekistan - it is the basis for the development of field crop rotations and providing all types of animals with protein feeds. The productivity of this crop, along with agrotechnical factors, mainly depends on the characteristics of the cultivated varieties and their seed quality. We have studied 14 hybrid combinations of the third generation of alfalfa.

The most valuable forms obtained from third-generation hybrids increased the number of productive and resistant plants; with free re-pollination, they increased their adaptability to the terrain. In terms of seed productivity, they exceeded such hybrid combinations as: Mexican x Indian, Tokmak x Afghan, Kherson x Tashkent-721 by 8-13%. Experimental data from our breeding works with alfalfa hybrids have proved that in most cases, success in breeding depends on the correct selection of the original parent forms, hybrids from which have a fairly high plasticity, providing better adaptability to the extreme conditions of the Aral Sea.

Assessment of Complex Valuable Economic Characteristics of Winter Bread Wheat (*Triticum aestivum* L.) under Drought and Hot Conditions of the Republic of Uzbekistan

Dilmurodov Sherzod¹, Oybek Amanov¹, Diyor Juraev¹, Akmal Meyliev¹

¹Southern Research Institute of Agriculture, Karshi-Beshkent yoli-Str. 3, 180100, Karshi city, Kashkadarya region, Uzbekistan

Correspondence to: s.dilmurodov@mail.ru

In the arid and hot climate of the southern regions of the Republic of Uzbekistan in the conditions of light gray soils, the complex valuable properties of local lines and varieties of winter bread wheat were assessed. To test under these conditions, 22 local bread wheat lines were selected from the selection nursery and compared with 3 local check varieties. Yaksart, Gozgan, Tanya varieties, which are grown in large areas in the dry and hot conditions of the southern regions of the Republic, were taken as local check varieties. During the experiment, soil composition, moisture, micro and macro elements were determined. Also, the effect of air temperature, relative humidity and rainfall on the valuable characteristics of varieties and lines was determined by determining the air temperature, relative humidity and rainfall in the year in which the experiment was conducted to determine the level of drought and heat. Development phases of winter bread wheat varieties and lines: sowing-germination days (SGD), germination-tillering days (GTD), tillering-shooting days (TShD), shooting-heading days (ShHD), germination-heading days (GHD), heading-maturing days (HMD), germination-maturing days (GMD) the effects of dry and hot conditions were determined. It also studied the variability of valuable properties such as plant height (PH), grain yield (GY), test weight (TW), protein content, gluten content, in dry and hot conditions. The results showed that the KR17-F6-BWYT-Plot-166 and KR17-F6-BWYT-Plot-171 lines were selected, which had a higher performance than the local check varieties and did not reduce their valuable properties in dry and hot conditions.

Specificity of rice breeding in Karakalpakstan

Abdullayev Bahodir¹, Abillaev Urazbay¹

¹Scientific and Production Association "Grain and rice", Krantau village, 230910, Nukus district, Republic of Karakalpakstan, Uzbekistan

Correspondence to: abdullayev.bakhadir@mail.ru

The rice massifs of Karakalpakstan are located in the lower reaches of the Amu Darya River on saline desert lands in a zone of sharply continental climate. Most of the irrigated lands in the north of Karakalpakstan are subject to severe and moderate salinization of chloride and sulfate type. The soil with low fertility is saline with groundwater, located close, unsuitable for the cultivation of cotton, wheat, etc. crops relatively insufficient duration of the thermal period in the spring - autumn period of the year. The presence of sharply differing complex natural stress factors, the shortage of irrigated water, especially its late arrival in the region significantly limits the possibility of expanding rice crops. All these factors dictate the need to create new rice varieties better adapted to local stressful conditions, imposes special requirements for the creation of highly productive ultra-ripe and precocious long-lake varieties valued on the world market. Using the latest achievements of modern methods of genetics, genomics and breeding on the basis of many years of breeding work, we have created ultra-ripe varieties "Sanam", "Gulistan" and "Almaz". These varieties are characterized by good plasticity and respond positively to the improvement of the growing condition. In production conditions, for 90-95 days of the growing season, these varieties form a yield of 65-75 kg / ha, on highly saline soil after the development of puffy salt marshes, they are able to produce 40-45 kg/ ha of grain yield.

Selection of rice varieties in extreme conditions of Karakalpakstan

Utambetov Duysenbay¹, Ganibay Khojambergenov¹

¹Scientific and Production Association "Grain and rice", Krantau village, 230910, Nukus district, Republic of Karakalpakstan, Uzbekistan

Correspond to: dusenboy1960@mail.ru

In our research, the direction concerns the selection of high-yielding, low-water-consuming, precocious varieties. During the growing season of rice, we conducted phenological observation on 5 varieties ("Gulistan", "Charm", "Innovator", "Alanga", "Azure") upon the onset of the phase of development of various rice varieties. If we analyze the duration of the growing season of the tested varieties, then the ultra-ripe variety "Gulistan", of local origin, ripened 4 and 7 days earlier than the precocious varieties "Charm" and "Innovator" of Russian origin. And in the late-ripening group, the "Alanga" and "Azure" varieties are 18-25 days late. This indicator, in turn, affects the flow of irrigation water. The data obtained show that the period of germination and the field germination of seeds are insignificant, i.e. within 0.9-3.9 pcs. m², and 0.2-0.7%, respectively. The tillering coefficient was from 1.32 to 1.42% for rice varieties. Based on the data obtained, it can be said that the precocious variety "Gulistan" ripens earlier than other varieties by 4-7 days and yields more by 2-9 kg / ha. With the saved water will be about 4.5 thousand m³ / ha. The variety "Gulistan" is offered for production as a re-sowing after winter wheat.

Analysis of heritability coefficients and correlation of some morphological characteristics of cotton varieties and lines

Ravshanov Azam¹, Malokhat Khalikova¹, Anastasiya Bakirova¹, Otabek Akhmedov¹

¹Cotton Breeding, Seed Production and Agrotechnologies Research Institute, University-Str., Kibrai district, Tashkent region, Uzbekistan

Correspondence to: kurbonov.abrorjon@mail.ru

Scientists, based on the genetic analysis of quantitative traits, propose to divide them into two groups: the characteristics of the first group (productivity and number of boxes) are influenced by numerous polygenes, showing a strong epistatic influence and the effect of dominant alleles. At the same time, paratypical variants are high, and heritability is low. The signs of the second group (the size of the box, the length and yield of the fiber, etc.) are controlled by small polygenes that have a weak effect. At the same time, the paratypical variation will be a little low, and the heritability will be a little high. From the data we have obtained, it can be seen that in different soil and climatic conditions, the level of balance of the trait is more variable and there is a change in the influence of genes on the development of the trait. Under the influence of adaptation to environmental conditions, there is a shift of indicators to the right side. The sign of the exit of the fiber from the environment of a set of polygenic traits has a relatively high heritability. According to the correlation between the precocity and the weight of raw cotton of one box, such a picture was also noted. In most cases, a weak or medium negative correlation was observed between the studied traits of varieties and lines.

Evaluation of breeding hybrid material for resistance to *Verticillium dahliae* Klebahn using phytoimmunity enzymes

Avtonomov Victor¹, Akhunov Ali², Khoshimova Nigora², Kayumov Umid¹

¹Research Institute of Breeding, Seed Production and Agrotechnology of Cotton Cultivation, 111218, University-Str., Kibray district, Salar city, Tashkent region, Uzbekistan

²A. Sadykov Research Institute of Bioorganic Chemistry, 100143, Mirzo Ulugbek-Str., Tashkent city, Uzbekistan.
Correspondence to: avtonovvik@mail.ru

As a result of a biochemical assessment using marker enzymes of phytoimmunity – peroxidase, semiofinoloxidase and phenylalanine ammonia-lyase, where the injection of seven-day seedlings by a group of aggressive *V. dahliae* strains isolated from a provocative field site was used as a stress load. Among 29 hybrids and breeding materials, the following are highly resistant to verticilliosis by fungus *V. dahliae*: F1 Sulton x Namangan-34, F2 136 x Namangan-77, F3 Carmen x Namangan-77, L-6207, L-6300, L-6386 with which it is advisable to carry out breeding work on infected backgrounds, in order to create highly resistant varieties to the above pathogen. At the same time, it is necessary to regularly use the monitoring system of the cotton resistance developed at the Institute of Biorganic Chemistry.

Prospects for the introduction of fine-fiber cotton varieties in the Republic of Uzbekistan

Boykhonova Gulniso¹, Avtonomov Victor¹, Ravshan Azam¹, Kayumov Umid¹, Elonov Muhammad¹

¹Research Institute of Breeding, Seed Production and Agrotechnology of Cotton Cultivation, 111218, University-Str., Kibray district, Salar city, Tashkent region, Uzbekistan

Correspondence to: gboykhonova@gmail.com

Within the framework of the innovative project I-KX-2021-493 in 2021, the variety of fine-fiber cotton Surkhan-103 was sown on an area of more than 1000 hectares in extreme conditions of the Surkhandarya region, where, according to the Presidential Decree, an elite seed farm was opened. It was found that the variety combines high precocity and the rate of return of the entire harvest of raw cotton no later than October 10, has a high fiber yield of +3.1% to the grade-standard Surkhan-14, surpasses it in total yield by an average of 2.6 c/ha, and in fiber by 3.2 c/ha, while it has precocity of 111-116 days, highly resistant to high daytime temperatures, harmsily – hot winds and lack of irrigation moisture during formation, accumulation of raw cotton and fiber quality at the level of the best Egyptian varieties. In connection with the above, the Surkhan-103 variety is recommended for introduction into production not only in Surkhandarya, but also Kashkadarya, Bukhara, Navoi and Namangan regions.

The degree of population variability of varieties of *G. Hirsutum* L. in different climatic conditions

Malohat Khalikova¹, Azam Erkinov¹, Viktor Avtonomov¹, Gulniso Boikhonova¹, Sharofiddin Karimov¹

¹Research Institute of Breeding, Seed Production and Agrotechnology of Cotton Cultivation, 111218, University-Str., Kibray district, Salar city, Tashkent region, Uzbekistan

Correspondence to: halikovamalohat@rambler.ru

To determine the degree of variability in the population of cotton varieties resulting from adaptation to different climatic conditions and the disclosure of the influence of this variability on the change in the phenotypic structure of the population, morphological characteristics of local cotton varieties of the species *G. hirsutum* L. in various soil and climatic conditions were studied. The dynamics of the variability of morphological traits of the studied varieties in different years has been determined, the degree of heritability and interrelation of morphological traits has been studied, the ratios of typical plants and modifiers (phenotypic groups) in the population of varieties and lines have been determined, the degree of variability has been comparatively analyzed. The object of the study were varieties Omad, Akkurgan-2, S-01, Bukhara-6, Bukhara-102 and lines L-001, T-100 of the cotton species *G. hirsutum* L. When growing varieties Omad, Akkurgan -2, S-01, Bukhara-6, Bukhara-102 and lines T-100, L-001 in different soil and climatic conditions for several years, there were differences in quantitative indicators of a number of morphological traits, their variability and the level of formation of atypical plants. It was revealed that when cultivating zoned varieties and new cotton lines in different soil and climatic conditions, their populations split into different biotypes according to morphological characteristics, i.e. there are different phenotypic groups that negatively affect their uniformity.

The effect of the drug Zem-M on the biochemical processes of the goose

Karimov Sharofiddin¹, Bakirova Anastasiya¹

¹Research Institute of Breeding, Seed Production and Agrotechnology of Cotton Cultivation, 111218, University-Str., Kibray district, Salar city, Tashkent region, Uzbekistan

Correspondence to: karimovsharofiddin78@gmail.com

From the nuts analyzed in our experiments, it was found that the activity of the peroxidase enzyme was high in the variants treated with Baikal EM1 and ground-M to Baikal 2,0 l/t during the coronation period and 2,0-2,0 l/h during the growing season. This is due to the fact that in the above variants, certain norms of biologically and physiologically active substances indicate the synthesis of the necessary phenolic compounds during the coronation of the larynx, as well as the acceleration of processes such as breathing, photosynthesis. It also increases the plant's resistance to various diseases and pests of insects. It was found that the activity of the peroxidase enzyme in the leaf samples obtained during the flowering period of the goose was higher than control in the variants used in the norms of Baikal EM1 and ground-M seeds 2,5-3,0 l/t and in the flowering-flowering-flowering periods 2,0-2,0 l/ha. It can be said that the studied ground-M stimulator increased the activity of the peroxidase enzyme and, in combination with the acceleration of the periods of honing and flowering of the goose, there was a decrease in the activity of such properties as mitigation of the negative effects of external environmental factors.

Large scale and effective in vitro clonal propagation of 45 famous world grapes

M.Inoyatova¹, U.Atabayeva², E.Strelkovskix³, **Alisher Touraev^{2,4}**

¹National University of Uzbekistan. 4 University street, Tashkent city, 100174 Uzbekistan
maftuninoyatova23@gmail.com

²Tashkent state Agrarian university. 2 University street, Tashkent region, Kibray district, 100020 Uzbekistan

³LTD "De Nova Agro", 2 University street, Tashkent region, Kibray district, 100020 Uzbekistan

⁴National Center of knowledge and innovation in agriculture. 2 University street, Tashkent region, Kibray district, 100140 Uzbekistan

Correspondence to: alishertouraev@yahoo.com

The research included the transplantation of young meristem tissues of 22 varieties of the most popular orchards and 23 varieties of vine grapes from different countries, which are considered the most valuable and unique in the world market. Alcohol, alkaline solution, distilled water were used for sterilization. The process was performed in laminar boxes in a special sterile environment. Sterilized implants were now transplanted into ½ MS containing artificial nutrient media and after 15 days of incubation, 1 mg/l -1 BAP and 0.1 mg/l -1 NAA hormonal nutrient medium in the MS medium were selected for the callusogenesis phase by experiments. For rhizogenesis, 4 experiments were also performed and 0.01 mg/l-1 BAP and 0.1 mg/l-1 IBA prepared in an MS environment. For elongation 0.5 mg/l-1 BAP and 0.1 mg/l-1 NAA nutrient medium were selected as the optimal option. During the process, it was intended to select common nutrient media as an alternative option for 45 different grape varieties and positive results were obtained as a result of the studies. Grapes planted to rhizogenesis were planted in a peat mixture for acclimatization with a 98% adaptation to the environment. So from our experience, the goal was achieved and we were able to get positive results.

Improvement of technology of selection and cultivation of seedlings of pecan nuts in the Surkhandarya region

Tulashev Fahriddin

Scientific Research Institute of Horticulture, Viticulture and Winemaking named after Academician M. Mirzaev, Chimkent tract-Str., 111118, Gulistan village, Tashkent district, Tashkent region, Uzbekistan

Correspondence to: bogdod92@gmail.com

In accordance with the results obtained from the experimental work carried out on varieties and samples of pecan nuts in the current year, the scientific Experimental Station of the Surkhandarya region, we have distinguished and achieved the intended goal of Pecan varieties "Kapalak", "Fahr" and "Gigant" with their resistance to diseases and pests, high yield, high yield of kernels, high content of precipitation and several in the budding of pecan seedlings, the use of well-developed two-year seedlings gives a good effect. Water is poured into the grafted seedlings, and as a result, the capture of hybridizations is accelerated. Hybridization works are considered a favorable time in the summer, if the 3-th decade of August and the 1-th decade of September, the 3-th decade of February in the spring and the 1-th decade of March. Grafted seedlings of quality pecan nuts will be ready in 3 year. The gardens formed from them begin to yield in 3-4 years. The possibilities of obtaining environmentally friendly natural pecan nuts are sought in the natural soil-climatic conditions of the same region with high fertile varieties and distinguished by selecting specific varieties suitable for the environment, and agrotechnology of the cultivation of fruits and seedlings is developed.

Useful model for eliminating salination and increasing soil fertility

Rakhmatov Bakhtiyor

Scientific Research Institute of Breeding, Seed Production and Agricultural Technology of Cotton Growing,
Bukhara Scientific Experimental Station, Bukhara, Uzbekistan

Correspondence to: BaxtiyorRaxmatov2306@gmail.com

The article provides data on the development of eliminating salinity and increasing soil fertility, by driving exhaust gas using the «USEFUL MODEL», the H_2CO_3 formed under the soil reduce the degree of salinity and improve the neutralization of the pH of the environment, improving the assimilability of plant phosphorus and soil fertility, while obtaining a high-quality crop. New ecological, water and resource-saving technologies have been developed with the help of the «Useful Model» for collecting exhaust gas in Uzbekistan, to improve the neutralization of the pH of the environment from 9.1 to 7.3, reduce the degree of salinity 35-40%, save rinse water by 35-40 %, increase soil fertility, assimilability of phosphorus, yield (+ 15 + 25.5 c / ha) and quality fibers.

The importance of groups in growing local and introduced peach varieties in Uzbekistan

Rasulov Jamshed¹, Sanaev Sobir¹, Amanturdiyev Ilhom¹, Isroilova Muhayo¹

¹Scientific Research Institute of Horticulture, Viticulture and Winemaking named after Academician M. Mirzaev,
Chimkent tract-Str., 111118, Gulistan village, Tashkent district, Tashkent region, Uzbekistan

Correspondence to: Jamshed.Rasulov.81@mail.ru

The cost of fruit of low-growing trees is lower than the cost of fruit of strong-growing trees, 1.5 - 2.0 times less labor is required to harvest 100 kg, and the yield is 1.5 - 2.0 times higher than that of ordinary orchards. The downside is that small fruit trees do not live very long, often. It is wrong to look at it from this point of view, because dwarf fruit trees produce the same amount of fruit as tall-fruited fruit trees, which live 45-50 years during their 25-30-year life cycle. At the same time, gardening, built from stunted fruit trees, allows you to quickly replace old varieties with valuable new varieties. If the trees do not produce a lot of fruit every year and the branches do not grow strong, the trunks of small trees will not grow and as a result will live a long, 25-30 years. Small tree orchards have a number of positive features, such as early harvest, high yield, quality of fruits, ease of care for trees, and so on. When connected to low-growing grafts, fruit seedlings are planted up to 2000–3000 bushes per hectare and yield up to 50-60 tons per hectare. Accelerated grafting of fruit crops is carried out mainly in the laboratory by vegetative means, i.e. by microclonal method of obtaining plant tissue. This process begins with the cultivation of healthy tissues isolated from the plant under sterilized conditions in a specially prepared nutrient medium. Laboratory-grown plants grown in a special nutrient medium are initially planted in cassettes designed for seedling cultivation. It is then grown in three stages in a climate-controlled nursery. In the first stage, the nursery will receive 14 hours of light per day, humidity 90-100%, air temperature 21-26°C for 21 days, in the second stage for 21 days, 14 hours of light per day and humidity in the nursery 70-85%, air temperature 24- In the third stage, it is grown in the nursery for 90 days with 14 hours of light per day, humidity of 50-55%, air temperature of 25-30°C. In our experiments, the aim was to determine the optimum air humidity and temperature when growing promising fruit grafts such as Garnem, GF-677, Myroblan C-29, which are used as grafts for cultivated fruit crops such as peaches, nectarines, plums and almonds.

Diseases faced by Lemon Plants

Boyjigitov Fozil¹, Tosheva Yoqutoy²

¹Scientific Research Institute of Horticulture, Viticulture and Winemaking named after Academician M. Mirzaev, Chimkent tract-Str., 111118, Gulistan village, Tashkent district, Tashkent region, Uzbekistan

²Scientific Research Institute of Quarantine and Plant Protection, Uzbekistan

Correspondence to: fboyjigitov80@mail.ru

During the observations, samples of lemon plants from its feathers and leaves infected with diseases were delivered to the laboratory. Fungi provoking fusarium, alternarium and anthracnose were isolated from damaged parts of lemon in the nutrient medium of potato-dextrose agar in laboratory conditions, and studies were conducted to determine their species composition. On a lemon plant, the disease provokes 1 type (Ascomycota), 2 classes (Sordariomycetes, Deuteromycetes), 3 families (Nectriaceae, Glomerallaceae, Pleororaceae), 3 categories (Fusarium, Colletotrichum, Alternaria), 3 units, i.e. Fusarium oxysporum f. citri, Colletotrichum gloeosporioides and Alternaria citri fungi were detected using a microscope. Among these diseases, the most dangerous are fusarium (gommosis, glue leakage), Ascomycota type, Sordariomycetes class, Nectriaceae family, fusarium belonging to the Fusarium f category. Citri has been proven to provoke fungi. Fusarium is also oxysporum f. high consumption was tested against the growth of the pathogen citri- the drug benomil 0.3% of the norm showed the highest result.

High-quality and high-yielding varieties of autumn wheat

Komilov Muhiddin¹, Siddiqov Ravshanbek¹

¹The Research Institute for grain and leguminous crops, Andijan street 39, Kuyganyor village, Andijan Region, Uzbekistan

Correspondence to: leomiha22@gmail.com

In the Republic of Uzbekistan, resource-intensive agricultural technology has been developed for growing high-quality grain crops from grain crops with an ear in a separate region for new varieties due to weather and climatic conditions. The following is recommended: The northern region of the Republic - the Republic of Karakalpakstan, Khorezm region, in the conditions of autumn frost-resistant, drought-resistant local Asr, Davr, Zvezda, Uzbekistan - 25 varieties belonging to the Krasnodar selection Thunder, Pervitsa, Krasnodar-99, Zimnitsa, Tanya, Alekseich, Antonina, Bezostaya-100, varieties of the herd; Southern region - early and mid-ripening, heat-resistant, drought-resistant, disease-resistant local Yaksart, Andijan-4, Chillaki, Yagdu, Gazgan, Turkestan, Elomon, Asr, Denov-1, Babur, Zvezda, Esaul, as well as related to the Krasnodar selection Krasnodar-99, Thunder, Winter, Bezostaya-100, Alekseich, Antonina varieties. Central region Tashkent, Syrdarya, Jizzakh, Samarkand, Navoi Bukhara regions, Andijan-2, period, Durdona, Chillaki, Andijan-4, Zvezda, Babur, matonat, Karadarya, Uzbekistan-25, Yaksart, Gazgan, Dostlik, Elomon and Yesaul, as well as related to the Krasnodar selection of Yesaul, Krasnodar-99, Grom, Alekseich, Antonina, Bezostaya-100, Herd, Pervitsa, Vassa, Brigade. Ferghana Valley is one of the local varieties in the Andijan, Namangan, Ferghana regions, ASR, Andijon-4, Bobur, Davr, Durdona, Zvezda, Chillaki, Egdu, Yzbekiston-25, Yaxart, Tanya, related to the Krasnodar selection Tanya, Thunder, Krasnodar-99, Pervitsa, Vassa, Gurt, Alekseich, Antonina, Bezostaya-100.

Biotechnological indicators of domestic and foreign soybean varieties

Siddiqov Ravshanbek

¹The Research Institute for grain and leguminous crops, Andijan street 39, Kuyganyor village, Andijan Region, Uzbekistan

Correspondence to: ddeiti-19@mail.ru

When studying the adaptability of local and foreign shade varieties in meadow soil conditions, there are from early Prudence to 18.5 c/ha, from Saline to 18.6 c/ha, from Pripyat to 18.7 c/ha, from Genetik-1 to 18.7 c/ha, from Menorah to 18.8 c/ha, from Sirelia to 19.5 c/ha, from the Memory of Fadeev to 19.6 c/ha, 23.2 c/ha, 25.7 c/ha from the Gavhar (Diamond) variety, 25.8 c/ha of the Slavia variety, 25.8 c/ha of the SK Veda variety, 30.2 c/ha of the Chara variety, 25.8 c/ha of the medium-maturation variety, obtained 26.6 c/ha of the Vilana variety, 32.0 c/ha of the Tamaris Man-60 variety. Of the mass of 1000 pieces of grain in foreign and local soybean varieties were 156 grams in varieties of precocious Prudence, 150 grams in varieties of Solena, 158 grams in varieties of Pripyat, 155 grams in varieties of geneticist-1, 160 grams in varieties of menor, 155 grams in varieties of Sirelia 165 grams in varieties of memory 150 grams, 192 grams in the medium-ripened variety, 135 grams in the Vilana variety, the Ta'maris man variety-60, 170 grams.

Creation of high-yielding local wheat varieties

Rahimov Tojiddin¹

¹The Research Institute for grain and leguminous crops, Andijan street 39, Kuyganyor village, Andijan Region, Uzbekistan

Correspondence to: tojiddin958@umail.uz

The main directions of the development of grain crop production in the republic were conducted research on the creation of new varieties of grain crops with high and high-quality grain yield, as well as the organization of their seed production and the development of cultivation technology. In recent years, a sharp change in weather conditions as a result of global climate warming in most regions of the world has led to a decrease in crop production in agriculture. However, the population's demand for nutritious, fertile products is steadily growing. In studies when brown rust disease was observed in the navel and new lines, the disease was not observed in the template Chillaki variety on the Peterson scale of plants affected by fungal impulses, in the Sharof-100 variety 15% were infected. In the case of brown rust, 25% of the AC-2012-D3 line and 30% of the AC-2013-D9 line were observed. On the other lines, the beetle is clearly infected with rust. According to frost resistance, it was found that the lines AC-2013-D30, AC-2013-D33 have 9 points - high resistance, in general, all plants in the field are in a healthy state.

In vitro propagation of *Ajuga turkestanica* (Regel) Briq. and *Codonopsis bacteriana*

Gafurov Gafur

National Center for Knowledge and Innovation in Agriculture, Sh. Rashidov-Str. 1, A. Yassaviy settlement, Yuqori Chirchiq district, Tashkent region, Uzbekistan

Correspondence to: g.gafurov@yahoo.com

Ajuga turkestanica (Regel) Briq. and *Codonopsis bacteriana* are a rare and medicinal plant endemic to Uzbekistan. The aim of this study was to achieve the restoration of the number of boths plants in nature using the meristematic parts of boths plants using an in vitro clonal reproduction system and to establish a plantation. To do this, *Ajuga turkestanica* and the apical meristem of *C. bacteriana* plants with young cells was collected from nature. Specially sterilized implants were transplanted in a sterile environment in laminar boxes in nutrient media free of ½ MS – containing hormones. For these implants, a 15-day incubation period was maintained in sterile conditions in special culture rooms with a temperature of 22° and 16 hours of light per day. Both plant transplants were then cultured in MS medium for callusogenesis and auxin in the amount of 0.1 mg/l⁻¹ NAA was added to each auxin 1mg/l⁻¹ BAP was inoculated into cytokinin containing media containing 2 mg/l⁻¹ BAP, 5mg/l⁻¹ BAP. These experiments were kept in incubation for 25 days and a culture medium containing 2 mg/l⁻¹ BAP with 0.1 mg/l⁻¹ NAA was selected as a most effective nutrient for the callusogenesis process from among the hormonal options. The next step was to determine the nutrient medium specific to the rhizogenesis process for the plants. The next stage is to plant the rooted plants in the mind for 1 moths, then in the greenhouse and finally in the open ground.

List of Poster Presentations

Poster number. Author and title	Page in Abstract book
Poster №1. Sinja Svetik Genetic tranformation of Cannabis sativa L.	19
Poster №2. Spela Mestinsek Mubi Comparison of transformation using plasmids or RNP complexes	19
Poster №3. Heidi Kaulfürst-Soboll CRISPR/Cas9 of GNTI to overcome previous limitations	20
Poster №4. Chantal Anders CRISPRing trees and maize for a climate-friendly economy	20
Poster №5. Kinga Maria Pilarska Properties of Sarracenia purpurea plants transformed with Agrobacterium rhizogenes.	21
Poster №6. Magdalena Wróbel-Kwiatkowska Transgenic flax with improved salt tolerance and resistance to fungi	21
Poster №7. Guillermo Sobrino Mengual The role of cis-acting elements in the control of gene expression: restoring functionality of the rice psy1 gene promoter to accumulate endosperm carotenoids	22
Poster №8. Guillermo Sobrino Mengual Engineering rice endosperm with an ectopic cytosolic MEP pathway induces profound metabolite and transcriptome changes	22
Poster №9. Ashwin Vargheese Introducing an algal Carbon Concentration Mechanism into rice to enhance photosynthetic efficiency and productivity	23
Poster №10. Ashwin Vargheese Introduction of bacterial glycolate catabolic pathway into rice to enhance photosynthetic efficiency and productivity	23
Poster №11. Wenshu He Towards the engineering of a novel ectopic oxygen scavenging pathway to enhance the photosynthetic efficiency of rice	24
Poster №12. Victoria Armario-Najera Glycosylation analysis of SARS-CoV-2 recept or binding domain (RBD) produced in rice	24
Poster №13. Victoria Armario-Najera Microbicidal activity of the Scytovirin domain 1 (SD1) produced in rice against HIV-1	25
Poster №14. Christina R Ingvarlsen Development of a platform for ornamental plant transformation and New Breeding Techniques (NBT)	25
Poster №15. Xin Huang Towards CRISPR/Cas9 mediated enhancement of β -carotene in maize endosperm	26
Poster №16. Martin Friberg Characterisation of PHO1 knock-out lines generated by CRISPR/Cas9	26

Poster №17. Maria Carelli Molecular, biochemical and physiological characterization of <i>Medicago truncatula</i> mutants in sapogenin pathway obtained by different genetic engineering strategies	27
Poster №18. Mattia Santoni SARS-CoV-2 RBD Plant Based Production for Serological Analysis	27
Poster №19. Fabio Pietrolucci Transient expression of five key genes of Artemisinin biosynthetic pathway to boost its content in <i>A. annua</i> plants	28

List of Participants

Henry Chen

Fujian Agriculture And
Forestry University
Fuzhou, China
henry37201@gmail.com

Amanov Oybek

Southern Research Institute of Agriculture,
Karshi-Beshkent yoli-Str. 3
Karshi city, Kashkadarya region
Uzbekistan
mr.amonov1984@mail.ru

Luka Biteznik

Biotechnical Faculty, University Of Ljubljana,
Slovenia Department Of Agronomy
Jamnikarjeva 101
Ljubljana, Slovenia
luka.biteznik@bf.uni-lj.si

Rasulov Jamshed

Scientific Research Institute of Horticulture,
Viticulture and Winemaking named after
Academician M. Mirzaev
Chimkent tract-Str., Gulistan village,
Tashkent region, Uzbekistan
Jamshed.Rasulov.81@mail.ru

Lidor Gil

Futuragene Israel Ltd.
2 Pekeris St.
Rehovot, Israel
lidor@futuragene.com

Ravshanbek Siddikov

The Research Institute for grain and
leguminous crops, Andijan street 39,
Kuyganyor village, Andijan Region,
Uzbekistan
ddeiti-19@mail.ru

Sergei Krasnyanski

Elo Lifesystems
3054 E Cornwallis Rd
Durham, United States
keltserg@yahoo.com

Rahimov Tojiddin

The Research Institute for grain and
leguminous crops, Andijan street 39,
Kuyganyor village, Andijan Region,
Uzbekistan
tojiddin958@uamail.uz

Juraev Diyor

Southern Research Institute of Agriculture,
Karshi-Beshkent yoli-Str. 3, Karshi city,
Kashkadarya region, Uzbekistan
di.yor@mail.ru

Andika Gunadi

JR Simplot Company
Simplot Plant Sciences
5369 W Irving St Boise
United States
andika.gunadi@simplot.com

Abdullayev Bahodir

Scientific and Production Association "Grain
and rice"
Krantau village, Nukus district
Republic of Karakalpakstan, Uzbekistan
abdullayev.bakhadir@mail.ru

Avtonomov Victor

Research Institute of Breeding, Seed
Production and Agrotechnology of Cotton
Cultivation, University-Str. 2, Kibray district,
Salar city, Tashkent region, Uzbekistan
avtonovvik@mail.ru

Andres Duque

Universidad Tecnologica De Pereira
Facultad De Ciencias Ambientales
Carrera 16 # 9-17
Pereira, Colombia
andduque@utp.edu.co

Komilov Muhiddin

The Research Institute for grain and
leguminous crops, Andijan street 39,
Kuyganyor village, Andijan Region,
Uzbekistan
leomiha22@gmail.com

Xavier Herman

UCLouvain
Louvain Institute Of Biomolecular Science
And Technology
Croix Du Sud 4-5
Louvain-la-neuve, Belgium
x.herman@uclouvain.be

Gafurov Gafur

National Center for Knowledge and
Innovation in Agriculture
Sh. Rashidov-Str. 1, A. Yassaviy settlement,
Yuqori Chirchiq district
Tashkent region, Uzbekistan
g.gafurov@yahoo.com

Fang-ming Lai

Pairwise Plants
110 TW Alexander Dr
Durham, United States
flai@pairwise.com

Jana MUROVEC

Fredy Altpeter

University of Florida
Agronomy
3085 Mccarty Hall
Gainesville, United States
altpeter@ufl.edu

Mária Škrabišová

Palacký University In Olomouc
Department of biochemistry
Šlechtitelů 27, Olomouc,
Czech Republic
maria.skrabisova@upol.cz

Karimov Sharofiddin

Research Institute of Breeding, Seed
Production and Agrotechnology of
Cotton Cultivation, University-Str.,
Kibray district, Salar city,
Tashkent region, Uzbekistan
karimovsharofiddin78@gmail.com

Lucie Fischerová

The Czech Academy of Sciences
Institute of Experimental Botany
Rozvojová 263
Prague, Czech Republic
fischerova@ueb.cas.cz

Boyjigitov Fozil

Scientific Research Institute of
Horticulture, Viticulture and Winemaking
named after Academician M. Mirzaev
Chimkent tract-Str., Gulistan village,
Tashkent region, Uzbekistan
fboyjigitov80@mail.ru

Bjorn Kloosterman

Keygene N.V.
Agro Business Park 90
Wageningen, Netherlands
evs@keygene.com

Shermukhammad Aminov

Scientific Research Institute Of
Vegetables, Melon Crops And Potato
Andijan, Uzbekistan
andspekiti@inbox.ru

Spela Mestinek Mubi

Biotechnical Faculty, University Of
Ljubljana, Slovenia
Department Of Agronomy
Jamnikarjeva 101
Ljubljana, Slovenia
spela.mestinek@mubi@bf.uni-lj.si

Catherine Navarre

UCLouvain Louvain Institute Of Biomolecular
Science And Technology
Croix Du Sud 4-5 L7.07.14
Louvain-la-neuve, Belgique
catherine.navarre@uclouvain.be

Przemysław Sitarek

Medical University of Lodz
Al. Kościuszki 4
Łódź, Poland
przemyslaw.sitarek@umed.lodz.pl

Rakhmatov Bakhtiyor

Scientific Research Institute of Breeding,
Seed Production and Agricultural Technology
of Cotton Growing
Bukhara Scientific Experimental Station
Omad street, house 18
Bukhara, Uzbekistan
BaxtiyorRaxmatov2306@gmail.com

Malgorzata Palusińska

University of Warsaw
Department of Plant Metal Homeostasis
1 Ilji Miecznikowa Str.
Warszawa, Poland
m.palusinska@biol.uw.edu.pl

Kalantarov Sanat

Research Institute of vegetable, melon crops
and potato
Keles-Str., 111106
Tashkent region, Uzbekistan
uzrivmcp@mail.ru

Sinja Svetik

Biotechnical Faculty, University Of Ljubljana,
Slovenia Department Of Agronomy
Jamnikarjeva 101
Ljubljana, Slovenia
sinja.svetik@bf.uni-lj.si

Malokhat Khalikova

Cotton breeding, Seed Production and
Cultivation Agrotechnologies Research
Institute
University Street
Qibray, Uzbekistan
halikovamalohat@rambler.ru

Chiara Fattorini

University Of Verona
Cà Vignal 1, Strada Le Grazie 15
Verona, Italy
chiara.fattorini@univr.it

Zuzana Kocsisova

CTC Genomics
1005 N Warson Rd
St Louis, United States
zuzana.kocsisova@ctc.com.br

Biotechnical Faculty, University Of Ljubljana,
Slovenia

Department Of Agronomy
Jamnikarjeva 101
Ljubljana, Slovenia
jana.murovec@bf.uni-lj.si

Yurong Chen

Bayer Crop Science
Plant Biotechnology
700 Chesterfield Parkway West
Chesterfield, United States
yurong.chen@bayer.com

Tatiyana Yagoutkin Ohana

Futuragene Israel Ltd.
2 Pekeris St.
Rehovot, Israel
tanya@futuragene.com

Utambetov Duysenbay

Scientific and Production Association
“Grain and rice”
Krantau village, Nukus district
Republic of Karakalpakstan, Uzbekistan
duzenboy1960@mail.ru

Edoardo Bertini

Università di Verona / Edivite S.r.l.
Quartiere San Mauro 30, San Pietro
Viminario
Padova, Italy
edoardo.bertini@univr.it

Mistianne Feeney

Tropic Bioscience Norwich Research
Park Innovation Centre Colney Lane
Norwich, United Kingdom
talisha.williamson@tropicbioscience.com

Fakhriddin Tulashev

The Academician Makhmud Mirzaev
Scientific-research Institute Of Horticulture,
Viticulture And Wine-making
Chimkent Street 16, Zangiota District
Zangiota, Uzbekistan
bogdod92@gmail.com

Bekmurat Turdishev

Karakalpak Research Institute Of Agriculture
Village Chimbay, Republic Of Karakalpakstan
Chimbay, Uzbekistan
tbekmurat64@mail.ru

Klaus Palme

ScreenSYS GmbH
Engesserstr. 4a
Freiburg, Deutschland
palme@screensys.eu

Bakhtiyor Kalandarov**Alisher Touraev**

National Center of knowledge and
innovation in agriculture.
University street 2, Tashkent region,
Kibray district, Uzbekistan
alishertouraev@yahoo.com

Nikolett Kaszler

University Of Szeged
Department Of Plant Biology
Szeged, Hungary
kaszler.n@gmail.com

Boykhonova Gulnisa

Cotton breeding, Seed Production and
Cultivation Agrotechnologies Research
Institute
University Street
Qibray, Uzbekistan
gboykhonova@gmail.com

Gabriella Pocsfalvi

National Research Council Of Italy
Institute Of Biosciences And Bioresources
Via P. Castellino, 111
Napoli, Italy
gabriella.pocsfalvi@ibbr.cnr.it

Subkhonberdi Rustamov

Scientific Research Institute of Rainfed
Agriculture
M. Omonov Street
Gallaorol City, Uzbekistan
subkhonberdirustamov@gmail.com

Oscar Bellon

Università degli Studi Di Verona
Verona, Italy
oscar.bellon@univr.it

Meyliev Akmal

Southern Research Institute of Agriculture,
Karshi-Beshkent yoli-Str. 3
Karshi city, Kashkadarya region, Uzbekistan
uzniizerno@yahoo.com

Gianluca Gambacorta

Università Degli Studi Di Verona
Biotechnology
Strada le Grazie 15
Verona, Italy
gianluca.gambacorta@univr.it

Sherzod Dilmurodov

Research Institute Of Agriculture In The
Southern Regions
Beshkent Yuli
Qarshi, Uzbekistan
s.dilmurodov@mail.ru

Vera Martínez-Barradas

Pontificia Universidad Católica De Chile
Santiago, Chile

Eliane Locali

Fundecitrus Biotechnology
Av. Dr. Adhemar Pereira de Barros, 201
Araraquara, Brazil
eliane.locali@fundecitrus.com.br

Rustam Nizomov

Scientific Research Institute Of
Vegetables, Melon Crops And Potato
Tashkent, Uzbekistan
uzrivmcp@mail.ru

Polyana Martins

GDM
Cambé – PR, Brazil
pmartins@gdmseeds.com

MukhtabarTashmatova

Uzbek-Hungarian Scientific Center
for Potato Growing under RIVMPC
Keles-Str. 119, Kuksaroy district
Tashkent region, Uzbekistan
muhtabar.alisherovna@bk.ru

Marta Leonor Marulanda

Universidad Tecnológica De Pereira
Facultad De Ciencias Ambientales
Carrera 16 No 9-17 Apto 403
Pereira, Colombia
mlmarulanda@utp.edu.co

Francisco Sanchez-rodriguez

Universite Paris Saclay
Ips2 Rue Noetzlin
Gif Sur Yvette, France
francisco.sanchez-rodriguez@universite-paris-saclay.fr

Fabio Pietrolucci

Università degli Studi di Verona
Verona, Italy
fabio.pietrolucci@gmail.com

Mengfan Li

Vib
Discovery Sciences
Zwijnaarde, Belgium
mengfan.li@vib.be

Stanton Gelvin

Purdue University
Biological Sciences 201 South University St.
West Lafayette, Indiana
United States
gelvin@purdue.edu

Laura Hernandez-Soriano

Cinvestav Genetic engineering department
Libramiento Norte Carretera Irapuato
Irapuato, Mexico
laura.hernandez@cinvestav.mx

Rice Research Scientific Institute
Tashkent Uzbekistan
baxtiyor1451@gmail.com

Ying Liu

Swedish University Of Agricultural Sciences
Department of plant breeding
Växtskyddsvägen 1
Alnarp, Sweden
ying.liu@slu.se

Esbosin Sadikov

Scientific and Production Association “Grain
and rice”
Krantau village, 230910, Nukus district,
Republic of Karakalpakstan
Uzbekistan
esbosin64@mail.ru

Cristina Pignocchi

Tropic Biosciences
Norwich, United Kingdom
cristina@tropicbioscience.com

Tobias Sieberer

Technical University Munich Plant Growth
Regulation
Liesel-Beckmann-Strasse 1
Freising, Germany
tobias.sieberer@tum.de

Linda Avesani

University Of Verona
Department Of Biotechnology
Verona, Italy
linda.avesani@univr.it

Goetz Hensel

Heinrich-Heine-Universität
Institute Of Plant Biochemistry
Universitätsstr. 1 Düsseldorf
Germany
goetz.hensel@hhu.de

Matthew Milner

Niab Transformation
93 Lawrence Weaver Road
Cambridge, United Kingdom
matthew.milner@niab.com

Lan-Ying Lee

Purdue University
Biological Sciences 201
S. University St. West Lafayette
United States
lee34@purdue.edu

Ewa Skala

Medical University of Lodz
Department of Biology and Pharmaceutical
Botany Muszynskiego 1
Lodz, Poland
ewa.skala@umed.lodz.pl

ybmartinez@uc.cl

Alessandro Giannetti

University Of Copenhagen
Plant and Environmental Sciences
Thorvaldsensvej 40\
København, Denmark
agi@plen.ku.dk

Azam Ravshanov

Cotton breeding, Seed Production and
Cultivation Agrotechnologies Research
Institute
University Street
Qibray, Uzbekistan
azamravshanov871@gmail.com

Sujit Tha Shrestha

CN Seeds Ltd
Plant Breeding And Research
18 Main Street, Pymoor
Ely, United Kingdom
sujit.tha@cnseeds.co.uk

Ralf Welsch

ScreenSYS GmbH
Engesserstr. 4a
Freiburg, Deutschland
ralf@screensys.eu

Ana Paula Ribeiro

GDM
Cambé-PR
Brazil
aribeiro@gdmseeds.com

Masudjon Sattorov

Rice Scientific Research Institute
Tashkent, Uzbekistan
garasha76@gmail.com

Victoria Armario Najera

Universitat De Lleida
Etsea / Departament Pvcf
Lleida, Spain
nuria.gabernet@udl.cat

Waseim Barriah

Al- Qasemi High School
Baka El- Garbiah, Israel
barriah@qasemi.net

Maneea Moubarak

Cnr IBBR Via Pietro Castellino 111
Napoli, Italy
maneea.moubarak@ibbr.cnr.it

Huw Jones

Aberystwyth University
Ibers Penglais
Aberystwyth, United Kingdom
Huw.jones@aber.ac.uk

Heidi Kaulfürst-Soboll

WWU Münster
Institute of Plant Biologie
and Biotechnologie
Schlossplatz 7, Münster
Germany
hfuerst@uni-muenster.de

Claudia Rosa Espinet

Universitat De Lleida
Etsea / Departament Pvcf
Lleida, Spain
nuria.gabernet@udl.cat

Andrea Saba Mayoral

Universitat De Lleida
Etsea / Departament Pvcf
Lleida, Spain
nuria.gabernet@udl.cat

Marcel Adriaanse

Bejo Zaden
Research Trambaan 2
Warmenhuizen, Netherlands
miranda.tenhoeve@bejo.nl

Chantal Anders

Ugent-vib Plant Systems Biology
Gent, Belgium
chantal.anders@psb.vib-ugent.be

Maria Carelli

Consiglio per la Ricerca in Agricoltura e
l'Analisi dell'Economia Agraria
Centro di Ricerca Zootecnia e Acquacoltura
Viale Piacenza 29 LODI, Italy
maria.carelli@crea.gov.it

Massimo Confalonieri

Consiglio Per La Ricerca In Agricoltura E
L'analisi Dell'economia Agraria
Centro Di Ricerca Zootecnia E Acquacoltura
Viale Piacenza 29 Lodi, Italy
massimo.confalonieri@crea.gov.it

Martin Friberg

Swedish University of Agriculture
Department of Plantbreeding
Växtskyddsvägen 3 Alnarp, Sweden
martin.friberg@slu.se

Guillermo Sobrino Mengual

Universitat De Lleida
Etsea / Departament Pvcf
Lleida, Spain
nuria.gabernet@udl.cat

Aranka van der Burgh

Wageningen University And Research
Plant Breeding, Droeendaalsesteeg
Wageningen, Netherlands
aranka.vanderburgh@wur.nl

Huang Xin

Universitat De Lleida
Etsea / Departament Pvcf
Lleida, Spain
nuria.gabernet@udl.cat

Nathan Geschiere

Enza Zaden R&D BV
Haling 1e Enkhuizen
Netherlands
n.geschiere@enzazaden.nl

Lennart Hoengenaert

UGent-VIB
VIB-UGent Center for Plant Systems Biology
Technologiepark-Zwijnaarde 71
Gent, Belgium
lennart.hoengenaert@psb.ugent.be

Inger Holme

Aarhus University Agroecology
Forsøgsvej 1, Slagelse, Denmark
inger.holme@mbg.au.dk

Yakubov Mirakbar

Scientific Research Institute of
Plant Genetic Resources,
University-Str. 2, 100140,
Kibray district, Tashkent region
Uzbekistan
mirakbardan@yahoo.com

Kinga Maria Pilarska

Wrocław University Of Environmental And
Life Sciences, Faculty Of Biotechnology And
Food Science
Chelmońskiego, Wrocław, Poland
kinga.pilarska@upwr.edu.pl

Magdalena Wróbel-Kwiatkowska

Wrocław University of Environmental
and Life Sciences
Norwida 25, Wrocław, Polska
magdalena.wrobel-kiwiatkowska@upwr.edu.pl

He Wenshu

Universitat De Lleida
Etsea / Departament Pvcf
Lleida, Spain
nuria.gabernet@udl.cat

Mattia Santoni

University of Verona - Diamante srl
strada le grazie 15
Verona, Italia
mattia.santoni@univr.it

Ashwin Vargheese

Universitat De Lleida
Etsea / Departament Pvcf
Lleida, Spain
nuria.gabernet@udl.cat

Stephan Wenkel

University Of Copenhagen
Frederiksberg ,Denmark
wenkel@plen.ku.dk

Christina Ingvarsdén

Aarhus University
Department Of Agroecology
Forsoegsvej 1, Slagelse, Denmark
christina.ingvarsdén@agro.au.dk

Huidong Liu

The University of Warwick
School of Life Sciences
The University of Warwick
Coventry United Kingdom
Huidong.Liu@warwick.ac.uk