

DAY 1

Scientific Tracks & Abstracts



5th Edition of International Conference on

Plant Genomics

June 13-14, 2019 | Berlin, Germany

DAY 1

June 13, 2019

Sessions
Cancer Science
Cancer Therapies

Session Chair

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Session Introduction

Title: Nutrition effects on synthesis of plant defense proteins and tolerance to metals

Ildiko Matusikova, University of St. Cyril and Methodius, Slovak Republic

Title: The development of a novel SNP genotyping assay to differentiate cacao clones

Jocelyn De Wever, Ghent University, Belgium

Title: Virulence of *Fusarium circinatum* is associated with perturbation of phytohormone homeostasis in *Pinus pinaster* seedlings

Laura Hernandez Escribano, National Institute of Agricultural and Food Research and Technology, Spain

Title: Establishment of regeneration protocol for Mongolian subendemic species *Oxytropis grubovii* Ulzij

Bolortuya Ulziibat, Institute of general and experimental biology, Mongolia

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Ildiko Matusikova, AJPSKY 2019, Volume 09

Nutrition effects on synthesis of plant defense proteins and tolerance to metals

Ildiko Matusikova

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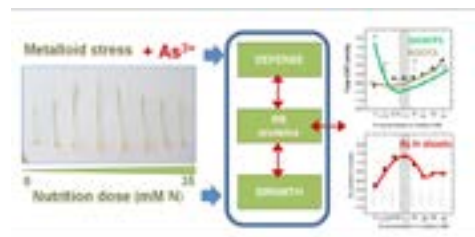
Under stress including metal toxicity plants synthesize defense components, including enzymes like β -1, 3-glucanases and chitinase. Since defense is costly the synthesis of these enzymes depends on nutrients availability. The situation is more complicated if nutrient dose itself represents stress several enzyme isoforms with peculiar response to either starvation or nitrogen excess have been identified. A comprehensive study on the impact of nutrition on defense enzymes under stress is missing. Therefore, responses of plants exposed to arsenic, combined with conditions of low, optimal as well as excessive N concentrations, were studied in more detail.

Methodology and Theoretical Orientation: Hydroponic wheat plants were grown in standard Hoagland media with different amounts of ammonium nitrate at the final nitrogen concentrations of 0, 0.75 and 5.25 mM N (suboptimal doses), 7.5 mM (optimum), and 15, 25, 30 and 35 mM N. After a week, As³⁺ at sub lethal dose was applied. The profile and activity of individual defense enzymes (β -1,3-glucanases and chitinases) as well as some morpho-physiological parameters were studied.

Findings: Nutrition conditions affect the responses of wheat plants to arsenic toxicity: Enzyme isoforms responsive to nitrogen concentration, metalloid, and to both were identified. Although (supra) optimal nitrogen concentrations positively activate the defense, the optimal dose appears not always the same for the individual parameters. Furthermore, at high doses of

nitrogen the plants accumulated less arsenic in the shoots; probably due to better ability to prevent the transport of toxic element to the aerial parts.

Conclusion and Significance: Nutrition availability affects accumulation and/or activity of defense-related compounds, and impacts uptake of arsenic by the wheat plants. Some chitinase and glucanase isoforms are candidates for screening of plants health in the context of fertilizer management and / or presence of toxic metals.



Biography

Ildiko Matusikova studies the physiology and biochemistry of, and gene expression changes in, stressed plants. She focuses on enzymes of chitinases and β -1, 3-glucanases in context of different scientific questions. Recently she extended her interests in studying the uptake, transport and accumulation of (toxic) metals in plants using radioanalytical approaches. She also does research on the molecular biology of *Drosera* and studies the role of hydrolytic enzymes in prey digestion by carnivorous plants.

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Jocelyn De Wever, AJPSKY 2019, Volume 09

The development of a novel SNP genotyping assay to differentiate cacao clones

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Plant genetic diversity studies are of high importance for efficient plant conservation and resource strategies (eg. tackling mislabeling, conserving valuable genetic material, parentage analysis, and genetic diversity studies) as they contribute to an increased knowledge on the genetic background and diversity of specific plants. These studies are most commonly analyzed through simple and effective genotyping methods making use of genetic markers, such as SSRs, however SNPs are gaining more interest. Recently, a cost-effective qPCR-based method has been proposed for SNP genotyping purposes, coined double-mismatch allele-specific (DMAS) qPCR as cheap alternative to other methods. It's an accurate and fast multi-sample and multi-locus method, based on straightforward readout of DNA-binding dye based qPCR technology. Its design, optimization, validation and application on *Theobroma cacao* L., an important cash crop involved in the chocolate production, has shown successful. It offered valuable knowledge on the background of cocoa which is often plagued by mislabeling and inefficient and limited management resources. The method, optimized here, showed 98.05% efficient in calling the right cacao genotype and identified 15.38% off-types and two duplicates in an internationally recognized cacao population (n=65), using a limited amount of markers (n=42). Furthermore, only 13 markers were needed to differentiate all analyzed accessions. Notably, the described method can easily be optimized and implemented in any molecular biology lab for a wide range of objectives and organisms e.g. mutation detection and to facilitate gene mapping and marker-assisted selection for breeding purposes.

Methodology and Theoretical Orientation: In this study, first the need for more genotyping in plant specific studies with focus on cocoa is elucidated, focused on the available markers and methods, together with their advantages and disadvantages. DMAS-qPCR SNP genotyping seems a cheap and reliable alternative for such analysis and has been analysed. In this study, the design (PrimerX1), optimization and validation (sequencing and database dependent) of the DMAS qPCR SNP genotyping method is pointed out specifically for cocoa. In addition, several genotyping models have been optimised, of which two can be automated, to translate the retrieved Cq values from DMAS qPCR assay to allele calls and finally a genotype. Thereafter, its applicability on cocoa genetic diversity and mislabelling studies, using GenAIEx v6.5, has been analysed and confirmed on a Vietnamese cocoa population.

Findings: Cocoa DMAS-qPCR based SNP genotyping method has been optimized and consist of 42 SNP markers, which showed 98.05% as efficient in calling correct genotypes. In addition 15.38% off-types and two duplicates have been identified in an internationally recognized cacao population (n=65). Furthermore, three genotyping models have been proposed, of which two could be used in an automated set-up starting from the qPCR data retrieved. Thereafter, key descriptive analysis of the markers, representing the applicability of this method in cocoa genetic diversity studies, using GenAIEx v6.5 has been described in more detail. From this analysis it has been concluded only 13 SNP markers from the DMAS- qPCR assay were needed to differentiate all accessions individually.

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Conclusion and Significance: In conclusion, we have developed a robust and accurate method for cacao genotype identification using a limited set of SNPs. The ease of use and cost-efficiency of the method without the need of specialized instruments can contribute to the adoption of routine-based genotyping to prevent mislabeling in germplasm collections and select optimal breeding parents in cacao and other organisms. The described method can easily be implemented in any molecular biology lab in the context of genotyping, genetic diversity studies, parentage analysis, mutation detection and to facilitate gene mapping and marker-assisted selection for breeding purposes.

Biography

Jocelyn De Wever is a, soon finishing, PhD student with synergistically combined technical (lab technician) and theoretical (bio-engineering) background on applied genetics. She developed a passion in trying to understand the encrypted secrets of flavor in the cocoa its genetics. Thereafter she gained several on-field experiences concerning the cocoa cultivar selection and post-harvesting treatment. Furthermore, she is the main actor on the optimized hands-on DNA and RNA extractions from the difficult and highly contaminated cocoa tissues, and aided in the development of several cocoa genotype and transcriptome based studies.

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Laura Hernandez Escribano, AJPSKY 2019, Volume 09

Virulence of *Fusarium circinatum* is associated with perturbation of phytohormone homeostasis in *Pinus pinaster* seedlings

Laura Hernandez Escribano

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Plants have developed complex molecular strategies to face the attack of a pathogen in order to maintain their survival, and phytohormones are known to play a crucial role in plant-pathogen interactions. The aim of this study is to elucidate the role of phytohormones in *Fusarium circinatum* virulence, the causal agent of pitch canker disease, known as one of the most important pathogens of conifers worldwide.

Methodology and Theoretical Orientation: For this purpose, by a dual RNA-sequencing approach, we determine the expression profiling of both organisms during the interaction at 3, 5 and 10 days post-inoculation.

Findings: *Pinus pinaster* showed moderate resistance at the early time points. This may be explained, at least in part, by the early recognition, the induction of pathogenesis-related proteins and the activation of complex phytohormone signaling that involves crosstalk between three main protagonists: Salicylic acid, jasmonic acid and ethylene. Moreover, we hypothesise the key steps where the pathogen could be manipulating host phytohormone balance to its own benefit, contributing to pathogen virulence. Upon examination of the pathogen

transcripts, we propose that *F. circinatum* prevents salicylic acid biosynthesis from the chorismate pathway by the synthesis of isochorismatase family hydrolase (ICSH) genes, perturbs ethylene homeostasis in the host by expression of genes related to ethylene biosynthesis, and could be blocking jasmonic acid signalling by COI1 suppression.

Conclusion and Significance: Targeted functional testing using *F. circinatum* mutants in future studies would be needed to support this hypothesis.

Biography

Laura Hernández Escribano is currently a PhD student in the National Institute of Agricultural and Food Research and Technology, Center for International Forestry Research (INIA-CI-FOR), working in the field of plant pathology with the thesis named "Fusarium circinatum – host interaction: Ecological and molecular aspects of the pathogenic and endophytic association". She has a degree in Biology and masters in "Applied Vegetable Biology", by the Complutense University of Madrid.

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Bolortuya Ulziibat, AJPSKY 2019, Volume 09

Establishment of regeneration protocol for Mongolian subendemic species *Oxytropis grubovii* Ulzij

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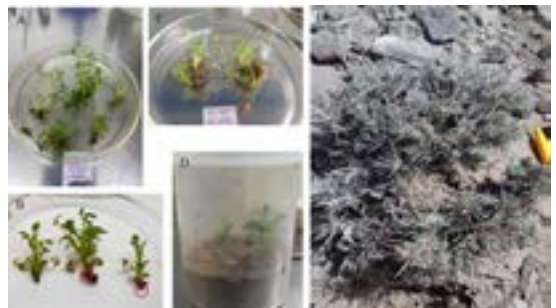
One of the Mongolian subendemic species, *Oxytropis grubovii* Ulzij., grows as a perennial woody shrub and registered in endangered plant list of Mongolia. It distributed in Mongolia gobi desert area with high content of underground wealth. Even it could be regenerated by seed and vegetative organs; it did not produce the seeds during last decade due to the lower rain-falling. Plants growing in a part of the gobi, desert ecosystem drastically damaged by off-road vehicles, mining and pollution depend on mining activity. Since, these native plants are endangered and difficult to propagate by conventional method, conservation and mass propagation in *in vitro* condition can play an important role in the rehabilitation of mining site.

In this study, our research group established tissue culture system for *Oxytropis grubovii* Ulzij. Contamination was highly occurred during the *in vitro* culture and general reagents for sterilization could not work on that woody shrubs. Washing in PPM solution together with supplemental PPM in growth medium was reduced the contamination rate until 60%. In the process of shoot development, medium with combinations of BAP and NAA, Kin and NAA, or BAP, TDZ and 2iP separately investigated step by step. In a result, shoots were effectively regenerated on the medium with 2iP (2.0 mg/l) and produced 15 shoots per explants within four weeks. For rooting of these proliferated shoots, only auxins of IBA, IAA, NAA or combinations of 2iP with IBA tested consistently and results revealed that roots were induced on the medium containing IBA and IAA (2.0mg/l) with 20-30 percent. Produced root's branching

in IBA supplemented medium were higher, however, root length was too short. Whereas, roots were effectively elongated when combining IBA with 2iP (5.0:1.0mg/l) on the medium and root induction percentage were about 50percents.

As indicated in these results, shoot and roots of *Oxytropis grubovii* Ulzij. Can be regenerate *in vitro* condition and this regeneration protocol is first time developed for the species. Preliminary test for *ex vitro* adaptation also executed a time, however, results not identified yet.

Regeneration steps of *Oxytropis grubovii* Ulzij. A. Proliferated shoots on the medium supplemented 2iP, B. IBA effect for shorter root generation, C. Combination of 2iP and IBA induced longer and actively elongated roots, D. Adaptation in *ex vitro* condition, E. Naturally growing plant.



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Biography

Bolortuya Ulziibat works as a researcher in the Plant biotechnology laboratory of Institute of General and Experimental Biology, Mongolian Academy of Sciences. She graduated her Ph.D course in Tohoku University, Japan in 2016. During her doctoral course, she conducted the research work to identify cold tolerance gene of Yunnan landrace 'Lijiangxintuanheigu' in rice during booting stage. Her current research work is tissue culture system development and gene

identification for rhizome development of Mongolian very rare, and endangered plants used in traditional and modern medicine *A. calamus* through the transcriptome analysis. She is also working on the project that identifies the possibility to establish micropropagation protocol for rare and endangered plant species in the mining field and to apply them for rehabilitation after mining exploitation.

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