International Conference

Plant Cells & Tissues *In Vitro*

Programme and Abstracts

Vienna, Austria

July 1 – 2, 2019

Organizing Committee

Local Organizing Committee	International Organizing Committee
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Welcome to the 3rd International Conference on "Plant Cells & Tissues *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 propagation, production of doubled haploids, secondary metabolites, genetic transformation, etc.

The **3rd International Conference on "Plant Cells & Tissues *In Vitro*"** to be held on July 1-2, 2019, in Vienna, Austria will discuss wide range of modern in vitro plant cell and organ culture technologies, fundamental aspects of plant cell totipotency, differentiation, regeneration, embryogenesis, and practical applications of in vitro technologies for crop improvement.
Vienna is located in the heart of Europe on the banks of the Danube River, and considered as one of the most important economic, cultural and touristic large cities of central Europe. Apart from providing top science, the Conference will capture the spirit of the city thanks to the central location of the venue offering a multitude of cultural events.

This two-days event will provide leading academy and industry scientists a platform to communicate recent advances in **"Plant Cells & Tissues *In Vitro*",** and an opportunity to establish multilateral collaboration.
The **3rd International Conference on "Plant Cells & Tissues *In Vitro*"** will cover the following research topics:

- ***Plant Cell Totipotency & Differentiation***
- ***Plant Cell Organogenesis Morphogenesis & 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 40 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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3rd International Conference on "Plant Cells & Tissue in Vitro" (July 1 - 2)

July 1 (Monday)

08.00 - 17.00	***Registration***
09.00 - 09.20	**Opening** Welcome address by **Alisher Touraev (Local Organizer, Austria)**
09.20 - 10.30	**Keynote Lecture:** **Klaus Palme (Germany):** From cellular structure to molecular response: phenotyping of single cells and multicellular organs deciphers signaling across scales in plants
10.30 - 11.00	***Coffee break***
11.00 - 12.30:	**Session I: Plant Cell Dedifferentiation & Totipotency**
Chairs	*Hongwu Bian (China) & Lin Xu (China)*
11.00 - 11.20 (+5)	**Hongwu Bian (China):** Different auxin-signaling pathways in callus formation in different species and different organs
11.25 - 11.45 (+5)	**Lin Xu (China):** Molecular Mechanism and Evolutionary Route of Plant Regenerative Abilities
11.50 - 12.05 (+5)	**Leor Eshed Williams (Israel):** The Molecular Mechanism Underlining Callus Cells Totipotency
12.10 - 12.25 (+5)	**Natalia Tikhenko (Germany):** Modifications of the Program in Embryo Development and Genome Structure of the Wheat-Rye Hybrids from Incompatible Crosses during Zygotic and Somatic Embryogenesis
12.30 - 14.00	***Lunch + Poster Session (all numbers)***
14.00 - 15.30	**Session II: Plant Cell Organogenesis & Regeneration**
Chairs	*Akira Iwase (Japan) & José Manuel Pérez-Pérez (Spain)*
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14.25 - 14.45 (+5)	**José Manuel Pérez-Pérez (Spain):** Regulation of Hormonal Control, Cell Reprogramming, and Patterning during De Novo Root Organogenesis
14.50 - 15.05 (+5)	**Fahriye Öcal Özdamar (Turkey):** In Vitro Investigations of Salt Stress Tolerance by H2O2 Pretreatment in Eggplant Calluses
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16.25 - 16.45 (+5)	**Václav Motyka (Czech Republic):** The Hormonome of in Vitro Developing Norway Spruce Somatic Embryos
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17.30 - 19.00	***Welcome Reception + Poster Session (all numbers)***
19.00 - 22.00	**Conference Dinner Party** Traditional Austrian food and wine, located in one of Vienna's famous 'Heurigen' **Cost: 50,- EUR**

July 2 (Tuesday)

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16.40 - 17.00	***Closing Ceremony & Conference Photo***

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ABSTRACTS OF ORAL PRESENTATIONS

From cellular structure to molecular response: phenotyping of single cells and multicellular organs deciphers signaling across scales in plants

Klaus Palme

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Biological signaling processes orchestrate the fate and function of cells and organs in plants. Key insights are now being elucidated at different scales by high-throughput systems biology approaches. Here, we discuss output from some of the latest technological advances in innovative imaging techniques as well as high-throughput phenotyping techniques that hold great promise for advancing our understanding of biological processes. We demonstrate the power of these techniques by showing how these integrated phenotyping approaches help to decipher in vivo cellular dynamic behavior of Arabidopsis organs and resolve the underlying intracellular hormone transport mechanisms at the systems level in single-cells.

Callus Initiation from Root Explants Employs Different Strategies in Rice and Arabidopsis

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Callus formation in tissue culture follows the rooting pathway and newly formed callus seems to be a group of root primordium-like cells. However, it is not clear whether there are multiple mechanisms of callus initiation in different species and in different organs. OsIAA11/OsIAA13 and its Arabidopsis homologue AtIAA14 are key players in lateral rooting. However, the AtIAA14-mediated pathway is not strictly required for callus initiation in the LR formation region in Arabidopsis. LRs can be initiated either through the AtIAA14-mediated or AtWOX11-mediated pathway in the Arabidopsis PR, therefore providing optional pathways for callus initiation. By contrast, OsIAA11/OsIAA13 is strictly required for lateral rooting in the rice PR, meaning the OsIAA11/OsIAA13 pathway is the only choice for callus initiation. Moreover, the OsIAA11/OsIAA13-mediated pathway is specifically and strictly required for callus initiation in the lateral root (LR) formation region of the primary root (PR) but not for callus initiation at the root tip, the stem base or mature embryo in rice. Meanwhile, by comparison of callus formations in suppression lines or mutants disrupting auxin signaling, we found that suppression of OsTIR1 and OsAFB2 (auxin receptor genes) blocked embryo-derived callus formation, but did not block root-derived callus formation; while gain-of-function iaa11 and iaa13 mutants blocked root-derived callus formation but did not block embryo-derived callus formation. In addition, suppression of OsTIR1 and OsAFB2 significantly inhibited the auxin-induced transcriptions of some of OsIAAs and OsARFs in embryo but not in root. These results demonstrate that different components of TIR1-Aux/IAA-ARF auxin signaling are required for callus initiations from different organs in rice. Our study suggests that multiple pathways may activate callus formation in different organs and different species.

Molecular mechanism and evolutionary route of plant regenerative abilities

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De novo root regeneration has wide applications in agriculture such as those related to cutting technology. We established a simple method to study de novo root regeneration by culturing Arabidopsis leaf explants on B5 medium without added hormones to regenerate adventitious roots. Wounding is the first event after leaf explant detachment. The JA-mediated wound signaling pathway activates auxin biogenesis in leaf explants within several hours after leaf detachment, and then auxin is polar-transported to regeneration-competent cells (i.e. procambium and some vascular parenchyma cells) near the wound site. Auxin accumulation in regeneration-competent cells activates WOX11 and WOX12, triggering the first step of cell fate transition from regeneration-competent cells to root founder cells. WOX11/12 promote expression of WOX5/7 and LBD16 for the second step of cell fate transition from root founder cells to root primordium. Overall, wound signaling, auxin accumulation and cell fate transitions are successively involved in regeneration of adventitious roots from leaf explants. In addition, analysis of WOX11 homologues in root founder cells of euphyllophyte plants suggests that auxin-mediated root initiation is a conserved mechanism during root evolution.

The molecular mechanism underlining callus cells totipotency

Leor Eshed Williams

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We combined molecular, genomic and genetic approaches to study the molecular mechanisms underlying cell totipotency and competency to regenerate in Arabidopsis.
By performing comparative analysis of mRNA-seq and chromatin landscapes between leaf differentiated cells and callus totipotent cells and between WT callus and calli derived from the emf2 mutant, exhibiting impaired regenerative capacity we revealed the following:
1. That callus cells express numerous genes of many developmental pathways such as root, leaf, embryo, shoot, meristem and seed. This suggests that one mechanism to allow rapid response to a signal is to maintain genes of all potential developmental pathways active, without the needs to release transcriptional silencing and to go through the intricate multistep process of transcription.
2. That key transcription factors that are sufficient to derive differentiation are silenced and marked by the H3K27me3.
3. That callus derived from the emf2 mutant which is impaired in setting the H3K27methylation, lost the capability to regenerate and that 78 transcription factors from which 18 regulate flower development, where up-regulated compared with WT callus.
Altogether our results suggest that competency to regenerate is achieved by keeping the chromatin of developmental genes active, and that upon a signal for cell fate switch, a mechanism to repress those genes is required to allow the one desired developmental pathway to dominate. When this mechanism is impaired the capacity to regenerate is decline.

Modifications of the program in embryo development and genome structure of the wheat-rye hybrids from incompatible crosses during zygotic and somatic embryogenesis.

Tikhenko N1-2*, **Rutten T1, Mascher M1, Senula A1, Rubtsova M1, Tsvetkova N3, Himmelbach A1, Boerner**

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The wild and cultivated relatives of the family Gramineae are a source of useful alleles for wheat (Triticum aestivum L.) improvement. Rye (Secale cereale L.) provides a vast genetic variation for commercially important traits not only for wheat but also for triticale. In case of triticale, embryonic lethality and hybrid sterility are well known postzygotic barriers, which significantly limit the possibility of obtaining primary triticale and greatly decrease the efficiency of hybridization of parental forms. The approach proposed here allows to overcome these barriers and to obtain highly fertile forms of primary triticale based on incompatible combinations. GBS analysis of genotypes of the offsprings from such incompatible crosses allowed it to evaluate the role of somaclonal and combinative variability in the stabilization of new forms and to classify the rearrangements of the genomes of both parents in offsprings with different fertility levels.

Wound-induced molecular network leading to plant regeneration

Akira Iwase

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Plants have a remarkable capacity to regenerate new tissues and organs upon stresses, especially wounding. Cellular reprogramming at wound sites serves as a first key step in organ regeneration via stem cell reformation but molecular mechanisms underlying this control remain largely unknown. We previously reported an AP2/ERF transcription factor WOUND INDUCED DEDIFFERENTIATION 1 (WIND1) and its close homologues WIND2-4 (WINDs) are induced by wounding, and promote formation of pluripotent cell mass, callus, and successive regeneration3, partly via another AP2/ERF transcription factor, ENHANCER OF SHOOT REGENRATION 1 (ESR1) in Arabidopsis and other crops. We also demonstrated that fully differentiated plant cell needs to epigenetically repress ectopic expression of reprogramming related genes, such as WINDs, in order to prevent dedifferentiation. To further uncover downstream events toward regeneration which are regulated by WINDs, we performed time-course transcriptome analyses, one is after ectopic WIND1 induction and another is after wounding where comparing wild type plants to plants expressing a dominant-negative form of WIND1, WIND1-SRDX. We found gene upregulation of many key regulators for stem cell maintenance and de novo organogenesis depends on WIND activity. Strikingly, we also observed defense-related genes are listed as putative WIND1 down-stream factors, predicting that WINDs function as master regulators for wound responses, not only for regeneration but also for protection against biotic stresses. Based on comparisons with other omics data relating to wounding and WIND1 function, i.e. ChIP-seq and metabolome, we will discuss molecular network underlying wound-induced regeneration.

Regulation of Hormonal Control, Cell Reprogramming, and Patterning during De Novo Root Organogenesis

Aurora Alaguero-Cordovilla1, Sergio Ibañez1, Ana Belen Sanchez-Garcia1, Joan Villanova1, Antonio Cano2, Manuel Acosta2, Jose Manuel Perez-Perez1

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Tomato is an attractive model to study the genetic basis of adventitious organ formation. Adventitious roots (ARs) are formed from non-root tissues in response to some abiotic stresses or after wounding. We investigated the temporal course of gene expression and of auxin and cytokinin accumulation along the apical-basal axis of hypocotyl explants during adventitious rooting. Quantitative histology allowed us to define the cellular dynamics during the early stages of AR initiation. We have initiated both forward and reverse genetic approaches to identify the molecular hubs required for de novo organ formation in tomato. On the one hand, the role of selected transcription factors of the AINTEGUMENTA-LIKE family during AR formation has been confirmed by transient inactivation. On the other hand, we are screening a large EMS mutant collection in search of novel gene functions related to de novo root organogenesis. The identification of the genetic networks involved in AR formation will contribute to our basic understanding of the molecular events leading to this complex developmental response.

Work in the laboratory of J.M.P.-P. (AGL2012-33610 and BIO2015-64255-R) was funded by the Ministry of Economy and Competitiveness and by European Regional Development Fund (ERDF) of the EC - "A way to build Europe“.

In vitro investigations of salt stress tolerance by H2O2 pretreatment in eggplant calluses

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Effects of H2O2 pretreatment on fresh weight, membrane permeability, MDA, proline, ion, inner H2O2 contents and antioxidant enzyme activities were investigated in the callus tissues of two eggplant genotypes (salt sensitive; Artvin Hopa and salt tolerant; Mardin Kiziltepe) cultured in saline conditions. For this purpose, calli were obtained from hypocotyl explants from 4-week-old seedlings germinated in vitro. Some of the calli were transferred to mediums containing control, 150 mM NaCl, 50 or 100 µM H2O2, and the others which were pre-treated with 48 hours 50 or 100 µM H2O2 were transferred to mediums containing 150 mM NaCl and all of them incubated during 24 hours. While fresh weight, K+ and Ca2+ contents were decreased on salt stressed groups, H2O2 pre-treatments reduced these negative effects of salt stress. Salinity increased Na+ contents, while H2O2 pre-treatments decreased Na+ contents and increased K+/Na+ ratio. Salinity increased membrane permeability, MDA and inner H2O2 contents, these negative effects were reduced by H2O2 pre-treatments. H2O2 pre-treatments enhanced antioxidant defence by increasing SOD and CAT enzyme activities and proline contents. The curative effects of H2O2 pre-treatment on parameters we investigated were found to be higher in the salt-sensitive genotype. It has been shown that the effects vary depending on concentrations and genotypes.

Keywords: Eggplant callus, hydrogen peroxide, in vitro, salinity

Cells of Viola species are highly tolerant to heavy metals – survive, accumulate, divide after metal treatment.

Klaudia Sychta, Aneta Słomka, Elżbieta Kuta
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Research on plant tolerance to heavy metals at the cellular level raises increasing interest in recent years. The model system of cell suspension culture provides crucial information how cells cope with stress induced by heavy metals. The main objective of this study was to establish single cell tolerance to Zn or Pb, both solely applied to cell suspension culture of selected Viola species with different metallophyte status: V. lutea ssp. westfalica – obligatory, V. tricolor – facultative, V. arvensis – accidental, and nonmetallophyte – V. uliginosa. Cell suspension cultures were treated with different Zn or Pb concentrations (200, 500, 1000, 2000 μM) for 72 h. Results of cell viability (alamarBlue), Zn and Pb accumulation efficiency (Atomic Absorption Spectrometry) and their deposition in cell structures (Transmission Electron Microscopy), cell ability to division and plant regeneration after Zn and Pb treatments (in vitro culture), programmed cell death induced by Zn and Pb (TUNEL) provide intriguing results. The conclusion is that cells of each Viola species reveal high tolerance to heavy metals and accumulate great amount of Zn and Pb in their vacuoles and cell walls. Cells after treatments survive, divide forming callus from which new metal tolerant plants regenerate.
Research was supported by the National Science Centre, Poland [grant: 2017/27/N/NZ8/00949].

A proteomic approach to investigate somatic embryogenesis in Triticum aestivum L. (wheat) variety Fielder

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Genetic improvement of wheat elite varieties through traditional breeding techniques and/or genetic transformation demands the continuous development and enhancement of the existing in vitro regeneration platforms. In this context, the regeneration of stable and fertile wheat plants through somatic embryogenesis is a crucial step of wheat improvement. In-vitro subcultured calli from the white spring wheat variety Fielder were used in a proteomic approach to identify protein candidates responsible for the induction of somatic embryogenesis. Among the subcultured calli, two distinctive population of calli were observed (i) uneven calli with only mitotic activity in the centre and (ii) smooth calli with a uniform mitotic activity. Smooth calli showed a high rate of shoot organogenesis compared to the uneven calli. Also, quantitative proteomics of the two calli populations reveals a small and specific set of proteins differentially expressed. Present research is focussed on specifically expressed proteins in somatic embryos with high shoot organogenesis properties.

The hormonome of in vitro developing Norway spruce somatic embryos

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Somatic embryogenesis (SE) is a developmental process during which somatic cells dedifferentiate and divide to initiate embryogenic development. It is tightly regulated by a complex network of metabolic and signaling pathways of plant hormones. Understanding of plant hormonome represents a powerful tool for control of SE and in vitro propagation of conifers. Changes in the hormonome including auxins, cytokinins, abscisic acid, jasmonates and salicylic acid during SE in Norway spruce (Picea abies) were analysed by HPLC-ESI-MS/MS. Our results revealed that the patterns and levels of particular phytohormone classes varied substantially between proliferation, maturation, desiccation and germination. A special attention was given to the involvement of phytohormones in the process of desiccation, when isolated somatic embryos were exposed to a high humidity treatment, with respect to a potential substitution of this stage by prolonged maturation. Our findings indicated close correlations between endogenous phytohormone profiles and particular embryo developmental steps and provided the currently most comprehensive summary of hormonome in somatic Norway spruce embryos. [Funded by Czech Science Foundation (19-12262S)].

Trichostatin A improves in vitro plant regeneration of barley (Hordeum vulgare L.)

Barbara Wójcikowska, Katarzyna Nowak, Monika Gajecka, Anna Elżbieciak, Anna Wójcik, Joanna Morończyk, Małgorzata Danuta Gaj
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Regeneration of plants from in vitro cultured explants is associated with extensive reprogramming of the somatic cell transcriptome and epigenetic modifications including histone modifications are believed to play a central role in this process. Thus, it is believed that modification of histone acetylation level in plant explants might impact their capacity for plant regeneration. In support, trichostatin A (TSA), an inhibitor of histone deacetylases, has been recently demonstrated to trigger the embryogenic response in Arabidopsis explants via activation of the genes that control somatic embryogenesis (Wójcikowska et al. 2018).
Here, we investigated the effect of TSA on barley explants derived from cultivars of a low capacity for plant regeneration in vitro (Morex, Dema, Krona, Scarlett) in comparison to the control cultivar, Golden Promise, of a high plant regeneration efficiency. Explants, the scutella derived from immature zygotic embryos were cultured for 1, 2 and 4 weeks on medium supplemented with different concentrations of TSA (0; 1; 2.5; 5; 7.5 µM). We found that the effect of TSA on regeneration efficiency of the explants was highly dependent on barley genotype and thus, the effective method of TSA treatment needs to be optimized for the genotype. Accordingly, the explants of Krona and Scarlett, the barley cultivars with the lowest capacity for plant regeneration, upon treatment with 7.5 µM of TSA for 4 weeks, regenerated plants with a threefold increase of efficiency that the control culture. The results show that the modification of histone acetylation level via TSA treatment of the explants might provide an efficient tool for the improvement of plant regeneration of barley cultivars recalcitrant for in vitro culture.
This work was supported by a research grant from the National Science Centre in Poland (OPUS13 2017/25/B/NZ1/01615)

Microspore embryogenesis: targeting the determinant factors of stress-induced cell reprogramming and totipotency to improve DH production in crop and forest species

Testillano P.S, **Berenguer E, Pérez-Pérez Y, Carneros E, Bárány I, Risueño MC**

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Stress-induced microspore embryogenesis (SIME) is used for production of doubled-haploids (DH) in breeding programs as source of new genetic variability, fixed in homozygous plants in one generation. We have recently characterized a number of determinant factors of SIME in crop and forest species. Autophagy and specific proteases are activated upon stress favoring cell death, while cell reprogramming and totipotency are regulated by hormonal and epigenetic mechanisms. Auxin biosynthesis, transport and action are required for microspore embryogenesis. SIME initiation involves genome-wide DNA hypomethylation, H3K9 demethylation, and H3/H4 acetylation. Cell wall remodelling, with pectin de-methylesterification and AGP expression, is necessary for embryo development. Pharmacological treatments with small molecule modulators of autophagy, proteases and epigenetic marks reduce cell death and enhance embryogenesis initiation, opening up new intervention pathways for improving in vitro DH production in breeding programs.
Supported by projects (AGL2014-52028-R, AGL2017-22447-R) funded by MINECO and ERDF.

Haploidization in carrot (Daucus carota L.) induced by wide pollination and ovule excision in vitro

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A doubled haploid (DH) plant results from doubling the chromosome set of a haploid plant. DH plants are of great significance in breeding programs, allowing for obtaining true homozygous plants serving as parents for F1 hybrids. The effects of pollination with parsley pollen, post-pollination treatments and culture conditions on the development of doubled haploid plants in carrot are discussed. Carrot ovules excised from the ovaries and cultured in vitro developed embryos or calli. The efficiency of embryo development was accession dependent. The highest frequency of embryo development was observed from ovules excised from ovaries approximately 20 days after pollination with parsley pollen. Medium supplemented with indole-3-acetic acid (IAA) was superior in our study and allowed the production of embryos restricting callus development. Genetic status of the regenerants was assessed at three independent loci based on isozyme and PCR analyses. Most of the obtained plants were homozygous diploids but haploids were also observed.
The research project was funded by the Polish Ministry of Agriculture and Rural Development, grant no. HORhn4040 dec—1/08.

Induced androgenesis in anther and isolated microspore culture of spelt wheat (Triticum spelta L.)

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Anther culture and isolated microspore culture was tested with four spelt genotypes ('Franckenkorn', 'GK Fehér', 'Mv Martongold', 'Oberkulmer Rotkorn'). The hybrids of genotypes were cultured also in anther culture. In isolated microspore culture, a lots of ELS were produced, but albinism hindered the production of large quantity green plantlets. However, the anther culture was proved efficient in spelt genotypes. The mean of green plantlets production has achieved high values (38.4/100 anthers) in anther culture which ranged from 13.8 to 85.0 depending on genotype. The number of green plantlets was higher in the mean of F1 genotypes than in anther cultures of the parental varieties.
This project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. The experiments were interlocked with scientific programme (project code: OTKA-K_16-K119835; Improvement of spelt wheat lines with low fermentable carbohydrate content (FODMAP) using modern and classical research methods) and GINOP projects (project number: GINOP-2.2.1-15-2016-00026).

Improvement of embryogenesis in anther versus shed microspore culture of rice (Oryza sativa L.)

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The androgenesis, has been reported as the preferred method of haploid induction/ DH production in many species such as rice. Both anther culture (AC) and shed microspore culture (SMC) were developed in three mutants and one Iranian cultivar of rice. The different concentrations of gum arabic (GA), as carrier of arabinogalactan proteins, were used as chemical enhancer. In AC, the highest number of induced embryos was obtained in MS medium supplemented with 2 mg/L Kin + 1 mg/L NAA + 10 mg/L GA. The highest number of induced embryos in SMC experiment was obtained when above B5 liquid medium contained 10 mg/L GA and lower B5 Agar-solidified medium contained 1% activated charcoal. The efficiency of SMC was more than AC in term of number of induced embryos. The established protocols are applicable for development of doubled haploid mutant lines in mutation and selection breeding programs.

Plant suspension cultures for industrial applications

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Plant cell suspension cultures are ideal platforms for production of valuable proteins or secondary metabolites with application in the food, pharmaceutical, and chemical industries. In addition, plant cell biomass, mostly derived from fruits, is used as ingredient in cosmetic products. Typically, cell cultures are grown in contained bioreactors under defined conditions, allowing process controls to regulate growth and product formation, thus ensuring season and geographically independent production, product consistency and avoiding environmental contaminations. The presentation will show novel strategies for process optimization, helping to increase product yields and process scalability and will present shaken bioreactors as efficient alternative to conventional stainless steel stirred-tank bioreactors. Economic data concerning the production process are provided to elucidate relevant parameters moving towards economic viability. Finally, the exploitation of plant suspension cells as starting material for the establishment of a novel cell-free production system is presented.

Na demethylation by 5-azacytidine promotes proliferation of somatic embryos in cork oak

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Cell reprogramming and somatic embryogenesis (SE) initiation involves changes in global genome organization in which DNA methylation plays a key role. SE is widely used for in vitro micropropagation, with limited efficiency in woody species. This work analyses the changes in global DNA methylation levels and nuclear distribution during SE in cork oak, by biochemical and immunocytochemical approaches, as well as the effect of the demethylating agent 5'-azacytidine (AzaC) in the process. Results showed low DNA methylation levels at early stages of SE and its progressive increase during embryo differentiation. AzaC treatment reduced global DNA methylation and promoted the proliferation of proembryogenic masses, and prevented embryo differentiation. Elimination of AzaC from treated cultures led to the formation of higher proportion of embryos compared with control cultures. Findings provide new insights into the epigenetic regulation of SE in woody species, opening up new intervention pathways for forestry breeding programs.

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Fluorine containing topolin cytokinins for Phalaenopsis micropropagation

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Phalaenopsis hybrids are among the most important ornamental pot plants, as their beautiful flowers and longevity ranked them as one of the most admired cut flowers of the world. However, because the monopodial growth nature, vegetative propagation is slow and difficult, and propagation through seed results in unwanted heterozygous types. Therefore there is a need for new tools to be developed. Here we illustrate the impact of second generation fluorine-containing topolin cytokinins during Phalaenopsis amabilis micropropagation. They exhibit cytokinin properties but do not exist in nature: 6-(3-fluorobenzylamino)purine (FmT) and its 9-β-D-riboside (FmTR). We show that FmTR should be preferred in Phalaenopsis tissue culture as an alternative to commonly applied cytokinin groups.

Chloroplast import of nuclear-encoded eGFP protein in rice driven by several transit peptides

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In an effort to identify and characterize minimal versions of transit peptides (mTPs) that work in crops, we have cloned four previously described TPs from the first methionine up to the predicted cleavage site by the Stromal Processing Peptidase, fused to the eGFP. For cloning, we adapted the MoClo system using a pUC57 vector backbone and the MoClo cloning cassette ligated within, optimizing for plant transformation via particle bombardment.
The resulting constructs were used for stable transformation of Oryza sativa embryos. Embryogenic calli were recovered from the bombarded embryos 2 weeks later by hygromycin B selection. We have analyzed the expression of eGFP by immunoblot analysis, confirming that several mTPs work in targeting protein accumulation to the rice calli pro-plastid.
For further experiments to determine mTPs effectiveness in targeting protein accumulation to mature chloroplasts, regeneration of rice plants is being carried out.

Micropropagation in Twenty-First Century: Increasing Efficiency and Reducing Costs

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Large-scale micropropagation continues to be a major source of clonal plantlets of many plant species with great commercial importance to global horticulture and forestry, also including sugarcane (Sacharum). The commercial viability of micropropagation is based on two main justifications: the limitations in seed reproduction (heterozigozity, long juvenile phase) or even related to the limited proliferation of new plants using conventional vegetative propagation, such as many ornamentals and banana (Musa); the presence of diseases of great economic impact in the culture and of which other techniques of control are not effective, such as in sugarcane and potato (Solanum tuberosum). The main challenges for commercial micropropagation in the Twety-First Century are: cost reduction of micropropagated plantlets, increasing efficiency of in vitro systems, the development and use of new and modern technologies and the combination of micropropagation with other cheaper vegetative propagation techniques. As example, the combination of light-emitting diodes (LEDs) with high photosynthetic photon flux, coupled with the technique of temporary immersion bioreactors (TIB) with CO2 injection could result in realistic photoautotrophic micropropagation, increasing the efficiency of the in vitro-plantlets production.

Predicting Optimal In Vitro Culture Medium for Pistacia Vera Micropropagation Using Neural Networks Models

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Specific computer software which combine several artificial intelligence techniques such as artificial neural networks (ANNs) with fuzzy logic (neurofuzzy logic) or with genetic algorithms (ANNs-GA) have been employed to model, to get insight, to predict and to optimize the effect of several independent factors on four growth parameters during Pistacia vera micropropagation. In this sense, the effect of any or all plant culture media ingredients (minerals, glycine, vitamins and plant growth regulators) on several growth parameters can be modelled simultaneously. Throughout simple rules (IF THEN) from neurofuzzy logic models, the positive and negative effects can be easily visualize and understand. Moreover, this technology allow to estimate the best combination of media ingredients and maximize the plantlets growth minimizing physiological disorders. In our opinion, the information obtained in this study is extremely advantageous to develop successful plant multiplication by using artificial intelligence technology to design plant tissue culture media with predictable and tailorable characteristics.

The relation between transpiration and growth in in vitro micropropagation, exemplified in Malus domestica 'Gala' plantlets

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Micropropagation enables vegetative production of large numbers of plantlets in a short period of time. One of several multiplication methods is the induced formation of adventitious buds and shoots; another is the forced outgrowth of axillary bud meristems. For growth, the conditions in vitro are far from 'normal' and mechanisms regulating growth are still largely unknown. A major question is how medium components are translocated to the areas of growth. Tissue culture experts generally believe that this translocation is brought about by diffusion, but according to Fick's second law of diffusion this is out of the question. We believe that most translocation occurs through xylem and phloem and is driven by transpiration. Our previous data showed that significant transpiration occurred in tissue-cultured plants. It is reasonably assumed that poor transpiration might be one of the reasons of poor performance in tissue culture. In our experiments, shoot cultures of Malus domestica 'Gala' were used to test the relationship between transpiration and growth. The hypothesis is that an increased transpiration potential might lead to improved growth of plantlets. High gas replacement filters stimulated the increase of plant weight, both fresh and dry weight, as shown by 'Gala' shoots growing in culture with various lid filters. Plants grown in reduced relative humidity by application of a small vial filled with a highly concentrated potassium chloride solution in the container exhibited an increased transpiration and higher fresh and dry weight. Opening the stomata also increased transpiration and biomass accumulation: apple shoots in medium treated with δ-aminolevulinic acid displayed enhanced stomata aperture and water loss, and also showed an increase in biomass compared to the controls. Our results demonstrated the potential of modulating transpiration to optimize biomass accumulation in vitro. A similar technique might also be applied to other species or cultivars growing poorly in tissue culture. These results in apple confirmed our earlier results obtained with Arabidopsis thaliana in vitro grown seedlings.

New regulators of somatic embryogenesis in Medicago truncatula

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WOX proteins are well-known regulators of plant meristem activity as well as embryo development. The aim of our study is the search of new regulators of somatic embryogenesis among WOX transcription factors and unraveling their mechanism of action. Previously, using Medicago truncatula as model object, we found that MtWOX9-1 can stimulate somatic embryogenesis in callus culture. Transcriptome analysis of calli with MtWOX9-1 overexpression allowed us to find several groups of genes which are probably stimulated by MtWOX9-1. These include several NF-Y genes, for example, LEC1 homolog MtNF-YB10, as well as several CLE genes. The expression levels of MtWOX9-1 and MtNF-YB10 correlate strongly, suggesting direct regulation. Now we are obtaining CRISPR mutants for investigated genes to analyze their impact on SE capacity.
This work was supported by Russian Science Foundation project no. 16-16-10011 and the grant from Russian Foundation for Basic Research no. 17-04-01708

Topolin cytokinins for micropopagation of elite tropical timber wood tree species

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The demand for tropical elite trees for timber wood production is increasing. Seeds are often difficult to obtain and the value of seedlings cannot be validated on short term. Therefore in vitro mass propagation is a good alternative. Nevertheless, a lot of species remain recalcitrant for the classical cytokinins such as benzyladenine. Modern cytokinins of the topolin family promise breakthroughs, as they follow different metabolic pathways. A number natural occurring meta-topolin derivatives such as mT, mTR, MemTR and MeoTR were compared with benzyladenine during micropropagation phase of Dalbergia retusa, Tabebuia guyacan, and Tectona grandis. Morphological observations were complemented by high-resolution multispectral imaging. The different reactions of the shoot explants will be discussed and put into perspective.

High content screening (HCS) to improve efficiency of plant protoplast regeneration

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Mature differentiated plant cells are capable to reprogram their cellular fate to an undifferentiated state under certain (physical or chemical) perturbations as a requirement for the initiation of proliferation. Arabidopsis protoplasts present a suitable model for studying the individual processes of reprogramming. We selected a variety of different reporter lines expressing histones, cell cycle as well as epigenetic markers in order to associate morphological cellular changes with epigenetic as well as proliferation-related processes during dedifferentiation. We established a systematic approach using automated microscopy in combination with high-throughput image processing and analysis which allowed us to characterize the developmental stages during reprogramming. Using this approach, we found that only a minor proportion of cells are capable for reprogramming and subsequently undergo microcolony formation. Tracking of individual cells through developmental stages allowed us to characterize the early events which distinguish dedifferentiating cells from cells which fail to proliferate. The system opens the door for future high-content screening procedures enabling to systematically screen various media compositions as well as chemicals including epigenetic regulators and to determine their effect on cellular dedifferentiation. Furthermore, results obtained will be applicable to protoplasts of other taxa in order to increase regeneration efficiency of agriculturally relevant species.

Factors increasing the yield embryoids of radish in culture of isolated microspore in vitro.

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One of the main problem of induction and cultivation of embryogenesis in the culture of microspores in vitro for radish - irregular cultivations of microspores inside of buds. Thats why, even at the optimum size of a bud just around 50 percent of its microspores are capable to an embryogenesis. Besides, ethylene is emitting in the course of death of microspores and pH level of medium is changing that leads to death of viable microspores and stops the cultivation of embryogenesis (Chun C. at all, 2011). For overcoming negative impact of these factors were used AgNO3 - a powerful inhibitor of ethylene, and a buffered solution 2-(N-morfolino) of etansulfonovy acid (MES) which promotes preservation of pH of medium (Beyer, 1976). Researches were conducted on two grades of a garden radish of RBC and Mokhovsky.
Addition of AgNO3 and MES in concentration of 0.05 mg/L-1 and 0.6 g/L-1 respectively to standard NLN-13 medium (Lichter, 1982) promoted increase in an induction of an embryogenesis at both grades.The quantity of the received yield of embryoids significantly exceeded control option (NLN-13 without additives) at statistically significant level (P0.05). According to preliminary data, effectiveness of embryogenesis can be increased by divisions of microspores into fractions before their introduction to the culture of isolated MICROSPORE IN VITRO by Percoll solution at the expense of a gradient of density.

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ABSTRACTS OF POSTER PRESENTATIONS

Poster №1: Transient Induction of Plant Regeneration

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

Poster №2: Effect of auxins on the production of cell suspension culture of peanut (Arachis hypogeaeL.)

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Phenolic compounds are synthetized by a large series of plants which have a crucial role in defense and adaptation to environment. Its potential application has been regarded as an important health promoting and has generated much interest in recent years. The present study reported the use of biotechnological technics applied to stilbenes production in peanuts and calli production from leaves explants in response to picloram and dichlorprop. Plant cell suspension cultures of Arachis hypogeae are a promising technology. They originate from calli and are established in 250 ml flasks. The growth curve of the cell suspension culture was measured by determination of the packed cell volume and cell viability was assessed with fluorescence diacetate. The effect of elicitation using methyl jasmonate and methyl-β-cyclodextrin was evaluated during the stationary phase.
Key words: peanut, callus, cell culture, elicitation

Poster №3: Somatic embryogenesis om mature cotyledons of Pistacia vera L.

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Induction of somatic embryogenesis from mature cotyledons of pistachio (Pistacia vera L. cv Mateur) has been studied with respect to light conditions and growth regulators. Explants were cultured on medium containing Murashige and Skoog with half concentration of NH4 NO3 and KNO3 salts (MS mod.3) and Gamborg vitamins. Kinetin was used in combination with different auxins : 2,4-D, 3.4 -, picloram, dicamba and dichlorprop. The experiment was executed in a photoperiod of 16 h/light and in darkness. The different responses of the explants to the type of auxins and light conditions will be discussed. Especially the reaction on the rarely used dichlorprop was remarkable. Key words : Somatic embryogenesis, pistachio, cotyledons.

Poster №4: Polyamines affected regeneration processes in Arabidopsis

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Over recent decades, various culture conditions have been established for plant regeneration. A common mode of plant regeneration is *de novo* organogenesis, the other way is somatic embryogenesis (SE). Polyamines (putrescine, spermidine and spermine) are known to play a vital role during plant regeneration processes. Our aim was to examine the effect of different polyamines in the induction of SE or organogenesis. To this end, we used a root culture based regeneration system which was previously established in our laboratory. The polyamine content was measured by a HPLC system and the activities of the enzymes involved in polyamine catabolism were determined by spectrophotometry. We have found that exogenously applied spermidine and spermine strongly improved the regeneration capacity of Arabidopsis roots. Our preliminary results indicate that exogenous polyamines promote the regeneration in *Arabidopsis thaliana* roots, mainly via the shoot organogenesis pathway.

This work was supported by grants from NKFIH (FK 128997).

Poster №5: **Somatic embryogenesis: inhibitory effect of shoot derived auxin on cytokinin-induced Arabidopsis roots**

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One of the type of asexual embryogenesis is somatic embryogenesis (SE). Until now, SE research in Arabidopsis was mostly limited to direct or indirect somatic embryogenesis from immature/mature zygotic embryos. Our aim was to establish a more efficient *in vitro* root culture based experimental system for studying SE in Arabidopsis. To induce embryogenesis auxin and cytokinin were used. Auxin induced the formation of lateral root primordia that started to transdifferentiate into shoot meristem in the presence of cytokinin. However, if the induced root explants were transferred onto hormone-free medium in time, the primordial were converted to somatic embryos. In this work, we have found that the same treatment failed to induce embryo formation in the case of whole seedlings. However, if shoot-derived auxin transport was inhibited, the embryogenic capability of the roots was retained even in whole seedlings.

This work was supported by grants from NKFIH (FK 128997).

Poster №6: **The Interplay Between Stress and Auxin during *in vitro* Embryogenesis**

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The positive relationship between stress and *in vitro* embryo induction is well documented. In *Brassica napus* (*B. napus*), immature pollen can be induced to form haploid embryos by exposing them to heat stress. Auxin plays an early and important role in this process, as the *DR5* auxin reporter is expressed shortly after the heat stress treatment, and specifically marks embryogenic cells that form differentiated embryos rather than the embryogenic callus and pollen that also develops in culture. Our current hypothesis is that microspore embryogenesis is a two-step process comprising i) a stress-related event needed for genome reprogramming to embryo development, followed by ii) an endogenous auxin-related event required for cell proliferation.

This project aims to dissect the role of stress and early auxin signalling during *B. napus* microspore embryogenesis. To this end, we will i) develop a toolbox of auxin reporters; ii) use *DR5* expression as a tool to capture the few-celled embryo transcriptome; and iii) use novel and existing auxin compounds to identify the role of this hormone in microspore embryo induction.

Poster №7: NF-Y genes in Medicago truncatula somatic embryogenesis

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Somatic embryogenesis (SE) is widely used in biotechnology to transform plants, obtain artificial seeds, to study the regeneration processes and zygotic embryogenesis. The main objective of this research is the analysis of MtNF-YB10 gene activity during somatic embryogenesis in Medicago truncatula. This gene is closely homologous to Arabidopsis thaliana LEC1. LEC1 has a lot of different functions, related to embryogenesis, for example, suspensor development, inhibition of germination or formation of embryonic traits in general.
MtNF-YB10 is one of NF-YB subunits, and it is probably can be a part of heterotrimeric transcription factor NF-Y, which includes NF-YA, B and C subunits and binds to the specific DNA motifs. Thus, it would be interesting to identify Medicago truncatula heterotrimeric complexes NF-Y, which function during the SE.
The interactions of NF-Y subunits, demonstrating high expression levels during SE were analyzed by yeast two-hybrid system. Our next step will be to confirm the revealed interactions in planta.
We also analyzed the SE capacity of mutant with MtNF-YB10 loss of function. It turned out that, most likely, ability of these mutants to develop somatic embryos depends on the chosen cultivation system.
This work was supported by Russian Science Foundation project no. 16-16-10011 and the grant from Russian Foundation for Basic Research no. 17-04-01708.

Poster №8: The 'WAR' complex specifies Arabidopsis root system formation

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Evolution of roots was an important step that enabled plants to migrate from aquatic to terrestrial habitats. Roots are responsible for anchorage, supplement of water and nutrients, and exchange of various growth substances with shoots. The root system of seed plants are usually comprised of three root types: primary root (PR), lateral root (LR) and adventitious root (AR).
Auxin regulates plant root initiation via the auxin response factors (ARFs). Genetic studies have shown that individual ARF controls distinct root initiation processes. For example, ARF5 is critically required for PR initiation, ARF7/19 specifically contribute to LR initiation, and ARF6/8 regulate AR initiation.
Recent studies showed that the transcription factor WUSCHEL-RELATED HOMEOBOX 11 (WOX11) upregulates LATERAL ORGAN BOUNDARIES-DOMAIN 16 (LBD16), which contributes to AR initiation. Moreover, the ARF6/8-dependent auxin signaling is required for the WOX11-LBD16 mediated AR formation pathway. In this process, ARF6/8 form a complex with WOX11 and its homologous protein WOX12 to regulate AR formation. Furthermore, we identified two other WOX proteins, WOX8 and WOX9, interact with ARF5, which is involved in hypophysis specification and PR formation. Our results showed that auxin signaling pathways accurately regulate different types of root formation through the 'WAR' complex (WOX-ARF) in Arabidopsis root system establishment.

Poster №9: Successful cryopreservation and post-thawing regeneration of wet habitat, endangered Viola stagnina Kit. (Violaceae)

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Preservation of plant material in liquid nitrogen is one of the methods of ex situ conservation. The crucial step in cryopreservation technique is to develop a protocol allowing to maintain viability of plant material and post-thawing regeneration. This technique is especially difficult for fragile species of wet habitats. In this study we successfully eliminated critical points of cryopreservation of Viola stagnina - integral to humid habitat and threatened with extinction. The size of adventitious shoots able to survive in LN, the most efficient cryoprotection methods and medium for regeneration were chosen. All post-thawing regenerated plantlets showed low genetic diversity and high conformity to the initial plant. Only one out of 44 recovered shoots had multiplied genome (2C=2.61 pg vs 2C=1.35 pg of mother plant and other regenerants). We developed the first protocol for cryopreservation of moist habitat violet with low dehydration tolerance.

Poster №10: Development of Tissue Culture and Transformation Protocols for Recalcitrant Plant Crops for Upcoming Gene Editing

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Our laboratory develops a systematic approach to advance genetic engineering in recalcitrant crops by combining tissue culture technologies and genome editing.
Our main subjects of research are:
Development of novel tissue culture protocols for recalcitrant plants
Histological characterization of tissue differentiation
Characterize the mode of regulation that controls transient and stable gene expression
Discovery of new genetic modules involved in regeneration and in somatic embryogenesis
Advance genome editing and cellular differentiation
To date, we have developed/adapted tissue culture protocols for tobacco, pepper, tomato, potato, chrysanthemum, gerbera, stevia, garden phlox (Phlox paniculata), star of Bethlehem (Ornithogalum dubium), Calla lily, and Saffron.
In parallel, we take advantage of type II restriction enzymes to develop a complete system of binary vectors that will facilitate highest gene expression in these target plants.
The combination of the two approaches will eventually permit gene editing in recalcitrant plants.

Poster №11: CLE Peptides in Medicago truncatula Somatic Embryogenesis

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Somatic embryogenesis (SE) resembles zygotic embryogenesis, furthermore they have many common transcription factors controlling their development. SE is used for studying gene cascades that are vital for regular ontogenesis. In previous research we showed that the gene MtWOX9-1 has influence on capacity of calluses to form somatic embryos. Because WOX genes are often regulated by CLE peptides, we assumed that a CLE peptide exists, upregulating the expression of MtWOX9-1. Previously we found that overexpression of MtWOX9-1 increases the levels of CLE06, CLE08, CLE16 and CLE18 mRNAs. WOX often have a feedback interactions with CLE, therefore we started to search MtWOX9-1 stimulator among these candidates. To perform this, we made transgenic calluses with overexpression of these CLE genes. In this study, positive correlation was found between expression of gene MtWOX9-1 and CLE06 and CLE18. We are planning to estimate the effect of chemically synthesized CLE06 and CLE18 peptides on SE capacity of Medicago truncatula 2HA and A17 lines that are highly different in SE capacity. We are also developing the suspension culture protocol for these lines.
This work was supported by Russian Science Foundation project no. 16-16-10011, the grant from Russian Foundation for Basic Research no. 17-04-01708

Poster №12: Implementation of ChIP and immunohistochemistry analysis to study a role of histone acetylation in somatic embryogenesis of Arabidopsis

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Epigenetic changes in the chromatin structure that control transcriptome activity involve DNA methylation and posttranslational histone modifications. The acetylation of histones via activation of the gene transcription is believed to play a key role in release of the embryogenic development in the in vitro cultured somatic cells of plants. However, the experimental evidences on the involvement of histone acetylation in regulation of somatic embryogenesis (SE) are limited.
Hence, in the present study we implemented the ChIP-qPCR and immunohistochemical (IHC) methods to investigate histone acetylation pattern at the gene and tissue level, respectively, in the embryogenic culture induced in a model plant of Arabidopsis. ChIP-qPCR was optimized to evaluate changes in a level of H3 histone acetylation in chromatin associated with the LEC2 gene of a key regulatory function in SE induction. In order to analyse the spatio-temporal pattern of histone acetylation in the SE-induced tissue, the polyclonal antibody against acetylated isoforms of H3 histone were used. The results demonstrate new opportunities that provide ChIP and IHC methods in studies on epigenetic regulation of developmental processes that are induced in vitro in somatic plant cells.
This work was supported by a research grant from the National Science Centre in Poland (OPUS13 2017/25/B/NZ1/01615).

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Efficiency of Improved Technology for Barley Haploid Production in Anther Culture in Vitro

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Evaluation the efficiency of improved technology applied to obtain haploids in genetically diverse spring barley varieties, lines and breeding hybrid material was carried out. When improved technology included such innovative elements as long-term spike cold pretreatment (Patent N 113261 Ukraine) and replacement of agar with new chemically modified starches was applied, the yield of green plants in model genotype exceeded 100 % of the total amount of cultivated anthers. Two-fold increase in the frequency of green plant regeneration was achieved in hulless barley varieties and hybrid material. A mechanism of morphogenesis improvement affected by chemically modified starches was revealed. Observations of androgenesis in vitro initial phases showed that in order to achieve high frequency of induction it is necessary to use medium containing a low weight component both with trophic and with osmotic activity. Gradual decrease in the gel water content and gel compression as well as providing developed androgenic structures with products of starch enzyme hydrolysis, which become additional sources of nutrition when more available maltose and sucrose exhausted, was very important for somatic embryo formation.

Overcoming difficulties in rooting and acclimatisation of saskatoon

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Saskatoon (Amelanchier alnifolia var. cusickii) is a fruit-bearing ornamental shrub native in the Northern hemisphere. Its edible berries are rich in biological compounds, such as flavonoids, vitamins and antioxidants, therefore is considered an attractive for consumers. Micropropagation of this species can result in efficient supply of stock material for further distribution, but the plantlets derived in vitro are often susceptible for dormancy after their transfer to pots. This study was focused on the examination of auxin, medium concentration, spermidine and special acclimatisation treatment with plant growth regulators for efficient rooting and acclimatisation of this species. The best rooting was achieved after the cultivation of saskatoon shoots on half concentration of MS medium with auxin naphthalene acetic acid. Spermidine addition did not enhance root formation. As for acclimatisation, the spray containing 6-benzylaminopurine (BAP) was more efficient in dormancy release compared to the spray containing gibberellic acid. Overall, more than 75% of plantlets survived the acclimatisation phase after being sprayed with BAP. These results can be especially valuable for micropropagation of plants that are difficult to root or to acclimatise.
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List of Poster Presentations

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