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Abstract Book

Plant Cell & Tissue Culture in Vitro IV

July 4 - 5, 2022, Vienna, Austria

International Conference

Plant Cell & Tissue Culture in Vitro IV

Programme and Abstracts

Vienna, Austria

July 4 - 5, 2022

Organizing Committee

Local Organizing Committee	International Organizing Committee
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Welcome to the 4th International Conference on "Plant Cell & Tissue Culture in Vitro"!

Plant cell, cultured in vitro, are able to regenerate into the complete fertile plants under the appropriate culture and environment conditions, e.g. plant cells are totipotent. Currently, the technology is not only the great tool in basic research, such as cell biology, genetics, biochemistry and biotechnology, but has also the direct commercial importance in the Mass Plant Micro-propagation, Genetic Transformation, Genome Editing, Speeding up Breeding Process via production of Doubled Haploids, Production of Secondary Metabolites, etc.

The **4th International Conference on "Plant Cell & Tissue Culture In Vitro"** to be held in **Vienna, Austria** on **July 4-5, 2022** will discuss wide range of modern in vitro plant cell and tissue culture technologies, fundamental aspects of plant cell totipotency, differentiation, regeneration, embryogenesis, and practical applications of plant cell in vitro technologies for crop improvement.

This two-day event will provide leading academy and industry scientists a platform to communicate recent advances in "**Plant Cell & Tissue Culture In Vitro**", and an opportunity to establish multilateral collaboration.

The **4th International Conference on "Plant Cell & Tissue Culture In Vitro"** will cover the following research topics:

- **Plant Cell Totipotency & Differentiation**
- **Plant Cell Organogenesis & Regeneration**
- **Somatic Embryogenesis**
- **Anther/Microspore Cultures (Androgenesis)**
- **Ovary/Ovule Cultures (Gynogenesis)**
- **Plant Cell Suspensions**
- **Plant Cell Bioreactors**
- **Protoplasts**
- **Micropropagation**
- **In Vitro Fertilization**
- **Plant Transformation**

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)

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4th International Conference on "Plant Cell & Tissue Culture in Vitro" (July 4-5)

July 4 (Monday)

08.00 - 17.00	***Registration***
09.00 - 09.10	**Opening** Welcome address by **Alisher Touraev (Local Organizer, Austria)** Welcome address by **Pedro Pablo Gallego (Conference Co-Chair, Spain)**
09.10 - 09.45 (+5)	**Keynote Lecture 1:** **Attila Fehér (Hungary):** Callus, Dedifferentiation, Totipotency, Somatic Embryogenesis: What These Terms Mean in the Era of Molecular Plant Biology?
09.50 - 10.25 (+5)	**Keynote Lecture 2:** **Klaus Palme (Germany):** Innovative technology platform for in vitro cell studies: advantages in research and industrial applications
10.40 - 11.00	***Coffee break***
11.00 - 12.30:	**Session I: Plant Cell Totipotency & Differentiation**
Chairs	*Klaus Palme (Germany) & Christophe Gaillochet (Belgium)*
11.00 - 11.25 (+5)	**Christophe Gaillochet (Belgium):** Control of plant cell fate transitions by transcriptional and hormonal signals
11.30 - 11.45 (+5)	**Lucie Fischerová (Czech Republic):** Regeneration of Cannabis sativa (L.) in-vitro
11.50 - 12.05 (+5)	**Vera Martinez-Barradas (Chile):** A Chilean common bean in vitro regeneration protocol to be implemented for CRISPR-Cas9 genetic edition
12.10 - 12.25 (+5)	**Maneea Moubarak (Italy):** Extracellular vesicles from cell suspension culture of different explants of Solanum lycopersicum L.
12.30 - 14.00	***Lunch + Poster Session (all numbers), Conference Photo***
14.00 - 15.20	**Session II: Plant Cell Organogenesis & Regeneration**
Chairs	*Pedro Gallego (Spain) & Nieves Vidal (Spain)*
14.00 - 14.20 (+5)	**Pedro Gallego (Spain):** Machine Learning and Plant Tissue Culture Tools for Medicinal Plant Valorization
14.25 - 14.45 (+5)	**Nieves Vidal (Spain):** Use of bioreactor systems in the propagation of forest trees.
14.50 - 15.05 (+5)	**Elisabeth Kopper (Austria):** Micropropagation and pathogen elimination i elderberry (Sambucus nigra L.)
15.10 - 15.30 (+5)	**Csaba Lantos (Hungary):** Utilization and improvement of In Vitro Androgenesis in Cereal Breeding of CR LTD
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16.00 - 17.35	**Session III: In Vitro Propagation**
Chairs	*Fredy Altpeter (USA) & Attila Feher (Hungary)*
16.00 - 16.25 (+5)	**Fredy Altpeter (USA):** Overcoming Recalcitrance of Energycane to Tissue Culture and Genetic Transformation

16.30 - 16.50 (+5)	**Tobias Sieberer (Germany):** Novel chemical strategies to boost shoot regeneration in recalcitrant crops
16.55 - 17.10 (+5)	**Marta Leonor Marulanda Angel (Colombia):** In vitro propagation and somatic embryogenesis in Guadua angustifolia Kunth
17.15 - 17.30 (+5)	**Josef Baltazar ŠENKYŘÍK (Czech Republic):** Astragalus membranaceus (Fisch.) – Artificial polyploidization: In vitro method for medical plants improvement.
17.35 - 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 5 (Tuesday)

08.00 - 17.00	***Registration***
09.00 - 10.30	**Session IV: Haploid Technologies**
Chairs	*Pilar Testillano (Spain) & Maria Pilar Valles (Spain)*
09.00 - 09.20 (+5)	**Pilar S Testillano (Spain):** Novel strategies with small molecules to improve microspore reprogramming to embryogenesis in crop and forest plants.
09.25 - 09.45 (+5)	**María Pilar Vallés (Spain):** How Trichostatin A affects developmental reprogramming of bread wheat microspores
09.50 - 10.10 (+5)	**Iwona Zur (Poland):** Reactive oxygen species and antioxidative defence as the determinants of microspore embryogenesis effectiveness
10.15 - 10.25 (+5)	**Yolanda Pérez Pérez (Spain):** Endogenous auxin and cytokinin play key roles in stress-induced microspore embryogenesis of Brassica napus
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11.00 - 12.30	**Session V: Plant Protoplasts: Modern Application**
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11.25 - 11.45 (+5)	**Ralf Welsch (Germany):** Determination of Protoplast Growth Properties using Quantitative Single-Cell Tracking Analysis
11.50 - 12.10 (+5)	**Andreas Schiermeyer (Germany):** Targeted insertion of large DNA sequences by homology-directed repair or non-homologous end joining in engineered tobacco BY-2 cells using designed zinc finger nucleases
12.15 - 12.35 (+5)	**Agnieszka Szopa (Poland):** Production of Schisandra type lignans in in vitro cultures of S. chinensis, S. rubriflora and S. henryi
12.40 - 14.00	***Closing Ceremony + Lunch***

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Abstracts of Oral Presentations

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Our understanding on in vitro plant regeneration in the era of modern plant biology

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An intriguing feature of plants that they often can be regenerated even from a single cultured somatic cell. This regeneration may follow various pathways of organogenesis or embryogenesis with or without callus formation. Furthermore, the initial conditions that can trigger regeneration in various species/explants are strikingly different, and some species explants are notoriously difficult to regenerate. Despite of decades of intensive research, our knowledge about the molecular processes underlying plant regeneration is rather scarce. Nevertheless, it is becoming more and more obvious that generalizations should be avoided. Therefore, the nomenclature of plant cell and tissue culture has to be revisited. Some of the basic terms such as “callus”, “dedifferentiation”, “totipotency”, “and somatic embryogenesis” might mean different things today than several decades ago when they were first used and defined. Moreover, their meaning might differ in various cases/pathways of regeneration.

In this lecture I attempt to review the meaning and use of the above traditional plant cell and tissue culture terms in light of current results of modern plant biology to avoid potential “terminology-raised” barriers in plant regeneration research.

Innovative technology platform for in vitro cell studies: advantages in research and industrial applications

Oleksandr Dovzhenko1, Patrick Schaub1, Olaf Tietz1, Qiuju Yu1, Hanna Lasok1, Maja Temerinac-Ott1, Sean Walsh1, Marcel Germer1, Markus Müller1, Kinan Azoulabi1, Ralf Welsch1,2, **Klaus Palme 1,2**
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New technologies for efficient exploitation of single plant cell in vitro reprogramming potential are highly demanded in plant biotechnology, breeding and research. Digitalization, automation and Artificial Intelligence (AI), which recognizably facilitate progress in other biomedical and agribiotechnology areas, are now being implemented by ScreenSYS to advance in vitro single plant cell research. Application of our technology platform was effectively demonstrated in haploid pluripotent stem cells called microspores as well as in totipotent somatic protoplast systems. The switch from pollen development towards embryo formation provides exceptional opportunities for “green” biotechnology, and an attractive model for studying the mechanisms of cellular reprogramming, totipotency, differentiation and development. This process is known as Doubled Haploid (DH) technology, which is of high relevance in plant breeding and green biotechnology. Similarly, highly efficient regeneration of fertile plants from protoplasts has been achieved.
Here we present a technology workflow for quantitative phenotyping and perturbation studies in mono- and dicotyledonous plants. Benefiting from our patented cell immobilization procedure for automated of automated cell handling and image-data generation, ScreenSYS developed high-content cell analysis studies driven by artificial intelligence and state-of-the-art computational approaches to quantitively study cellular reprogramming in vitro.

Control of plant cell fate transitions by transcriptional and hormonal signals

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The characterization of gene regulatory networks and their subsequent engineering allows the creation of plants with enhanced traits. To this end, it is critical to obtain a deeper knowledge of genes and developmental mechanisms controlling plant growth together with having a toolset in hand that allows precise and efficient genome editing.

In the first part of my talk, I will present the role of HECATE (HEC) genes in regulating developmental trajectories of shoot stem cells of Arabidopsis thaliana. HEC function stabilizes cell fate in distinct zones of the shoot meristem thereby controlling the spatio-temporal dynamics of stem cell differentiation and lateral organ production. This activity is concomitant with the local modulation of cellular responses to cytokinin and auxin, two key phytohormones regulating cell behavior.

Secondly, I will show how the ITER platform (Iterative Testing of Editing Reagents) can be used to accelerate design-build-test-learn cycles and develop new gene editing reagents. The platform is based on arrayed protoplast transfections and high-content imaging, allowing one optimization cycle –from design to results– within three weeks. We validated ITER in wheat and maize protoplasts and used it to develop an improved LbCas12a-ABE system.

Regeneration of Cannabis sativa (L.) in-vitro

Lucie Fischerová, Zuzana Vondráková, Jana Pavlíčková, Kateřina Eliášová, Jaroslav Nisler, Petre I. Dobrev, Václav Motyka, Tomáš Moravec
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In recent decades, Cannabis sativa (L.) has been the subject of extensive research namely due to its pharmaceutical value. Modern genome editing tools such as CRISPR Cas9 open up the possibility of altering the metabolic pattern of Cannabis and represent one of the main goals of current research. A prerequisite for this is the establishment of a reliable regeneration protocol, as the lack of robust and reproducible in vitro regeneration system is the main obstacle for the transformation of this species.

Here we present a comparative study in which we tested different media compositions to induce plant regeneration and/or multiple shoot formation. Explants were isolated from both aseptically germinated seedlings (5 cultivars) and surface-sterilized greenhouse-grown plants (2 cultivars). We compared the effect of selected cytokinins (m-topolin, zeatin, thidiazuron, isopentenyladenine) in combination with auxin 1-napthaleneacetic acid and described the changes in organogenesis after application of novel cytokinin oxidase/dehydrogenase inhibitors. Monitoring of the morphological response of the explants was accompanied by analyses of endogenous phytohormones.

A Chilean common bean in vitro regeneration protocol to be implemented for CRISPR-Cas9 genetic edition

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Common bean (Phaseolus vulgaris) in vitro regeneration represents a limitation for its genetic transformation. P. vulgaris is considered a species recalcitrant to regeneration because regeneration protocols are cultivar-specific and a general regeneration protocol for the species does not exist. In the present work we developed a regeneration protocol for ´Zorzal´ a highly cultivated Chilean cultivar using as explants embryo axes and embryo apical meristems. Our regeneration protocol is developed in the context of a P. vulgaris genetic edition project where we pretend to improve drought tolerance by generating knock-out plants from the PvMYB60 gene. To the moment we have implemented a whole regeneration system from the mentioned explants and achieved seed obtention from regenerated plants successfully. Regarding genetic edition we have identified the P. vulgaris MYB60 gene in silico, cloned the sequence corresponding to 'Zorzal' cultivar and designed sgRNAs and plasmid constructions for its genetic edition. Current results will be presented. VMB was supported by CONACyT (Mexico) 739582, and ANID (Chile) 21200394 PhD scholarships.

Extracellular vesicles from cell suspension culture of different explants of Solanum lycopersicum L.

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Cells actively shed nanometer-sized membrane-enclosed vesicles, called extracellular vesicles (EVs) into the environment. They represent a new paradigm in cell biology and medicine due to their specific ability to transfer complex functional biomaterial such as proteins, RNAs, lipids to recipient cells. Tomato fruit has been shown to be a valuable source for high-yield production of nanovesicles resembling to EVs [1]. Moreover, EVs released by tomato roots show antifungal activities [2]. Here, we aimed to combine the established cell suspension culture with the production of EVs.
M82 and Microtom tomato varieties were used to establish fine suspension cultures from leaves, stem and roots explants. Conditions of callus and suspension cultures were optimized. EVs were isolated by gradient ultracentrifugation (gUC). Physical, morphological and molecular characteristics were analysed by nanoparticle tracing analysis, Qubit assay and SDS-PAGE analysis. Densities of the EV containing fractions were determined by iodixanol gUC.
The small-scale batch suspension cultures yielded from 2.4×10-5 to 8.2×10-6 µg of EV proteins per cell. M82 leave culture produced the highest EV amount (15µg protein/mL medium). GUC resulted in a single visible band in the density between 1.10-1.14 g/mL. EVs showed a size distribution 103 nm±6nm and were characterized by complex protein profiles. Our results show that cell suspension culture could be a promising source of EVs generated by plant cells.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant, agreement No 895579, acronym "greenEV".

Machine Learning and Plant Tissue Culture Tools for Medicinal Plant Valorization

Pedro Pablo Gallego*, Manuel Ayuso*, Pascual García-Pérez*, Eva Lozano-Milo*, Mariana Landín**, Mª Esther Barreal*

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Machine learning allows modeling very complex processes, being a powerful tool for decision-making and the study of unknown facts and hidden patterns between independent variables (factors) and dependent variables (response parameters) (Gallego et al., 2011). Among the various ML algorithms, artificial neural networks combined with fuzzy logic (neurofuzzy logic) and genetic algorithms (ANNs-GA) have been employed to model, learn, predict and optimize seed germination and dormancy (Ayuso et al., 2019) and plant tissue culture (PTC) of medicinal plants. ML tools have been applied to identify the essential factors involved in mineral nutrition imbalances during plant tissue culture (Garcia-Perez et al., 2020a), the most critical factors guiding both direct shoot regeneration and indirect organogenesis (Garcia-Perez et al., 2020b), to maximize the production of phenolic compounds (Ayuso et al., 2020; Garcia-Perez et al., 2020c). In our opinion, the information obtained in these studies is very advantageous for developing successful procedures for plant cell differentiation, organogenesis, regeneration, embryogenesis, haploids, protoplasts, in vitro cell suspension culture and micropropagation using machine learning and artificial intelligence technologies.

Use of bioreactor systems in the propagation of forest trees

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Bioreactors were developed to allow automation and enhance large-scale propagation. They use liquid media and frequently incorporate aeration. Increased absorption of nutrients, and controlled air exchanges can improve the physiological state of the explants, reducing costs and increasing yield. Mature forest trees are often recalcitrant conventional to vegetative and micropropagation techniques. Bioreactors have been used for culturing axillary shoots and somatic embryos of several tree species, with the aim of obtaining vigorous plantlets with high photosynthetic capacity and functional roots for improved transitioning to ex vitro conditions. While proliferation, rooting and acclimation may be improved, contamination and hyperhydricity are still the main hindrances limiting the widespread use of this technology. To solve these obstacles, it is necessary to investigate parameters such as the type and size of bioreactor, the mineral and hormonal media composition, including frequency and duration of immersion, vessel environment and physiological conditions of the explant. New developments in LED lighting and 3D printers make these promising tools to design new bioreactors better adapted to the specific characteristics of tree species.

Micropropagation and pathogen elimination in elderberry (Sambucus nigra L.)

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To date, more than 75% of Austrian elderberry acreage is planted with cultivar 'Haschberg'. This leads to high costs during harvest, since most of the berries have to be harvested at the same time and need to be frozen within hours after picking. In order to improve the spectrum of cultivars, an attempt was made to optimize micro-propagation of six elderberry cultivars. To meet the requirement for certified planting material, thermotherapy and chemotherapy (ribavirin), followed by meristem tip culture, were applied to eliminate various plant pathogens from elderberry plants. Virus detection was carried out by RT-PCR, and confirmed by electron microscopy. Additionally, plants were analysed for the presence of Xylella fastidiosa and Verticillium dahliae. Several media of meristem-tip culture and early stages of shoot development and proliferation were tested. A micropropagation protocol was developed using the cytokinin meta-Topolin for multiplication as well as for rooting of Sambucus nigra L. plants. To improve the process of ex-vitro-acclimatization of micropropagated elderberry plantlets, two commercial arbuscular mycorrhizal inoculants were evaluated. In a two-step-experiment under greenhouse conditions plantlets rooted in vitro, were inoculated at two different times during acclimatization. To assess the potential for elderberry root colonization, the impact on growth and survival of treated and untreated plants was determined. Both inoculants significantly enhanced root colonization levels and survival rates and shoot length of elderberry plants.

Utilization and improvement of In Vitro Androgenesis in Cereal Breeding of CR LTD

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The increasing importance of doubled haploid (DH) plant production methods are incontrovertible in modern plant breeding and research programmes.
In some cereal species (wheat, spelt wheat, triticale and barley), more thousands of DH lines are produced year by year in our laboratory. The well-established anther culture method (AC) is applied to accelerate our breeding programs. 'GK Déva', new DH winter wheat variety was protected in 2021. The AC is also used in Hungarian rice breeding program to produce new DH lines for the special conditions of Carpathian Basin (cold- and salt tolerance).
However, the in vitro androgenesis induction remained a challenge in einkorn (Triticum monococcum L.). Androgenesis was induced in in vitro anther culture of einkorn. Green and albino plantlets were regenerated from the microspore – derived ELS. The haploid ploidy level of the regenerated green plantlet was determined by flow cytometric analyses.
The research programmes was supported by scientific projects (TKP2020-NKA-21, OTKA-K_21-K138416, OTKA-FK_21-FK138042, GINOP-2.2.1-18-2018-00005).

Overcoming Recalcitrance of Energycane to Tissue Culture and Genetic Transformation

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Although sugarcane and energycane share the same progenitors, the relative contribution of these progenitors to their complex genome makes them look like two distinct species. Sugarcane has a small number of thick stems with high sugar and low fiber content, while elite energycane cultivars have been selected for very high biomass accumulation attributed to many thin stems with a higher fiber and lower sugar content than sugarcane. Energycane is far more recalcitrant to tissue culture and genetic transformation than sugarcane and exhibits heavy visual browning of the cultured explants followed by tissue necrosis and cell death. Visual browning of newly excised explants is a major hurdle that needs to be overcome to establish efficient somatic embryogenesis in support of a genetic transformation protocol for this target species. Several media supplements and their combinations were tested in tissue culture and evaluated for reduction in visual tissue browning, induction of embryogenic callus, and plant regeneration response. Some of the evaluated combinations of media supplements significantly reduced visual tissue browning while increasing the number of regenerating plantlets from embryogenic energycane callus. The optimized protocol was applied to introduce multigene constructs into energycane by biolistic gene transfer.

Novel chemical strategies to boost shoot regeneration in recalcitrant crops

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De novo shoot organogenesis is a prerequisite for numerous applications in plant research and breeding but is often a limiting factor, for example, in genome editing approaches. HD-ZIP III transcription factors have been characterized as crucial regulators of shoot specification, however up-stream components controlling their activity during shoot regeneration are only partially identified. Notably, HD-ZIP III proteins also contain putative ligand binding domains for unknown small molecule regulators, indicating additional layers of post-translational control.

In an attempt to identify novel mechanisms of HD-ZIP III regulation, we performed a reporter based small molecule screen for compounds, which increase the activity of HD-ZIP III in planta. We identified a novel plant growth regulator that promotes HD-ZIP III function by stimulating their transcriptional expression. Application of the compound during tissue culture enhances the shoot regeneration response of Arabidopsis in an HD-ZIP III-dependent manner. We further provide evidence that the regenerative function of the compound is based on its ability to limit polar auxin transport. Finally, we show that chemical modulation of auxin transport can significantly enhance shoot regeneration in sunflower (Helianthus annuus L.), an important oil crop, in which genetic transformation and genome editing is currently difficult to achieve due to its poor shoot formation capacity in tissue culture.

In vitro propagation and somatic embryogenesis in Guadua angustifolia Kunth

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Colombia has more than 51,000 plant species and is considered one of the five megadiverse countries in the world. It is the second country in bamboo diversity in the Americas — after Brazil — with 18 genera, 105 species, and five varieties, of which 24 are endemic species, 69 are woody bamboos and 36 are herbaceous bamboos.
The vast majority of studies conducted on bamboo species have been aimed at propagation by different routes. Some in vitro propagation methods have been described for Guadua angustifolia in Colombia, however, there is no known project for mass propagation of the species by in vitro methods of economically relevant varieties in the country.
Somatic embryogenesis has been the technique used since it is the way of cellular development where somatic cells give rise to organizations similar to zygotic embryos without the fusion of gametes. In this way, somatic cells can generate complete plants, although, since the initial cell is not the product of a process of recombination and fusion of gametes, the genotype of the donor plant (cloning) is preserved in its entirety.
Based on these considerations, the objective of this work focused on the production of somatic embryos of Guadua angustifolia for purposes of mass propagation of the species. The materials obtained in vitro have been successfully established in the field.

Potential of artificial polyploidization in medicinal plants improvement

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In vitro techniques generally improve cultivated plants, including medicinal and aromatic ones. The improvement of medicinal plants is focused on the modification of the content of secondary metabolites not only in quantity but also in qualitative characteristics. One of these techniques is the induction of artificial polyploidy. Here we find an effective protocol for artificial polyploidization for different medicinal plants like Thymus vulgaris, Ajuga reptans, Hyssopus officinalis, and Astragalus membranaceus. This successful in vitro technique uses oryzalin as the polyploidization reagent and balanced culture media for explants cultivation, regeneration, multiplication, and rooting. Multiplicated clonal strains were analyzed for ploidy levels by flow cytometry. Selected clones and species undergo analyses on morphological and transcriptional levels. Together with biochemical assays done in the past, it could establish different morphotypes and chemotypes. Regenerated polyploids are possible to transfer to greenhouse or field conditions.
This research was supported by the grant QK1910103 (NAZV, Ministry of Agriculture, Czech Republic)

Novel strategies with small molecules to improve microspore reprogramming to embryogenesis in crop and forest plants

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More efficient technologies for in vitro plant embryogenesis and propagation are urgently needed to accelerate breeding programs, using double-haploids through microspore embryogenesis, to clonally propagate elite genotypes, and to convert gene editing or transformation events into plants with improved traits. Knowledge gained in recent years has revealed that initiation and progression of microspore embryogenesis involve a complex network of factors, whose roles are not yet fully understood (Testillano 2019).
Recent advances in chemically-controlled reprogramming of mammalian cells have shown the enormous potential of synthetic small molecules to regulate cellular reprogramming. We have initiated a pioneer line of research, in collaboration with experts in chemical biology to screen novel small molecules from chemical libraries of the pharmaceutical field, to identify new chemical promoters of in vitro plant cell reprogramming and regeneration. Here, we report a new strategy using synthetic small molecule inhibitors of mammalian glycogen synthase kinase 3β (GSK-3β), never used in plants (Patent PCT/EP2020/083316), that enhance embryogenesis initiation rates and embryo production in three species, microspore embryogenesis of Brassica napus and Hordeum vulgare, and somatic embryogenesis of Quercus suber. We found that the compounds inhibited GSK-3 activity in plant cell cultures, as it does in mammalians. The small molecules increased expression of embryogenesis-genes FUS3, LEC2 and AGL15, and activated brassinosteroid signalling pathway, probably by targeting plant GSK-3 kinase BIN2 (Berenguer et al. 2021). Promising results have been obtained with other families of small molecules, also in recalcitrant systems. These findings open the way for the development of new strategies and technical innovations using novel small molecules, as GSK-3β inhibitors, that could be extended to other species for improvement of in vitro microspore embryogenesis.
Work supported by Project grant PID2020-113018RB-I00, funded by MCIN/AEI/ 10.13039/501100011033.

How Trichostatin A affects developmental reprogramming of bread wheat microspores

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Microspores can be developmentally reprogrammed by the application of different stress treatments to initiate an embryogenic pathway leading to the production of doubled haploid plants. Epigenetic modifications are involved in this microspore reprogramming. To increase the efficiency of microspore embryogenesis in bread wheat, the effect of the histone deacetylase inhibitor trichostatin A (TSA) has been studied in two cultivars with high and mid-low response to microspore embryogenesis. Out of the different strategies adopted, the application of 0.4 µM TSA simultaneously with a 0.7 M mannitol treatment for five days produced the highest efficiency, resulting in a four time greater number of green doubled haploid plants than mannitol. Diverse studies have been conducted to characterize the mechanisms involved in the effect of TSA on microspore embryogenesis in wheat.

Reactive oxygen species and antioxidative defence as determinants of microspore embryogenesis effectiveness in triticale

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Induction of microspore embryogenesis (ME) requires a precisely balanced stress treatment, strong enough to induce microspore reprogramming but not exceeding cell stress tolerance threshold. As each stress is accompanied by the generation of reactive oxygen species (ROS), an efficient antioxidative defence (mainly enzymatic antioxidants) was suggested as the first prerequisite for effective ME induction. However, detailed studies using anther and isolated microspore cultures of triticale (×Triticosecale Wittm.) showed that too intense elimination of ROS suppresses the switch to embryogenic development. It seems that ROS accumulation creates the signal necessary for microspore reprogramming but it has to be accompanied by efficient protection against cytotoxic effects of their generation.
Research financed by National Science Centre, Poland; project 2015/18/M/NZ3/00348

Endogenous auxin and cytokinin play key roles in stress-induced microspore embryogenesis of Brassica napus

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In vitro, isolated microspores can be reprogrammed by stress treatments, becoming totipotent cells and producing haploid and doubled-haploid embryos and plants, widely used in plant breeding, but still a process highly inefficient in many crop species.
In the present work we have analyzed the dynamics and role of endogenous auxin and cytokinin during stress-induced microspore embryogenesis in Brassica napus. We analyzed gene expression, localization and concentration patterns of endogenous auxins and cytokinins during microspore embryogenesis. Functional analyses with auxin and cytokinin inhibitors revealed the involvement of both phytohormones in microspore embryogenesis initiation and progression. The results indicated that biosynthesis and signalling of auxin and cytokinin were activated and required at different developmental stages of the process. Findings reveal complementary patterns for both hormones, being auxin necessary in cell reprograming and embryogenesis, whereas cytokinin is required mainly at advanced developmental stages.
Supported by Project grant PID2020-113018RB-I00, funded by MCIN/AEI/ 10.13039/501100011033.

Composition of the reconstituted cell wall in protoplast-derived cells of Daucus is affected by phytosulfokine

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Re-synthesis of the cell wall is one of the most important key steps in protoplast development preceding mitotic divisions and initiating establishment of a protoplast-to-plant system. Over the years many systems of protoplast culture including different variants of chemical nursing have been applied to over 400 species. Recently, phytosulfokine (PSK) - a peptidyl plant growth factor, has been recognized as a promising intercellular signaling molecule involved in cellular proliferation and dedifferentiation. Since PSK had been shown to cause an increase in efficiency of somatic embryogenesis, it was reasonable to check the distribution of selected chemical components of the cell walls during the protoplast regeneration process. Three Daucus taxa were analyzed during protoplast regeneration to specify what pectic, arabinogalactan protein (AGP) and extensin epitopes are involved in the reconstruction of the wall in protoplast-derived cells. The obtained results indicate diversity in the chemical composition of the cell walls in the control and the PSK-treated cultures.

Determination of Protoplast Growth Properties using Quantitative Single-Cell Tracking Analysis

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Although quantitative single-cell analysis is frequently applied in animal systems, similar applications on plant single cells are underexploited. We applied quantitative microscopic image analysis on plant single cells using totipotent tobacco leaf protoplasts as system. High-throughput automated microscopy coupled with the development of image processing pipelines allowed to quantify multiple parameters of thousands of individual protoplasts at different time steps of culture using our patented multiwell immobilization method. We compared cell expansion parameters of wild-type tobacco cells with cells expressing the antiapoptotic protein Bcl2-associated athanogene 4 from Arabidopsis (AtBAG4) which was reported to mediate increased resilience to various stress responses and improved cellular survival rates if overexpressed in various plants. Interestingly, AtBAG4-expressing protoplasts showed responses at the single-cell level which allowed to interconnect phenotypic observations made with transgenic plants. The possibility to associate plant phenotypes with single-cell properties, extracted with the single-cell processing and analysis pipeline developed, allows to envision novel biotechnological screening strategies able to determine improved plant properties via single-cell analysis.

Targeted insertion of large DNA sequences by homology-directed repair or non-homologous end joining in engineered tobacco BY-2 cells using designed zinc finger nucleases

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For efficient screening and identification of targeted DNA integration in tobacco BY-2 cells, an engineered transgene integration platform (ETIP) has been developed. In order to easily screen for targeted integration events, the ETIP contains truncated versions of marker genes at each end of the construct. The markers encode the neomycin phosphotransferase II and the red fluorescent protein DsRed, respectively. Upon targeted integration of a donor DNA, mediated by zinc-finger nuclease DNA double strand breaks, the marker genes are complemented and those events are identified by their growth on selection medium and their red fluorescent phenotype. This strategy enables the swift identification of targeted events and greatly reduce the number of clones that need to be characterized on the molecular level. To endow the ETIP with maximum flexibility, the coding regions of both marker genes are flanked by intron sequences. This molecular architecture enables the integration into the target site either by homologous recombination (HR) or via non-homologous end joining (NHEJ). For HR the donor DNA is equipped with homology arms flanking the sequence of interest, while for the NHEJ-mediated mechanism the donor DNA is flanked with short intron sequences providing the missing splicing sites for proper mRNA formation. Using this system targeted integration of large DNA constructs carrying up to 25 kb payload has been demonstrated.

Production of Schisandra type lignans in in vitro cultures of S. chinensis, S. rubriflora and S. henryi

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Dibenzocyclooctadiene lignans are the main secondary metabolites specific to the Schisandra genus of e.g. hepatoprotective, antioxidant, and anti-inflammatory activities [1].
Our studies aimed to investigate lignan production in S. chinensis, S. chinensis cv. Sadova, S. henryi C. B. Clarke, and S. rubriflora (Franch.) Rehd. et Wils microshoot cultures using different comprehensive and globally innovative biotechnological methods.
The maximum contents of dibenzocyclooctadiene lignans in S. chinensis and S. ch. cv. Sadova agar, agitated, and PlantForm bioreactor cultures were 237.86, 195.15, 546.98 and 574.42, 375.11, 313.51 mg/100 g DW, respectively [2].
The presence of dibenzocyclooctadiene, aryltetralin, dibenzylbutane, tetrahydrofuran, and neolignans has been confirmed in cultures of S. henryi and S. rubriflora male and female lines. The total amount of the detected lignans were 873.71, and 250.92 and 220.70 mg/100 g DW, respectively. The main compounds were schisantherin A, B, and angeloylgomisin H, O [3,4].
All the presented results document the high biosynthetic potential of cells from in vitro cultures of different Schisandra species and cultivar.
Funded by National Science Centre, Poland (2016/23/D/NZ7/01316 and 2020/37/N/N27/02436), and Polish Ministry of Science and Higher Education (N42/DBS/000273).

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Abstracts of Poster Presentations

July 4 - 5, 2022, Vienna, Austria

Poster №1:

Impedance flow cytometry early predicts embryo yields in wheat (Triticum aestivum L.) microspore cultures

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Haplomethods are key biotechnological tools allowing to quickly produce perfectly homozygous lines, resulting in speeding up plant breeding programs. Under specific stress conditions, microspores are reprogrammed toward sporophytic pathway leading to embryo formation. Various endogenous and exogenous factors affect embryo yield in androgenesis, so the improvement of androgenesis efficiency requires the development of early, reliable and robust reactivity markers. During the last decade, numerous cytological, cellular and biochemical approaches have been carried out to finely characterize microspore development and fate during androgenesis. However, the different available markers are often species-dependent, and their development or application are time-consuming and cumbersome. Here we show the suitable use of impedance flow cytometry (IFC) to develop new robust, reliable and strong markers of androgenesis reactivity in wheat allowing (i) routine monitoring of the viability of heterogeneous cell cultures, (ii) quick and simple evaluation of stress treatment efficiency, (iii) early prediction of embryo yields from microspore suspensions. IFC technology becomes a very useful tool to track and characterize wheat microspores in androgenesis.

Poster №2:

Modification of the N-glycosylation profile of pharmaceutical glycoproteins produced in Nicotiana tabacum BY-2 suspension cells

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Production of recombinant glycoproteins gives rise to different N-glycosylation profiles depending on the heterologous system used and culture conditions. N-glycosylation is also responsible for a huge heterogeneity of the final product as each N-site can bear different N-glycans. This heterogeneity is challenging for batch-to-batch reproducibility and represents an issue for pharmaceutical glycoproteins since the presence or absence of N-glycan structures affects their properties.
Our research aims at generating a set of Nicotiana tabacum Bright Yellow-2 (BY-2) suspension cells with specific and well-defined glycosylation profiles using CRISPR Cas9 genomic edition of glycosyltransferase genes.
A BY-2 cell line producing humanized N-glycans was obtained through the inactivation of XylT and FucT genes. Next, to generate a BY-2 cell line producing glycoproteins without complex N-glycans, the N-acetylglucosaminyltransferase I (GnTI) genes were inactivated. No complex N-glycans were detected but traces of alpha(1,3)-fucose residues were identified. A second genome editing targeting both GnTI and FucT genes was achieved to tackle this issue. Finally, a BY-2 cell line expressing glycoproteins with a single N-linked GlcNAc at each N-site was also generated through the ectopic expression of EndoT from Hypocrea jecorina in the GnTI/FucT-KO line.
Antibodies and viral antigens were successfully produced in these glyco-engineered cell lines. Glycoproteomic characterization will be reported.

Poster №3:

In vitro propagation of Heliconia plants

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In Colombia, floriculture has become an important economic activity. The cultivation of Heliconia plants and related species has great potential since American, Asian and German markets have a growing demand for their flowers. One of the objectives of this work is the development of an in vitro mass propagation protocol and the field evaluation of the morpho-agronomic development of ten cultivars of Heliconia plants of commercial interest in central-western Colombia.
Propagation of Heliconia plants from floral buds requires more time in the establishment and multiplication stages compared to vegetative buds, however, it is a method with a higher percentage of survival and contamination below 50 %, which offers promising results to be applied to a diverse number of Heliconia plants of commercial interest. The results of this work were evaluated in the laboratory, nursery, and field plots, for which aspects related to the taxonomy, botanical description, floral biology, genetics, and breeding were analyzed. The form of propagation and environmental conditions, their influence over growth and flowering, as well as the cultural tasks necessary to achieve better production quality, were decisive.

Poster №4:

Borago officinalis L. – artificial polyploidization

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Borage (Borago officinalis L.) is a hairy annual herb that is cultivated for medicinal and culinary uses. The plant is known for its antispasmodic, antihypertensive, antipyretic, and diuretic properties. Significant secondary metabolites include for example tannins and resins, and, unfortunately, unsaturated pyrrolizidines alkaloids including amabiline. There are many methods to quantitatively and qualitatively influence the composition of secondary metabolites. One method is artificial polyploidization, which affects genetic and epigenetic information without the need for genetic modification. The process of artificial polyploidization uses chemical compounds called antimitotics. In addition to anatomical changes such as increased cell size, change in leaf shape and size, and change in habit structure, polyploidization also achieves changes at the level of secondary metabolites and potentially increased resistance to stresses. In our experiments, the derivation of mixoploid (12% success) and polyploid plants (5% success) was achieved by in vitro artificial polyploidization using a medium containing 20 µmol oryzalin. These plants were evaluated on morphological and cytological levels.
This research was supported by the grant QK1910103 (NAZV, Ministry of Agriculture, Czech Republic).

Poster №5:

Regeneration of microspores and protoplasts of Brassica species with or without PSK-alpha peptide

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Microspores, immature pollen grains, are haploid cells that have the ability to regenerate into whole plants when exposed to appropriate culture conditions and stimuli. Protoplasts are isolated cells obtained by mechanical removal of the cell wall or by the use of enzymes. They can be isolated from leaves, hypocotyls, cotyledons, and roots. In this study, young leaves of different Brassica species were used for protoplast isolation and immature flower buds for isolation of microspores. Phytosulfokine (PSK) is a small disulfated peptides consisting of five amino acids. The peptide PSK-alpha added to the medium is known to stimulate of cell proliferation and embryo regeneration. With this study, we aimed to evaluate the effect of the peptide PSK-alpha in the culture medium on the regeneration of microspores and protoplasts of different Brassica species. The results showed a positive response to the use of PSK-alpha peptide. Protoplast regeneration increased with the use of PSK-alpha, and PSK-alpha also had a positive effect on microspore regeneration, as evidenced by a higher percentage of regenerated embryos compared to the control.

Poster №6:

Micropropagation of ten genotypes of Cannabis sativa L.

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Cannabis is a multipurpose plant grown for fiber, seeds, flowers, or cannabinoid extraction. Micropropagation is a way to propagate plants or simply to maintain valuable genotypes in a sterile environment. It is also used when there are many genotypes to preserve and not enough space to keep them in the greenhouse. Some research has already been done on cannabis micropropagation, but data from the literature clearly shows that there are significant differences in the responsiveness of different genotypes. Therefore, our aim was to establish an efficient protocol for micropropagation and preservation of germplasm used in our cannabis breeding program. We included seven different genotypes of medical cannabis and three different varieties of industrial cannabis in in vitro cannabis culture experiments.
Six different media were tested, based on Murashige and Skoog or DKW basal media with vitamins. The hormone TDZ (0.1 mg/l), the hormone meta-topolin (0.5 mg/l) and charcoal (500 mg/l) were added in six different combinations. Cultures were initiated by inoculation of nodal explants and multiplication through several subcultivation steps before acclimatization of the plants. All genotypes and cultivars grew successfully and produced an average of one shoot per nodal explant. Highly valuable genotypes were maintained in vitro and were diseases free. DNA was isolated from mother plants grown in the greenhouse at the beginning of the study and from propagated plants grown in vitro and after acclimatization. The effect of in vitro plant tissue culture on the epigenome of cannabis was assessed using the methylation-sensitive amplification polymorphism.

Poster №7:

Establishment of a DNA-free genome editing and protoplast regeneration method in cultivated tomato (Solanum lycopersicum)

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Tomato is an important agricultural crop with high commercial value. So far, most reports about CRISPR/Cas9 applications on cultivated tomato were based on Agrobacterium tumefaciens-mediated transformation with the risk of the integration of foreign DNA. An alternative method is to use Ribonucleoprotein (RNP) based CRISRP/Cas9 to generate DNA-free plants, which might be considered as non-GMO. However, the major bottleneck of this technology is the shoot regeneration and only a few old reports published on the successful shoot regeneration on cultivated tomato from unedited protoplasts and one very recent report on wild tomato from edited protoplasts. In this study, the implementation of a transgene-free breeding method for cultivated tomato was achieved by RNP based CRISRP/Cas9, including the optimization of protoplast isolation and solving the challenge of shoot regeneration from transfected protoplasts. We have successfully obtained mutant plants targeting at SELF PRUNING (SP) and SELF PRUNING 5G (SP5G) simultaneously with a high mutation rate up to 60% in at least one allele in either SP or SP5G genes.

Poster №8:

The possible role of tobacco PAO during protoplast isolation and maintenance

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Plant protoplasts are valuable tools for genetical and biotechnological utilization. Treatment with cell wall-degrading enzymes results in the formation of a large population of protoplasts. During this process complex metabolic rearrangement, for example generation of reactive oxygen species (ROS), occur. Polyamine oxidases (PAOs) are able to orchestrate polyamine (PA) level by PA catabolism. Alteration of PA has effect on protoplast cell growth and cell death. However, the background, how protoplast regeneration is affected by PA catabolism, is hardly known. Therefore, our aim was to determine the role of PAOs at cellular response of protoplast isolation and maintenance.

This work was supported by grants from National Research, Development and Innovation Fund (Grant no. FK 128997). Katalin Gémes was supported by János Bolyai Research Scholarship of the Hungarian Academy of Sciences (Grant no. 00580/19/8) and UNKP-21-5-SZTE-587 new national excellence program of the ministry for innovation and technology.

Poster №9:

Polyamine oxidase 5, ethylene, NO: Do they cross-talk to inhibit direct shoot formation?

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Vegetative plant propagation can be achieve by several pathway. One of them is de novo shoot meristem formation. Lateral root primordia can be directly converted to shoot meristem (SM) by exogenous cytokinin induction. Beside cytokinin, importance of ethyelne, polyamines and polyamine catabolism enzyme, PAO5, was shown during direct shoot organogenesis of arabidopsis. Moreover, polyamines can induce NO synthesis, which may also implicate in this process. During this work we have found that ethylene induced NO production which decreased PAO5 expression and direct conversion of LRP to SM in arabidopsis.

This work was supported by grants from National Research, Development and Innovation Fund (Grant no. FK 128997). Katalin Gémes was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (Grant no. 00580/19/8) and UNKP-21-5-SZTE-587 new national excellence program of the ministry for innovation and technology.are a first step in understanding how SnRK1 modification can contribute in sustainable resistance against this important disease.

Poster №10:

Role of Arabidopsis E2F transcription factors in the direct conversion of LRP to SM

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Durum wheat (Triticum turgidum ssp. durum) is extremely susceptible to Fusarium head blight (FHB). Biocontrol agents such as yeasts, including Debaryomyces hansenii, use to suppress F. graminearum, could pose a viable alternative to fungicides. The aim of this study was to evaluate the expression of the gene encoding chitinase in Triticum turgidum ssp. durum during infection with F. graminearum after biocontrol treatment. The mRNA transcript levels were normalized based on the mRNA transcript levels of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and RNA-binding heterogeneous nuclear ribonucleoprotein Q (hnRNPQ). The expression of the chitinase gene increased 6-fold between the first and second day after spike inoculation with the spore suspension of F. graminearum. The biocontrol treatment that involved the application of yeast cell suspension to wheat spikes contributed to a 14-fold increase in chitinase gene expression between 24 and 48 hours of F. graminearum infection. The biocontrol treatment exerted an inhibitory effect on the expression of selected genes involved in the defense responses of durum wheat during F. graminearum infection.

Poster №11:

Using dormant axillary buds to improve organogenesis of Damask rose (Rosa x damascena Mill) in vitro

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Damask rose (Rosa x damascena Mill) is an important ancient scented hybrid rose. A direct organogenesis protocol was developed for in vitro propagation using dormant axillary buds. We tested the effect of Murashige and Skoog (MS) and Woody Plant Medium (WPM) supplemented with benzyladenine (BA), kinetin, and thidiazuron at 0.2, 0.5 and 1 mg/L. Shoot induction occurred from 40% of sterilised explants. WPM produced longer shoots and more leaves than MS. Optimal shoot multiplication occurred on WPM supplemented with 1 mg/L BA, 3% sucrose and 0.7% phytagar. In vitro rooting (50%) was obtained when shoots were transferred to WPM with half strength macronutrients containing 2% sucrose and 1 mg/L indole-3-butyric acid. Rooted plantlets were transferred to soilless media. The use of a growth chamber with controlled humidity during the first days of acclimatization increased plant survival (93%). Plantlets were subsequently grown to maturity in the glasshouse.

Poster №12:

Effect of iron on the rooting response of hazelnut shoots cultured in RITA® bioreactors

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European hazelnut (*Corylus avellana* L.) is an economically important, sustainable crop and a multipurpose tree. In this study we micropropagated hazelnut axillary shoots using semisolid medium and temporary immersion in RITA bioreactors. The factors investigated were explant type, cytokinin concentration and iron (source and concentration). In liquid medium, basal explants were superior to apical explants in all the growth responses including shoot number, shoot length, multiplication coefficient, largest leaf per explant, rootable shoots. The bioreactors allowed the cytokinin use to be halved for a better response than on semisolid medium. Explants cultured in RITA produced more and larger shoots than in semisolid medium and had fewer chlorotic leaves. Use of Fe-EDDHA up to 400 micro molar enhanced the rooting response. Using Fe-EDTA over 200 micro molar reduced shoot length and the multiplication coefficient. This study showed both iron and culture environment affect the performance of hazelnut shoots in vitro.

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Publications

July 4 - 5, 2022, Vienna, Austria

Results of testing Hungarian varieties in the soil and climatic conditions of the Republic of Uzbekistan and future steps

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Potato (Solanum tuberosum L.) is a crop of world importance that produces tubers of high nutritional quality. It is considered one of the promising crops to overcome the challenges of poverty and hunger worldwide. However, potato is a rather sensitive crop to biotic and abiotic stresses: Virus Y, Nematode, Virus X, Erwinia, Leaf roll virus, Fusarium, Phytophthora and Nematode, that can cause significant losses in production. Consequently, the choice of the cultivar and quality of its seed potato is more important than in case of other major food crops. It has much higher influence on yield and quality then production technology itself. Potato has a high importance in food security and self-sufficiency (90.000 ha) - massive need for high quality seed potato (from import, 20.000 tons) in the Republic of Uzbekistan. Main tasks of the Centre helping to increase productivity of Uzbek potato production in cooperation by: I adaptation Hungarian seed potato production technology, II putting Hungarian varieties into production, III initiation of common breeding and science research programmes. For example, it was created the conditions for seed propagation of these varieties I propagation of 600.000 in vitro plantlets of 5 the varieties, II production of 600.000 highest quality PBTC minitubers, III production of 150.000 second generation tubers of the 5 varieties, IV will ensure the freedom the produced seed potato from any pathogens or pest, by strictly keeping and operating quality control methodology. Expected results is the ability to operate a complex seed potato production technology with internal quality control system and availability of three level of propagation material of seed potato production (in vitro plants, first and second generation minitubers – which will be ready for planting in 2023.

Methods for determining the mass fraction of raw oil in soybean varieties

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The test is carried out according to the method of implementation of this standard in the Soxhlet extractor on the mass fraction of raw oil on the degreased residue. Before the analysis, flasks of approximately 110 x 90 mm in size are prepared from the filter paper for extraction. The prepared flasks are degreased in a Soxhlet extractor for one hour, then dried in glass beakers at a temperature of 105℃ in an oven for one hour, and cooled in a desiccator. Soybean oil is widely used in the manufacture of soap and lacquer paints. Soybean oil makes up 25% of the vegetable oil used by the world's population. The most important ingredient in soybean oil is irreplaceable linoleic acid (50-60%). However, its amount is directly correlated with linolenic acid (2-3%), which gives the oil a specific odor and causes the oil to decompose rapidly.

Influence of different sowing methods on the amount of conditioned seeds yielding from the Iskandar variety of rice

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The research was conducted in 2020-2021 on the experimental field of the Rice Research Institute. In the experiment, the amount of conditioned seed yield was calculated from 4 types of rice sowing methods: manual sowing of rice seeds, sowing of rice seeds in dry soil with the help of seeders, manual transplanting, transplanting by mechanism. Experiments are included 4 options. The area of each option was 9 m^2 in 4 options and the total experimental area was 9x16 = 144 m^2. Placement of laboratory and field experiments in scientific research, calculations, observations were carried out on the basis of "Methodological manual of the State Commission for Variety Testing of Agricultural Crops" (Tashkent 1994), "Methods of field experiments" (UzPITI 2007). net profit and other economic indicators in the method of VN Polozhi (Tashkent 1976), statistical analysis of experimental results "Methodology of field experience" (Kolos 1985) was performed by BA Dospekhov. The research used medium-ripe rice "Iskandar". In our study, sown rice seeds by broadcasting were selected as a control option. In this method, the sowing norm of seeds per hectare is 160-180 kg. In the option of rice planted in the soil and in the broadcasting method, low yields and conditioned seeds were caused by immature, rotten and semi-rotten grains in physiologically immature or dormant seedlings. Due to the fact that the rice seedlings were biologically fast maturing and fully mature, the yield of selected seeds was 25-26% higher than that of manual dry sowing and broadcasting methods. When the rice was planted by the seedling method, as a result of ensuring full ripening of the crop, it was possible to get 85-86% of yellow seeds from the harvest.

Methods for determining the mass fraction of raw oil in soybean varieties

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The test is carried out according to the method of implementation of this standard in the Soxhlet extractor on the mass fraction of raw oil on the degreased residue. Before the analysis, flasks of approximately 110 x 90 mm in size are prepared from the filter paper for extraction. The prepared flasks are degreased in a Soxhlet extractor for one hour, then dried in glass beakers at a temperature of 105℃ in an oven for one hour, and cooled in a desiccator. Soybean oil is widely used in the manufacture of soap and lacquer paints. Soybean oil makes up 25% of the vegetable oil used by the world's population. The most important ingredient in soybean oil is irreplaceable linoleic acid (50-60%). However, its amount is directly correlated with linolenic acid (2-3%), which gives the oil a specific odor and causes the oil to decompose rapidly.

Introduction to in vitro culture of various fruit and berry plants

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In order to develop and promote modern technologies in the field of crop production and plant biotechnology laboratory was established on the basis of the Research Institute of Plant Genetic Resources. The laboratory is working on the study of the basic laws of the processes of plant morphogenesis in vitro, microclonal reproduction and production of high-quality planting material of promising introduced species, forms and varieties of sparsely distributed and hard-rooted by traditional methods of agricultural crops. The main competitive advantages of microclonal reproduction are the high reproduction are the high reproduction rate of plants, which is especially important when propagating new and rare species and varieties. At the same time, the production of planting material takes place all year round, and does not depend on the time of year. It is also important to significantly save the areas necessary for growing planting material, as well as the possibility of mass reproduction of plants that are difficult to propagate by traditional methods. In this regard, the laboratory is working on the introduction of grapes, blueberries, blackberries and strawberries into culture in vitro. As a result, we get the primary material for the microclonal reproduction of identical mother plants free of viruses, fungi and bacteria. Plants are the exactly identical to their mother plants.

Scientific works presented by the research institute of vegetable, melon crops and potato

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In 2022, the Institute will introduce the results of scientific research conducted in recent years, along with more than 20 scientific developments on resource-saving, water-saving, intensive technology cultivation of vegetables, melon crops and potatoes. Including:
- Technology of sowing the seeds of melons with various drugs before sowing;
- Technology of ecologically pure tomato production;
- Technology of growing okra from non-traditional vegetable crops;
- Technology of growing melon varieties on newly irrigated lands of Zarafshan valley;
- Technology of cultivation and drying of melon varieties in different technologies on newly irrigated typical gray soils;
- Pumpkin (Cucurbita pepo L.) cultivation technology;
- Technology of growing tomatoes in the greenhouse and in the open field with grafted seedlings;
- Technology of growing lettuce and leafy turnips from fresh vegetable crops;
- Technology of growing sweet peppers in the second period;
- Technology of growing vegetable shade on the gray soils of Uzbekistan;
- Technology of growing high quality seeds from garlic;
- Accelerated primary seed technology of early and medium ripening potatoes has been introduced in the Republic of Uzbekistan.

PHENYLETHANOID ACCUMULATION IN LIQUID CULTURES OF SALVIA VIRIDIS

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Salvia viridis is an annual herb which has been used in traditional Turkish medicine as an inflammatory and antiseptic agent. Several classes of secondary metabolites have been detected in its aerial parts including phenolic acids, phenylethanoids and flavonoids. The aim of the study was to obtain liquid cultures of S. viridis shoots, which in a short time would provide a significant amount of the raw material rich in bioactive compounds. The cultures of the sage shoots were carried out in various liquid systems: agitated medium, stationary medium without support and with cellulose or polyurethane as support, and, for comparison, on a solid (agar) medium.

I was found that S. viridis is sensitive to continuous immersion in the medium and shaking, and requires support materials for effective growth in a liquid medium. The medium, when cellulose was used as supported material, turned out to be the most beneficial for the proliferation of shoots of this species; 8.7 buds/shoots was obtained from a single explant. The liquid system with cellulose also proved beneficial for the production of phenylethanoids including predominant verbascoside. Their total content under these conditions, amounting to 21.4 mg/g DW and 18.2 mg/g DW for verbascoside, was about 5 times higher than that found in the control shoots grown on the solid medium.

Organization of primary seed production of non-traditional and export-oriented vegetable crop turnip leaves

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Today, 38,835,235 tons of turnips are grown on 1,368,358 hectares of land around the world, with an average yield of 28.4 tons per hectare. Turnip is one of the main crops in the vegetable industry of the USA, Japan, China, Ireland, Israel, Russia, Sweden, England, Belgium and other European countries. Darmon varieties of leaf turnip L-356RSR were created and introduced to the conditions of our country on the basis of individual selection and study of generation. The total yield of Darmon variety of turnips was 21,3 t/ha, and the yield of commodity turnips was 21,2 t/ha. The duration of development periods of leaf turnip plants also varied depending on planting times. The longer the planting period, the longer the growth period. Sowing times significantly affect the manifestation of morphobiological signs of leaf turnip plant. Leaf length was 65,6 cm in the first term and 45,5 cm in the fourth term. A similar situation was observed in the width of the leaves.

Rich collection of melons of Uzbekistan

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Uzbek melons have a unique sweet taste, pleasant aroma and various shapes. Varieties have early, middle and late varieties, as well as varieties that are stored for a long time in the winter. Uzbek melons have many domestic and foreign buyers. Varieties resistant to transportation, with an average weight of 2-2,5 kg are being created for export. At the same time, in recent years, varieties of melons resistant to diseases, especially flour dew, are being created. In 2000, the Uzbek Institute of Botanical Research conducted a scientific expedition on the project "Increasing the use of genetic resources of melons in Uzbekistan through their storage and storage on farms" melon varieties grown during the period were collected and described. As a result, more than 100 local melon varieties were collected and based on them, the atlas book "Melons of Uzbekistan" was prepared. It should be noted that the Uzbek Research Institute of Vegetable, melon crops and potato in 1970-1980 collected a collection of local varieties of all melons of the country, in 2022, the institute has a collection of more than 350 local melons.

The results of studying new bread wheat varieties and lines for rainfed conditions in Uzbekistan

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The bread wheat early maturing varieties have more grain yield than late maturing in drought condition of dry lands. Yellow rust (Puccinia striiformis West f.sp. tritisi et Henn) is main disease of wheat in mountain and foothill areas of rainfed. Rainfed areas of Uzbekistan require growing of widely adapted, high yielding wheat varieties that combine high yield potential with diseases resistance, drought tolerance and high quality. In 2021 more than 650 varieties and new lines of bread wheat from ICARDA (International Center for Agricultural Research in the Dry Areas) and local varieties have been estimated by different traits as early maturing, heat and drought tolerance, resistance to rust diseases, grain quality and high yielding.The new varieties which Kizildon, Nushkent were selected as a high yield with high gluten quantity, early maturity.

Tissue-specific expression of NtZIP5 in tobacco (Nicotiana tabacum)

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Expression of NtZIP5 gene (encoding a protein involved in Zn and Cd uptake in tobacco plants) depends on Zn and Cd concentration. q-RT-PCR analyses in tobacco roots showed strong induction of NtZIP5 expression by Zn deficiency (compared to control conditions).
To determine in which tissues NtZIP5 expression is localized, transgenic tobacco plants with GUS reporter gene under the control of NtZIP5 promoter were generated. Selected transgenic lines were grown under Zn-deficiency and the presence of Cd.
The GUS staining was detected in all tissues of the apical root part above the quiescent center and in the epidermis and cortex of the middle and basal main root part. The presence of Cd caused a loss of NtZIP5 expression in the apical part which suggests protection of the meristematic tissues from the toxic effects of Cd. On the other hand, in the middle part of the main root NtZIP5 expression was upregulated by Cd presence. It may suggest the participation of NtZIP5 in the accumulation of Cd excess and contribution to the regulation of long-distance Cd transport.

Funding: project OPUS-8 no. 2014/15/B/NZ9/02303 supported by National Science Centre, Poland.

Cooperation for the development of scientific research

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The institute cooperates with foreign scientific centers, research institutes and universities to create varieties of agricultural crops, develop agricultural technologies, develop methods of disease and pest control, determine the avirulence and virulence of rust diseases, improve the skills of young scientists. Since 2008, the Institute, in cooperation with the international organizations ICARDA and CIMMYT, has been working to improve the skills of young scientists in the creation of varieties of winter wheat, durum wheat, barley, spring wheat, peas and lentils. In addition, more than 20 varieties of soft wheat, 8 varieties of durum wheat, 2 varieties of peas and 2 varieties of barley were created and more than 10 young scientists underwent internships in Germany, Turkey, Mexico, Morocco, Korea, Australia, Kazakhstan and Tajikistan. In collaboration with the FAO, research is being conducted on "Integrated management of natural resources in the arid and saline agricultural landscapes of Central Asia and Turkey" as a result of extreme climate change. Work is underway with the University of Bonn of Germany, to create varieties of wheat resistant to salinity, and to increase the knowledge of young professionals. In addition, cooperation has been established with the Kazakh Academy of Sciences, the University of Sydney, the Moroccan Academy of Agricultural, the Tajik Academy of Agricultural, the Russian Academy of Agricultural, the Turkish Academy of Agricultural and the Ukrainian Academy of Agricultural Sciences.

Effectiveness of using in vitro method on wheat in Uzbekistan

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It is known that it takes an average of 10-12 years to create wheat varieties with traditional selection methods. Therefore, it is important to develop and improve modern methods of biotechnology and to study the effectiveness of their application in genetics and selection of varieties. Nowadays it is possible to accelerate the process of creating homozygous genetically stable lines by growing microspores under in vitro condition which is considered one of the most effective methods of biotechnology. The method of obtaining haploids from microspores is an effective and alternative method of creating stable genotypes on valuable traits and shortening the selection process. There are currently three main methods used to obtain haploids: proliferation of male gamete cells under in vitro condition; reproduction of maternal tissue organs; breeding hybrid offspring. Based on the all abovementioned, scientists of the Southern Agricultural Research Institute in Uzbekistan have planned to conduct research on the study of the isolation of microspores in wheat, study of the development and formation of multicellular structures from microspores, embryos and creation of regenerant-sustainable varieties of wheat. As a result, using the haploid selection method, sustainable wheat forms with a positive set of valuable economical traits will be created and recommended for the selection-breeding and production process.

Assessment of grain productivity and quality of new local lines in winter bread wheat

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Currently, global climate change, rising temperatures and drought are negatively affecting the quality of wheat (Triticum aestivum L.) grain grown in the southern regions of the Republic of Uzbekistan. To solve this problem, 23 local lines of common wheat, created by hybridization, were studied by comparing grain quality indicators with local check varieties Grom and Gozgon. The main goal of studying bread wheat lines in the control nursery is to select high-yielding, high-quality lines adapted to irrigated areas, as part of the creation of new varieties of bread wheat and transfer to agro-ecological competitive variety testing. The selected standard varieties have been planted in the largest area in the southern regions this year. In the experiment, varieties and lines were placed in 3 replicates, and the data obtained were statistically analyzed. Important indicators of the quality of grain varieties and lines, such as protein content, gluten, IDC index and grain vitreousness were evaluated. Lines of genetically high quality grain were selected and transferred to the next stage. Of the cultivars and lines studied in the control nursery, 4 lines were selected for testing in the agroecological variety nursery. The selected KRBW 18-14, KRBW 18-18, KRBW 18-21, KRBW 18-29 lines were selected due to the fact that the yield was 80 c/ha, grain weight per 1000 grains was 45 g., grain nature was higher than 800 g/l, grain quality was higher than local check varieties.

Southern Research Institute of Agriculture (SRIA) and ICARDA – an example of outstanding cooperation

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ICARDA and SARI have collaborated in a number of agricultural researches in Uzbekistan. ICARDA have supported research work at SARI through exchange of mor than 20000 accession of improved germplasm of winter wheat, spring wheat, durum, barley, chickpea, lentil, fababean, and grasspea. Another important area of collaboration includes capacity building of the young researchers at SARI. ICARDA has been supporting SARI since 2008 in capacity building through in-country training and workshops, opportunity of training abroad at ICARDA's and CIMMYT's research facilities in Mexico, Syria, Morocco and Turkey. A number of young researchers received scientific support from ICARDA scientists in completing their post-graduate thesis research. The research work done collaboratively by SARI and ICARDA have been published in a number of international journals. The scientists of SARI and ICARDA have participated and presented several research papers at national, regional and international conferences. ICARDA and SARI completed a number of collaborative research projects funded by international donors. The long-term collaboration between SARI and ICARDA have resulted in development of more than 20 varieties of bread wheat, durum, barley, and chickpea. Yellow rust resistant wheat varieties have saved the farmers money spent on spraying fungicide, besides protecting the environment. The wheat variety "Gozgon" identified in 2009 as yellow rust resistant remains undefeated by the highly variable fungus in Uzbekistan. The above extensive collaborative activities between SARI and ICARDA have set a prominent example of international partnership in agricultural research. SARI has become a center of excellence for international crops research. New constraints in the form of biotic and abiotic stresses keep emerging, Therefore, development of stress tolerant and further higher yielding crop varieties would be needed from SARI-ICARDA collaboration to increase crop productivity to meet the ever-emerging food demand of the growing population in the country. The deliberations during the present seminar will address some of the emerging challenges.

Improving the use of land and water resources and increasing their productivity in conditions of water scarcity

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Currently, the agriculture of the republic is in urgent need of the development of scientifically-based techniques that contribute to increasing the productivity of rice and other crops. Increasing the productivity of agriculture by increasing the utilization rate of arable land is particularly important for the conditions of the Republic of Karakalpakstan, where there is almost often a shortage of irrigation water. The results of a three-year study showed that increasing the utilization rate of irrigated arable land by saturating the crop rotation wedge with other dry grain crops has a beneficial effect on increasing the productivity of the entire complex. At the same time, rice in the proposed variant is reduced to 40%, but grain in the crop rotation wedge increases to 80% or more. In the end, the stability of crop yields and an increase in net profit in general are ensured. The reduction of rice to 40% in the proposed scheme, on light loamy soils at the irrigation rate recommended for this zone, allows saving 25-27% of irrigation water. Due to the saved irrigation water, it will be possible to additionally cultivate 300-325 hectares of dry grain crops at an irrigation rate of 1200 m3 ha.

Inheritance of a sign of precocity inhybrids, depending on the genealogy of parental forms

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Straumal B.P. studying the inheritance of precocity in F1 cotton hybrids notes that when crossing cotton varieties close in length of the growing season in F1, heterosis in precocity usually manifests itself. In the case when the parental forms differed significantly on this basis, the hybrids inherited intermediate precocity. In our study, the nature of inheritance and the variability of the sign of precocity by components in F1 hybrids were studied. The starting material was grades C-4727, C-9070, Kirghiz-3, Chimbay-3104, Namangan and 175-F. In studies, the level of heterosis of F1 hybrids was estimated by the value of the dominance coefficient (hp) according to the Wright formula. According to the flowering phases, the best indicators were obtained in the precocious varieties of Cimbai - 3104 and C-4727. The late-ripening variety 175-f bloomed four days later than the precocious varieties. The effect of heterosis on germination-flowering was obtained in hybrid combinations C-9070 x 175-F (hp =-2.0), C-4727 x Kirghiz-3, C-4727 x Namangan, C-9070 x Namangan and 175-F x C-9070 (hp =-1.0). The duration of the growing season varies from 100 to 106 days. In the breeding process, the periods of germination – flowering and the height of the first sympodium are important components that determine early maturity, but in this study it is clear that the sign of 50% maturation is the best criterion for this indicator. Based on the analysis of the phenophases of the studied parent varieties and hybrids, it can be noted that the duration of the interphase periods of flowering and maturation in cotton is a structurally complex feature of the polygenic type of inheritance.

Development of new cotton varieties resistant to verticillium and fusarium wilt

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The harmful effects of seed and seedling damage are exacerbated by the fact that 80% of the future cotton yield is determined within 30 days from the date of seedling emergence. The genotyping results, especially those for three primer pairs (BNL4003, CM209, TMB0161), showed that the best prospective hybrid was F_{11} [F_6 (L-101 x L-105) x L-106] because the largest number of individuals within this line were identified as having candidate linked to wilt resistance traits. Based on a biochemical analysis using marker proteins such as the activity of peroxidase enzymes, hydrogen peroxidase as well as a cytochemical study of the roots of various cotton lines, the hybrids F_{11} [F_6 (L-101 x L-105) x L-106] and F_7 [Zharkurgon x Namangan-34] were characterized as the most resistant to V. dahliae. This line was separated from the complex, interlinear hybrid combination F_{11}[F_6(L-101 x L-105) x L-106]. L-1077 produces fibre that meets the type IV fibre quality standards, produces high fibre yields and is highly resistant to V.dahliae and Fusarium. Moreover, an application (NAP 2020 0007 from 31.01.2020) for a patent for this line has been issued by the Intellectual Property Agency of the Republic of Uzbekistan under the cotton variety name C-6602.

Selection of winter soft wheat in Karakalpakstan

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The conditions of cultivation of winter soft wheat in Karakalpakstan are much more complicated and require more material costs and labor than in other regions of Uzbekistan. Currently, the scientists of the region face an urgent task not only to mitigate the ecological situation of the region, but also to develop grain production, i.e. to improve the reclamation condition of irrigated soils, to improve agrotechnological techniques using modern resource-saving innovative technologies. The main tasks of grain breeders are to create varieties of winter soft wheat adapted to the agroecological conditions of the region, with high yield potential and good baking quality of grain, characterized by winter hardiness, drought resistance, salt resistance, disease resistance and responsiveness to high agrophone. In Karakalpakstan, agriculture is of the irrigation type and requires intensive agricultural production. Therefore, the creation of grains combining high yields with good baking properties corresponding to strong and valuable varieties of winter soft wheat in a complex resistant to stress factors of the region is a very difficult, but solvable task. In recent years, plant breeding, as a branch of science, has been in constant dynamic development, enriching itself with the latest methods and examples using modern achievements of non-traditional selection methods. They make it possible to quickly create the necessary source material and contributes to the construction of new desired plant forms, therefore, the selection process is facilitated and accelerated. Using and improving modern breeding methods, we have created and in 2020 entered into the state register a new relatively salt-resistant variety of winter soft wheat "Utkir". Currently, primary seed production of the variety is underway and the area of sowing in farms and clusters of the Republic of Karakalpakstan is rapidly expanding.

Setting standards and deadlines for feeding soybeans varieties with mineral fertilizers

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Today, it is required to use new effective methods of soil tillage processing to maintain and increase soil fertility, to ensure its biological activity, to achieve the active development of living organisms, to apply scientifically based advanced technologies for the care of agricultural crops, to plant crops with high yields, to choose varieties with high levels of fast-growing and grain quality. For this, deep research work with wide coverage is required. Proceeding from these tasks, the scientific research institute of grain and leguminous crops at the "Central" Experimental site and Karakalpakstan scientific-experimental Center carried out research on the application of various norms of mineral fertilizers in the nutrition of the varieties of soybeans Ta'maris man-60, Ayjamol, Selekta-201, Amigo, the study of irrigation periods. In a 3-year scientific study conducted in 2018-2020 years, the average grain yield indicators obtained for 3 years on variants from soybeans varieties planted in the main fields were as follows.

Efficiency of application on cotton of new environmentally safe fertilizer Ecophosphoazotine

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The studies were carried out according to the methodology adopted at Scientific Research Institute of Breeding, Seed Production and Agricultural Technology of Cotton Growing, to determine the total content of humus, samples in layers of 0-30, 30-50 cm of soil were taken before application and at the end of the growing season according to the method of I. V. Tyurin. Nitrogen and phosphorus were determined by the method of I. M. Maltsev and L. N. Gritsenko. Nitrate forms of nitrogen - by an ionometric device, mobile phosphorus - by B.P. Machigin and exchangeable potassium by a flame photometer. The amount of gross and mobile forms of humus and nutrients (NPK) in the soil of the experimental field was averaged by sampling for each option from 0-30, 30-50 cm soil layer "Methods of "[31], "Methodology for conducting field research".These yield results were dispersively analyzed according to the method of B. Dospekhov "Methodology of field experience". The distance between the rows is 90 cm.The area of each plot is 270 m^2. The depth of groundwater is -2-2.5 m. The mechanical composition of the soil is of medium weight.

Evaluation of the source material and creation of highly resistant F1 hybrids to Verticillium dahliae Klebahn

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As a result of a biochemical study of the activation of the enzymatic activity of three enzymes peroxidase, semifinoloxidase and phenylalanine ammonia-lyase – when infecting seven-day cotton seedlings with strains of the V.dahliae fungus isolated from the soil of a natural, mixed provocative background of the Research Institute of Breeding, Seed Production and Agrotechnology of cotton cultivation, it was found that among the cotton varieties of Uzbek selection, the following should be considered highly resistant: Namangan-34, Gulbakhor-2 and Andijan-35, and F1 hybrid combinations created with the participation of the above-mentioned varieties: Namangan-34 x Sulton, Namangan-34 x Gulbohor-2, Namangan-34 x AN-16, Sulton x Namangan-34, Gulbohor-2 x Sulton, Gulbohor-2 x Andijan-35, Andijan-35 x Namangan-34, Andijan-35 x Sulton, AN-16 x Namangan-34, AN-16 x Sulton, AN-16 x Andijan-35, which are recommended for the creation of highly pathogen-resistant breeding material and a new variety of cotton.

The results of the competitive variety testing of L-279 in the conditions of Surkhandarya region

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As a result of the evaluation in the competitive variety testing of L-279 created within the framework of the applied project A-2021-317 in extreme soil and climatic conditions of the south of Uzbekistan characterized by high daytime temperatures during the formation and accumulation of the harvest of raw cotton 500C and more accompanied by prolonged hot, sandy winds 3-5 days and a shortage of irrigation water. It has been established that, in relation to the standard grade, L-279 has advantages in the precocity of 3 days, in the staple length of the fiber 0.03 inches, in the microneur 0.1, in the specific breaking length 1.2 g. s/tex, by the yield of 1.2% fiber, by the yield of raw cotton by 30.09 - 3.3 c/ha, and by the yield of fiber by 1.6 c/ha, that is, the above-mentioned line is recommended for transmission in the form of a new variety Surkhan-107 for evaluation in the state variety testing of the Republic of Uzbekistan.

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

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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 VL 77 and albite stimulants on the spilling of the elements of porous formation

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As a result of the application of porous nutrition, processing of a number of intervals, irrigation and other measures, in the processes of growth-development and harvest of the plant, honeycombs, flowers, knots are formed, but due to unfavorable weather conditions, shortcomings in agrotechnical activities, morphobiological features of the varieties, spilling of the harvest elements is observed. Based on this, in experiments, it was found out how the level of spilling of the harvest elements in the goose affects the VL 77 stimulants. In the experimental options, the number of harvest elements poured in each goose Bush during the ripening period of the goose (8.09.2015) was determined. VL 77 stimulator when yield elements in options treated with norms 0,5-0,7 l/t pour into the seeds 11,9-12,1%, VL 77 when applied to 0,5-0,7 l/T during the flowering-flowering period 10,1-13,3%, VL 77 when poured into the seeds 0,5-0,7 l/t and VL 77 in the flowering-flowering it was found that it spilled 3-6 times less.

Cultivation of sweet corn as a vegetable crop in Karakalpakstan

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Scientists around the world note that it is correct to consider sweet corn as a vegetable crop, since it occupies a special place in the group of food crops. Vegetable corn still occupies an important place among vegetable crops. Therefore, in the conditions of Karakalpakstan, the cultivation of sweet corn is important. The results of studying the germination of seeds of varieties and hybrids of vegetable (sweet) corn, the duration of the growing season, plant height, biometric indicators in order to study the suitability of the Karakalpak soil for climatic conditions were as follows. After sowing seeds of 8 studied varieties and 8 hybrids of vegetable (sweet) corn, the duration of the germination period was 7-9 days. Relatively early germination between varieties and hybrids of Ground, Can. Pedro 2 was noted 7 days after sowing in hybrids Inta, Berys, Sweet star F1, Sentinel F1 Relatively late germination was noted 9 days later in hybrids SPV 1022, Leonard's Early, Honey Bontam F1, Spirit F1, Megaton F1, Baron F1, Soyan F1 , Hybrid F1 Germination and leaf formation (7 leaves) 13-16 days, formation and leaf formation (7 leaves) 23-33 days, flowering to flowering period 4-6 days, flowering and flowering period 9-15 days. In these studied varieties and hybrids, the ripening period was 13-18 days, and the period of milky ripeness was 4-8 days. The study of the duration of development phases in varieties and hybrids showed that the growing season from germination to milky-wax ripeness is 75–85 days. Early maturation was noted in hybrids Baron F1, Sweet star F1, Spirit F1, Soyan F1, growth period 73-77 days, relatively early maturing Ground, Sentinel F1, SPV 1022, Osnova 209, Hybrid F1, Can Pedro 2 Inta, Clx3349ys in varieties and hybrids , such as the slaus, have been reported at 79–81 days of age.

Introduction of pomegranate varieties into culture in vitro conditions

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In our studies, the effect of nutrient media in the introduction of pomegranate varieties into the culture was studied by the method of in vitro. The exoplanets grow and develop under the influence of macro-and micro-elements, amino acids, growth-controlling substances contained in the nutrient medium. The nutrient medium was adjusted in different concentrations of growth-controlling substances (BAP, Kin and GA3). Sterilized plants were placed in a feed environment where 50 ml of Ms control (Murasige and Skug, 1962), DKW (drive and Kuniyuki, 1984), Mstak (Murasige and Skug improved) and BAP (benzyl amino purine), Kinetin (kinetin), and GA3 (gibberillin) growth-controlling substances with different content and concentrations were added to the feed medium. The pH of the nutrient medium was administered via 5,8 index 1N (normal) li HCl and NaOH. To tighten the nutrient medium, agar and gelzan were added, and in the autoclave 20 minutes were sterilized. The esplants were planted in ready-made feed environments.

Technology of microclonal reproduction of lemon plant in vitro conditions

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Increasing the yield of lemon plants, improving its quality, creating new varieties, growing them on the basis of innovative technologies, as well as reproduction and widespread introduction of virus-free seedlings in vitro is one of the urgent tasks today. In vitro microclonal reproduction of lemon plants has a number of advantages over traditional methods, allowing to produce a large number of genetically identical plants and obtain healthy plants in a short period of time. When these plants are grown in the field, their genetic diversity is due to the factor of interclonal variability, or more than one plant is obtained from the exhibits. Under in vitro conditions, the stages of sterilization, introduction into culture, branching during reproduction of Meyer lemon varieties were studied. Different proportions of the MS nutrient medium, different concentrations of auxin and cytokine affect the production of lemon extracts. The best indicator was when sterilizing Meyer navi exhibits for 15 minutes in a solution of 0.1% sodium hypochloride (NaOCl), there were 30 buds included in the culture, 28% damaged shoots and 72% surviving shoots. When introduced into the culture of the Meyer variety, the substances germinating in the MS nutrient medium were BAP – 3 mg / l and IBA – 0.01 mg / l under the influence of branched explants 76.2%, the flow of explant into the culture was 3.25 pieces, the length of the explant was 2.65 cm, and the number of explants was 0.01 mg / l. The number of leaves was noted that the number of branches was 5 grains, and the length of the branches was 4.79 cm, and the number of branches more than 4 pieces and the length of the branches were 3.79 cm higher than in the control version.

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

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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 22o 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.

Economic and biological indicators of sunflower varieties and hybrids

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When growing meadows of foreign varieties and sunflower lines as a repeat crop in a field cleared of bile, in conditions of porous soils, sunflower grows from the Umnik variety to 28.9 c/ha, from the Buzuluk variety to 27.8 c/ha, from the Natalie Duragai variety to 25.0 c/ha./ha, from Mercury to 25.6 c/ha, grain yield was obtained from Nord to 24.3 c/ha, from Revenge to 25.8 c/ha, from Typhoon to 26.5 c/ha, from Arneb duragayı to 27.1 c/ha, from Aris to 26.7 c/ha/ha. In the conditions of irrigated soils of the Republic of Uzbekistan, sunflower sowing is effective as a re-crop for grain crops in fields cleared of bile, the most favorable period is sunflower sowing from June 15 to July 15. At the Research Institute of Grain and Leguminous Crops, 12 varieties and hybrids of sunflower from the Russian Federation were planted in a field of loose soil in the climatic conditions of the irrigated soil of Uzbekistan. The sunflower variety Umnik has a plant height of 188 cm, the Buzuluk variety has a plant height of 180 cm, Mercury has the following natal hybrids 180 cm, Torch 186 cm, Elion 193 cm, Epsilon 190 cm, Nord 170 cm, Revenge 179 cm, Typhoon and Arneb 175 cm, in Aris 180 cm . In general, it was noticed that all varieties and hybrids in the test have completely passed the growth and development phases.

Test results of newly created autumn wheat varieties and their competitive hybrids

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The following results were reached by comparing the newly created 14 varieties and hybrids of Chillaki and Krasnodarskaya-99 varieties selected from a control plant for 2014-2016 with yield, biometric and technological indicators of grain quality. The average yield for three years in the variety "Chillika" was 60.2 cents per hectare, and in the variety "Krasnodar-99" - 71.6 cents per hectare. In our studies, it was found that the highest yields in newly created autumn varieties are 0.6-12.2 cents higher than in template varieties, while in newly created autumn varieties the yield reaches 74.2 quintals in Uzbekistan - 25 varieties, 74.4 quintals in AC-2004D48 hybrids, 73.1 quintals in as-2004D82 hybrids and 72.0 hundredweight hybrids as-2004-D-33-1. Biometric analysis of plants shows that the length of the ear is 7.5-8.8 sm.ni by agreement, this figure template is 8.8 cm for the Chillaki variety and 99 cm for the Krasnodar-99 variety sm.ni the highest rates are in the varieties Chrome, Vassa, Top 8,6-8,7 cm ni reconciled. Newly created Uzbekistan - this indicator is 8.7 in 25 varieties sm.ni it was established that this was organized. The indicator of the number of spikelets in one ear was in the range of 15.3-18.7 pieces, in the Chillaki variety the pattern was 15.3 pieces, and in the Krasnodar variety-99 - 17.7 pieces. In the newly created varieties "Uzbekistan-25", the number of ears in one ear averaged 18 pieces. The number of grains in one ear was determined by the results of the analysis by 38.0-41.8 units in the varieties and hybrids studied in the experiment. In newly created hybrids and varieties studied in seedlings, the grain weight of 1000 pieces averages 37.3-43.4 g, the highest indicator is Grom, AC-2004D48 G in varieties and hybrids 43.4 g. Template, Chillaki and Krasnodar-99, 40.7-43.0 gr.ni in the newly created varieties Uzbekistan-25, this indicator is 42.0 grams, and the lowest indicators of AC-2004d82 are 37.3 gr.ni and in the brigade class 38.6 gr.ni reconciled. The amount of protein, one of the main indicators determining the quality of grain, was 13.0-12.9 percent in Chillaki and Krasnodarskaya varieties-99, and the amount of protein in newly created varieties AC-2004-D62 (Uzbekistan-25) was 14.3 percent, and in AC-2005S364 hybridization this indicator was 13.4 percent. Despite the fact that the varieties and hybrids studied in the experiment had a high gluten content index, i.e. 27.3-31.2%, its IDK index and its class mainly belonged to Class II. The results of the analysis showed that this indicator meets the requirements of the I-st class in the newly created varieties of Uzbekistan-25.

In vitro propagation of local grape varieties in Uzbekistan

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For the experiments, seedlings were selected for in vitro microclonal propagation from local cultivars Rizamat, Mersedes, Black Husayni, Black Kishmish, Isabella, Sogdiana, Toyfi, White Kishmish. The implants were sterilized and transplanted into a special artificial feed. After a 15 day incubation period the implants were transplanted to hormonal nutrient media for the callusogenesis phase after adaptation to artificial nutrient media. In this case, an artificial nutrient prepared with 1 mg/l $^{-1}$ BAP and 0.1 mg/l $^{-1}$ IBA hormones in an MS environment was found to be effective for callusogenesis. Optimal nutrient media were selected for both rhisogenesis and elongation stages. During the rhizogenesis stage, the effectively rooted plants were planted in peat mixture in order to adapt to the soil and a gradual acclimitization process was carried out and at the end of the process, seedlings grown in the greenhouse were planted in field fields. The final conclusion is that today in Uzbekistan there is an in vitro propagation of local varieties of grapes.

Factors of abundant and high-quality crop growth in the autumn on the lands exposed to irrigation erosion

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Protection of soils exposed to irrigation erosion, agrotadbir (methods of soil tillage before planting autumn, norms of fertilization and feeding) applied for the production of high quality grain from autumn were determined to increase the amount of water given for irrigation to the agrophysical, agrochemical hosses of the soil, increase the amount of water absorbed into the soil. When processing with a cultivator at a depth of 12-14 CM before planting in autumn on the lands subjected to irrigation erosion, the amount of water absorbed into the soil in accordance with the norms and mineral fertilizers was 2105,9-2222,8 m3/ha, in the field plowed at a depth of 28-30 CM increased to 52,5-227,6, processing with a cultivator at a depth of 12-14 cm between the rows of buds, the norm of fertilization is 4 million rubles.6 million from the grain. Grain yield increased by 10,4 ts/h when increased to grain, With an increase in the planting norm in the fields when plowing at a depth of 28-30 cm, the grain crop was reduced to 6,8-13,8 ts/ha, while the grain crop was increased from n150p105k75 kg/ha to N250P175K125 kg/ha, depending on the thickness of the seedlings and methods of soil tillage 2,3-12,3. In the lands exposed to irrigation erosion, the fall seed among the range of the goose is 4 million hectares. The level of profitability of sowing units is 8,0%, the norm of fertilization is 5 - 6 million. With the increase in grain yield is 4 million hectares, plowing at a depth of 27.6-35.7 %, 28-30 cm. When fertilized, mineral fertilizers are used in the norm up to N200P140K100 kg/ha, which is 53,4%.

Getting of sterile material depending on the growing conditions of the source material

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Modern viticulture and horticulture in Uzbekistan should be based on the production of certified planting material that meets the modern requirements of intensive viticulture and horticulture, free from all known viruses, which is the basis for the durability and profitability of perennial plantings. It is also important to preserve the existing gene pools of plants for vegetatively propagated, including grapes. The most convenient form of genebank is a biotechnological collection. Vegetative reproduction of fruit, berry, ornamental plants contributed to the accumulation of pathogens spread with planting material. During the cultivation of plants, viruses and bacteria accumulate in them, which affect the yield and viability of plants. In this regard, we have started the introduction into in vitro culture and restoration of wild forms of grapes, which is growing in Uzbekistan, and varieties traditionally cultivated in Uzbekistan. Wild forms of grapes are the primary material for obtaining grape varieties resistant to various stress factors. At the moment, the collection of wild forms of grapes is on the verge of extinction and therefore requires rehabilitation and restoration. For the introduction of grapes into culture in vitro, a comparative analysis of the production of sterile viable material was carried out between grapes harvested from old plantations and grapes harvested from new plantations. To obtain nodal explants, young spring shoots of 6-year-old table grapes of the “Rizamat” variety and young sprouts of two forms of 50-year-old wild grapes were used.

The results showed that the material of 50-year-old plants requires additional use of antibiotics, whereas the sterile material of 6-year-old plants can be obtained by standard methods. As a result, it was shown that the yield of sterile viable explants in the “Rizamat” variety is 85% and in wild forms it does not exceed 25%. The solution to these problems is based on the introduction of it into culture in vitro.

The use of biotechnological methods will make it possible to obtain planting material free from fungal, bacterial and viral infections in a short time and in sufficient quantity.

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