EUROBIOTECH

9th Central European Congress of Life Sciences

27-28 June 2024 | Krakow, Poland

BOOK OF ABSTRACTS

PLENARY LECTURES

Title:

EU-SAGE (European Sustainable Agriculture through Genome Editing): the role of scientists in the new genomic techniques regulation in Europe

Author:

Oana Dima[1]

Abstract:

Did you know that there are already more than 900 genome-editing applications in crops published in peer-reviewed articles? However, the European Commission concludes that all organisms resulting from new genomic techniques including genome editing fall within the scope of the EU GMO legislation. Consequently, a crop with a genetic change produced by a conventional mutagenesis technique is exempted from the provisions of the GMO Directive, whereas a crop with the same genetic change obtained with genome editing is not, which limits the potential of genome editing for the development of improved plant varieties in Europe.

In 2023 the European Commission (EC) published its legislative proposal on plants derived from new genomic techniques (NGTs) to enable the potential of genome editing for crop improvement to contribute to sustainable food systems. The EC legal proposal on NGTs could have a considerable impact on the European plant research and breeding sector, seed companies, farmers, and vegetable growers. The EU is now at a critical juncture to choose which innovative technologies will be available to help farmers to meet the challenges of climate change, to improve sustainability, and to protect biodiversity while leaving no one behind. The upcoming policy decisions in Europe will determine how genome editing in plant breeding can contribute to the green transition.

Key words:

genome editing; plant breeding; legal proposal; Europe; agriculture

1 VIB-UGent Center for Plant Systems Biology Ghent University Technologiepark-Zwijnaarde 71 - 9052 Ghent - Belgium

Title:

Progress in protein structure prediction. What's next?

Author:

Krzysztof Fidelis[1]

Abstract:

Recent advances in modeling of protein structure, attained with the AlphaFold algorithms from DeepMind, and by others, may lead to the conclusion that the long-standing Protein Folding Problem has now been solved. The folding challenge, so elegantly stated by Christian Anfinsen (Nobel Prize in Chemistry, 1972), puzzled researchers in this area for the last fifty years. But has the folding problem really been solved? Not everyone agrees, for example "The protein-folding problem: Not yet solved", Science, 375(6580):507. We will examine the recent progress in modeling of protein structure from the point of view of CASP, the Critical Assessment of Structure Prediction experiments. We will also take a closer look at what was accomplished so far, and what's ahead.

Note:

Krzysztof Fidelis is directly involved in the Critical Assessment of Structure Prediction (CASP) program, dedicated to identifying state-of-the-art in modeling of macromolecular structure. CASP accomplishes this through independent assessment of structure modeling methods, with approximately 100 research groups worldwide taking part. He heads the CASP Protein Structure Prediction Center since 1996, instituted to support the organization of CASP, infrastructure, evaluation of predictions, and dissemination of results.

1 Univeristy of California Davis

Session 1

STRUCTURAL VIROLOGY

L1.O1

Title:

From Repression to Expression: Promoter Liberation in Toxin-Antitoxin Modules.

Author:

Grzegorz Grabe[1]

Abstract:

Transcription factors control gene expression; amongst these transcriptional repressors must liberate the promoter for derepression to occur. Toxin¬-antitoxin (TA) modules are bacterial elements that autoregulate their transcription by binding the promoter in a toxin:antitoxin ratio dependent manner, known as conditional cooperativity. The molecular basis of how excess toxin triggers derepression remained elusive, largely because monitoring the rearrangement of promoter¬-repressor complexes, which underpin derepression, is challenging. We dissected the autoregulation of the Salmonella enterica tacAT3 TA module. Using a combination of DNA binding and promoter activity assays, and structural characterization, we determined the essential TA and DNA elements required for transcriptional control and reconstituted a repression-¬to¬-derepression path. We demonstrate that excess toxin triggers molecular stripping of the repressor complex off the DNA through multiple allosteric changes causing DNA distortion that ultimately leads to derepression. Thus, our work provides important insight in the elucidation of the mechanisms behindconditional cooperativity.

1 University of Gdansk

L1.O2

Title:

Integrative Structural Biology in Bioscience Research: Halfway to hypusine. Molecular basis of (deoxy)hypusination.

Author:

Przemysław Grudnik[1]

Abstract:

Hypusination, a key post-translational modification of the eukaryotic translation factor 5A (eIF5A), aids in resolving ribosome stalling at polyproline stretches. Deoxyhypusine synthase (DHS) initiates hypusination by forming deoxyhypusine, but the precise molecular mechanism is poorly understood. Recent associations of patient-derived DHS and eIF5A variants with neurodevelopmental disorders highlight the importance of this process. We have determined the structure of the eIF5A-DHS complex using cryo-EM at 2.8 Å resolution and captured DHS in the reaction transition state. Variants of DHS linked to diseases affect the complex formation and hypusination efficiency, providing new insights into this crucial cellular process

We also examine the regulatory role of DHS through its interaction with ERK1/2. Cryo-EM reveals that ERK2 obstructs substrate access to the DHS active site, inhibiting deoxyhypusination in vitro. Alanine scanning pinpointed key residues involved in this interaction. Notably, activation of the Raf/MEK/ERK pathway decreases DHS-ERK1/2 interaction while enhancing DHS-eIF5A association, with ERK1/2 kinase activity regulating DHPS and eIF5A expression. This underscores dual kinase-dependent and independent regulation of deoxyhypusination.

Furthermore, we discuss efforts to identify new inhibitors targeting human DHS. Crystallographic fragment screening identified potential ligands, aiding in the rational design of specific inhibitors. These tools hold promise for hypusination-focused research, helping to elucidate its role in cellular processes and diseases, and contributing to the development of novel therapeutic strategies.

1 Malopolska Centre of Biotechnology (MCB), Jagiellonian University

L1.O3

Title:

Structures of bunyaviral polymerases – how can we use them in drug design

Author:

Piotr Gerlach[1]

Abstract:

Bunyaviruses are large and under-studied group of segmented negative-strand RNA viruses. Several bunyaviruses are human pathogens, causing either inflammations of the central nervous system, or high fatality rate haemorrhagic fevers. WHO listed three of the latter ones – Lassa virus, Rift Valley fever virus, and Crimean-Congo virus, as potential pandemic threats. In recent years, in light of the global warming, these arthropod-borne viruses started to appear in regions of the world they did not belong to previously, including Central Europe. Yet, we do not have specific countermeasures, like broadly approved vaccines or antivirals, to fight them back.

Bunyaviruses are equipped with specialized RNA-dependent RNA polymerases (also known as the L proteins), decorated with several functional domains and responsible for both transcription and replication of the viral genome. Following the seminal atomic structure of the La Crosse orthobunyavirus polymerase (Gerlach et al. 2015), cryo-EM structures of polymerases from other bunyaviruses, captured at different functional states, have been determined (Malet et al. 2023). Available structural insight, combined with in vitro enzymatic assays and in cellulo reporter assays, provides solid ground for the design of novel antiviral drugs, targeting bunyaviral polymerases. This could either be small molecules, specifically inhibiting selected functional domains, or short RNA oligos mimicking viral RNAs naturally interacting with these proteins.

References:

1. Gerlach P, Malet H, Cusack S & Reguera J. Structural insights into bunyavirus replication and its regulation by the vRNA promoter. Cell (2015) 161: 1267–1279, doi: 10.1016/j.cell.2015.05.006
2. Malet H, Williams HM, Cusack S, Rosenthal M. The mechanism of genome replication and transcription in bunyaviruses. PLoS Pathog (2023) 19(1): e1011060. doi: 10.1371/journal.ppat.1011060

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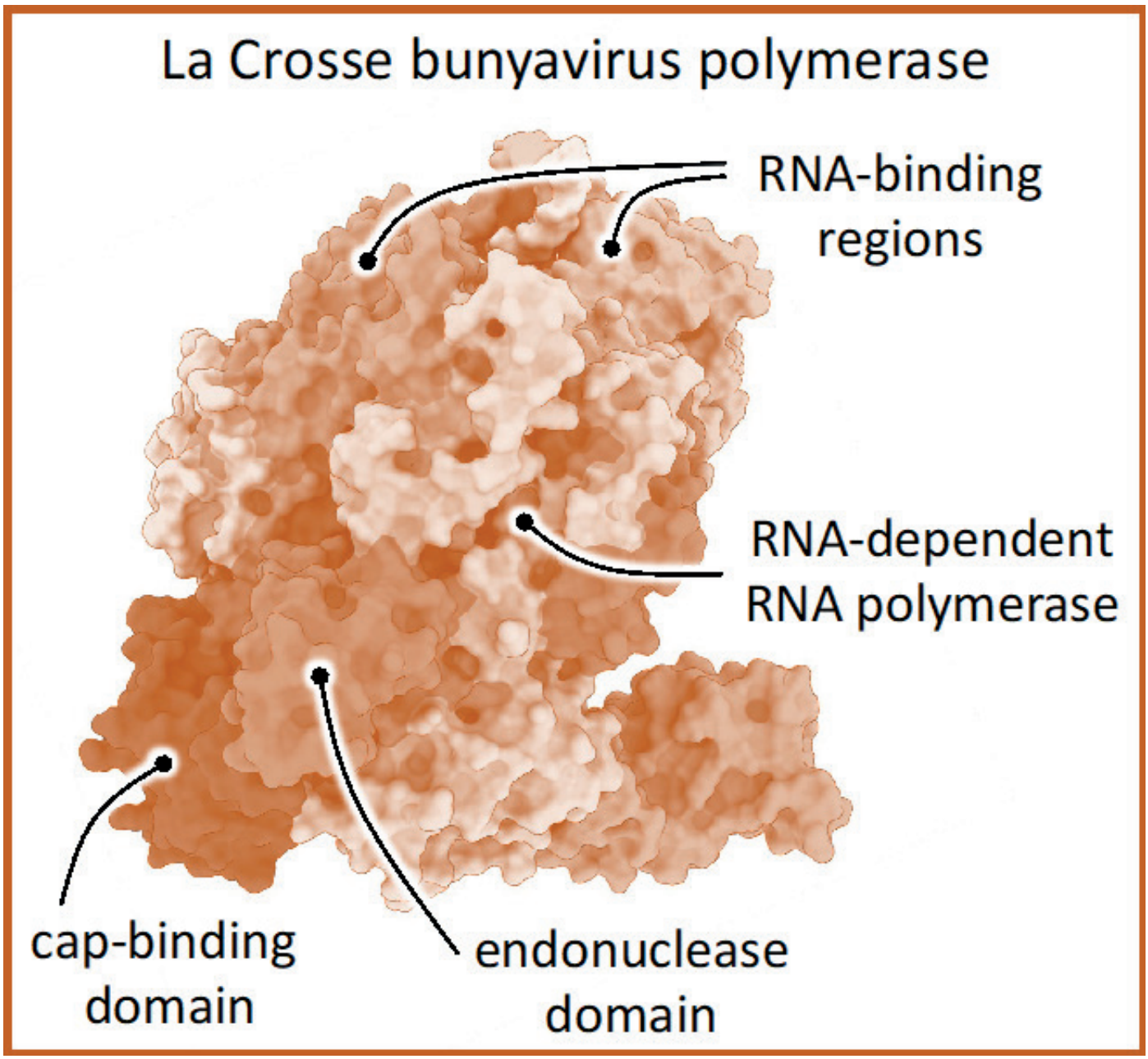
Keywords:
bunyavirus, polymerase, structure, RNA, drug design



Title:

Designed, Programmable Protein Cages Utilizing Diverse Metal Coordination Geometries Show Reversible pH- Dependent Disassembly

Author/Authors:

Norbert Osiński[1], Karolina Majsterkiewicz[1], Yusuke Azuma[1], Artur Biela[1], Szymon Gaweł[1], Jonathan Heddle[2]

Abstract:

Trp RNA-binding attenuation protein (TRAP) is a 74 AA protein that natively forms a ring-like structure consisting of 11 individual subunits. It comes from the bacterial species Geobacillus stearothermophilus.

When artificially introduced metal-binding sites are located on the TRAP ring's external rim, the protein gains the ability to form nanometric cage-like structures. We originally demonstrated this using engineered variants of TRAP containing cysteine residues. These proteins formed cages upon addition of Au(I). Preference for other metals can be engineered into the protein based on an understanding of their coordination geometries.

In the current work, two other TRAP mutants were used, namely TRAP S33H K35H and TRAP S33H K35C. The former makes cages with cobalt (II) and zinc (II) ions, the latter with zinc (II) and gold (I) ions. We have characterized these cages both using Cryo-EM and biophysically, showing them to be highly stable. We have found that some cages can be disassembled with addition of EDTA and readily disassembled at pHs below approx. 7.

Keywords:

TRAP, protein nanocage, metal coordination

1 Jagiellonian University
2 Durham University

Session 2

GENETICS AND GENOMICS

L2.O2

Title:

Unraveling the complex interplay between the human gut microbiome, diet, and cardiometabolic health

Author:

Francesco Asnicar

Abstract:

The human gut microbiome is a complex community of microbes within our intestines that significantly impacts our health. The human microbiome has been associated with many diseases, but we still do not know the precise role played by the many specific bacterial and non-bacterial species. Shotgun metagenomics is the most advanced tool that allows us to analyze the genetic makeup of microbial communities, providing an unbiased and in-depth view and revealing which species are present and their functional potential. Shotgun metagenomics data also revealed many previously unknown species in the community. Computational methods were pivotal in improving the resolution of analysis methods and uncovering stronger links between the microbiome and host health.

Title:

Benefits and challenges for the development of molecular testing in oncology patients

Author/Authors:

Paulina Kliszewska-Krems[1], Ewelina Szczerba[2], Katarzyna Kamińska[2], Janusz Kowalewski[1], Marzena Anna Lewandowska[1]

Abstract:

Introduction

The integration of molecular testing into oncology patient care has revolutionized diagnosis, treatment, and prognosis. This advancement offers numerous benefits, including personalized treatment strategies and improved patient outcomes. However, along with these advantages come significant challenges, such as a broad range of kits or technical complexities. Exploring both the benefits and challenges is essential for understanding the full impact of molecular testing in oncology patient management.

Aim

To explore the benefits and challenges associated with the development and implementation of different approaches to molecular testing in oncology patients.

Material and Methods

4 DNA samples from FFPE tissue of breast cancer patients and 2 DNA+RNA samples derived from FFPE of external quality assessment. Automatic(Onco Core Gene Tissue Kit or BRCA1/BRCA2 Gene Mutation Detection Kit, 3DMed Diagnostics) or manual preparation of libraries(Devyser BRCA CE-IVD or FusionPlex Lung v2, Archer).

Results

Both kits detected the EML4 (exon 13)–ALK (exon 20) fusion. TIER II variant in KRAS detected using the automatic library preparation. Lack of reads, and quality control failed using manual method. We used a germline-dedicated kit for preparing libraries from FFPE material (RUO). It gave consistent results as a competitive kit dedicated to FFPE (for commonly known variants in BRCA 1/2). The automatic detection method performed better with more challenging material, whereas in the competitive kit, the result was non-diagnostic due to a lack of coverage in some regions.

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2 The F. Lukaszczyk Oncology Center, Molecular Oncology and Genetics Department, Innovative Medical Forum, Bydgoszcz, Poland

Conclusions
Both automatic and manual methods for preparing NGS libraries from FFPE material yield accurate and consistent results. Manual kits sometimes lead to non-diagnostic or false negative outcomes, potentially due to variations in human work compared to machine uniformity. Laboratory automation reduces the risk of analytical errors caused by human factors, particularly in high-workload situations.

References:

1. Śledzińska P, Bebyn MG, Furtak J, Kowalewski J, Lewandowska MA. Prognostic and Predictive Biomarkers in Gliomas. Int J Mol Sci. 2021 Sep 26;22(19):10373. doi: 10.3390/ijms221910373. PMID: 34638714; PMCID: PMC8508830.
2. Śledzińska P, Bebyn M, Szczerba E, Furtak J, Harat M, Olszewska N, Kamińska K, Kowalewski J, Lewandowska MA. Glioma 2021 WHO Classification: The Superiority of NGS Over IHC in Routine Diagnostics. Mol Diagn Ther. 2022 Nov;26(6):699-713. doi: 10.1007/s40291-022-00612-3. Epub 2022 Sep 2. PMID: 36053463; PMCID: PMC9626418.
3. Epub 2022 Sep 2. PMID: 36053463; PMCID: PMC9626418.

Keywords:

next generation sequencing, automation, library preparation, oncology

S2.O2

Title:

Strigolactone insensitivity affects differential shoot and root transcriptome in barley

Author/Authors:

Magdalena Korek[1], R. Glen Uhrig[2], Marek Marzec[1]

Abstract:

Strigolactones (SLs) are plant hormones that play a crucial role in regulating various aspects of plant architecture, such as shoot and root branching. However, the knowledge of SL-responsive genes and transcription factors (TFs) that control the shaping of plant architecture remains elusive. Here, for the first time, transcriptomic analysis was conducted using the SL-insensitive barley mutant hvd14.d (carried mutation in SL receptor DWARF14, HvD14) and its wild-type (WT) to unravel the differences in gene expression separately in root and shoot tissues. This approach enabled us to select groups of SL-dependent genes exclusive to each studied organ or those not tissue-specific. The data obtained, along with in silico analyses, found several TFs that exhibited changed expression between the analysed genotypes and that recognized binding sites in promoters of other identified differentially expressed genes (DEGs). In total, 28 TFs that recognize motifs over-represented in DEG promoters were identified. Moreover, nearly half of the identified TFs were connected in a single network of known and predicted interactions, highlighting the complexity and multidimensionality of SL-related signalling in barley. Finally, the SL control on the expression of one of the identified TFs in HvD14- and dose-dependent manners was proved. Obtained results bring us closer to understanding the signalling pathways regulating SL-dependent plant development.

Acknowledgments:

This study was supported by the National Science Centre, Poland (2020/37/B/NZ3/03696).

Kerywords:

barley, Hordeum vulgare, root, shoot, strigolactones, transcriptome

1 University of Silesia
2 University of Alberta

Title:

Temperature-Induced Changes in Sex Expression, Morphology, and Transcriptome Profiles in Six Different Cucumber (Cucumis sativus) Lines

Author/Authors:

Aparna Aparna[1], Agnieszka Skarzyńska[1], Szymon Turek[1], Wojciech Plader[2], Magdalena Pawelkowicz[2]

Abstract:

Cucumber (Cucumis sativus) is an important vegetable crop that produces male, female, and hermaphrodite flowers. Temperature critically influences various cellular processes, including the modulation of sex ratio, with high temperatures favoring male flower development and low temperatures favoring female flower development. In this study six cucumber lines - B10, 2gg, Gy3, Hgy3, 2667, and 859 were evaluated under high (H) and low (L) temperature treatments to assess sex changes, as well as morphological and transcriptomic responses. Morphological parameters, such as plant height and internodal length, were documented to determine the effect of temperature on cucumber development. Tissue samples were collected from the shoot apex under both temperature conditions for RNA sequencing (RNA-seq). Significant differences in plant height, internodal length, and sex expression were observed across all six lines due to temperature variation. Notably, flowers of the B10 line were smaller when grown under high temperatures. Transcriptomic analysis revealed significant changes in gene expression associated with temperature differences, identifying differentially expressed genes (DEGs) in growth cones in all compared lines. In total, 1845 DEGs were identified in male lines (B10, 859), 5306 in female lines (2gg, Gy3), and 1237 in hermaphroditic lines (2667, Hgy3). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses highlighted key pathways involved in defense mechanisms, carbohydrate metabolism, glycolysis, carbon metabolism, and cutin, suberin, and wax biosynthesis. These findings provide insights into the molecular mechanisms underlying temperature-dependent sex determination in cucumbers, advancing our understanding of plant reproductive biology and offering practical implications for crop management and breeding strategies.

1 Warsaw University of Life Sciences,Institute of Biology, Department of Genetics, Breeding and Plant Biotechnology

2 Warsaw University of Life Sciences,Institute of Biology, Department of Genetics, Breeding and Plant

References:

1. Lai, Y. S., Shen, D., Zhang, W., Zhang, X., Qiu, Y., Wang, H., ... & Li, X. (2018). Temperature and photoperiod changes affect cucumber sex expression by different epigenetic regulations. BMC plant biology, 18, 1-13.
2. Miao, M., Yang, X., Han, X., & Wang, K. (2011). Sugar signalling is involved in the sex expression response of monoecious cucumber to low temperature. Journal of Experimental Botany, 62(2), 797-804.

Acknowledgments:

The work is funded by the NCN research project OPUS-19 (UMO 2020/37/B/NZ9/00586)

“Integration of cucumber Multi-omics data to identify sex determination mechanisms and their climatic determinants”

Keywords:

Temperature, Cucumber, Sex-Determination, Yield, Flower Formatio

P2.1

Title:

Agrobacterium engineering: characterization of phenolic-inducible promoters

Author/Authors:

Joanna Potwardowska[1], Krzysztof Michalski[1], Slawomir Sowa[1]

Abstract:

Agrobacterium tumefaciens is a well-known bacterium used as a tool in genetic engineering. It is due to its rare natural ability to transfer DNA into plant cells. In our study, we focused on characterizing phenolic-inducible promoters within the Agrobacterium genome, with an aim of enhancing the precision and efficiency of gene expression in this organism during plant transformation. The Ti (tumor-inducing) plasmid of Agrobacterium tumefaciens has a virulence (Vir) region containing virulence genes that can be indirectly activated by phenolic compound like acetosyringone (As). In the present study, we isolated the Ti plasmid from Agrobacterium tumefaciens AGL-1 strains and used as a template for amplification of Vir promoters. Then, we cloned the amplified fragments into a vector encoding mCHERRY marker gene, so we could compare its expression and also activity of Vir promoters under non-inducing conditions and after induction with acetosyringone.

Keywords:

agrobacterium, virulence, plant transformation, genome editing, CRISPR/Cas9

1 Plant Breeding and Acclimatization Institute (IHAR) – National Research Institute

P2.2

Title:

Application of thermostable ligases for SNP detection

Author/Authors:

Klaudia Bernacka[1]

Abstract:

Single nucleotide polymorphism (SNP) analysis is an important technique successfully used in diagnostics in: medicine, pharmacology and agrobiotechnology. The most common detection methods are based on sequencing and various types of PCR analysis. These techniques may vary in sensitivity, specificity and precision. For the identification of mutations in genetically modified organisms (GMOs), sequencing is not routinely used by the official control laboratories. On the other hand, PCR methods are sometimes insufficiently sensitive and precise for single-nucleotide mutation detection. For this purpose, a ligase chain reaction (LCR)-based analysis can be used. However, in case of small number of copies of the target sequence, this method doesn't meet required minimum performance parameters. To improve sensitivity we combine ligase-based detection with the classical PCR amplification of the target sequence with SNP. In the case of ligase-based analysis, the choice of enzymes is crucial. The chosen ligase should not exhibit any activity in mismatched sequence to minimize the risk of false positives due to the possibility of target-independent ligation. The performance of the three thermostable ligases was compared in the SNP variations observed in rapeseeds.

1 Plant Breeding and Acclimatization Institute

Title:

Computational workflow for analysis of qualitative differences of transcriptomes in multifactorial experiments

Author/Authors:

Maria Nuc[1], Michał Stanoch[1], Barbara Naganowska[1], Paweł Krajewski[1]

Abstract:

We consider methods of comparing datasets of nucleotide sequences. The Mash distance [1] expresses the differences between data sets in the qualitative sense, that is, in terms of common and disjoint elements, which are represented by k-mers. This work presents the MASHt toolkit, which uses Mash distance during the analysis of transcriptomic data in multifactorial experiments.

The proposed analysis consists of computing pairwise Mash distances between data sets obtained for all experimental samples, performing principal coordinate analysis on the distance matrix, and application of univariate and multivariate analysis of variance to principal coordinates of samples. Computations can be performed for the whole datasets or their subsets related to functional annotation of sequences.

We illustrate functioning of the workflow using data obtained in an experiment performed to study reaction of barley forms to elevated temperature [2]. Qualitative differences between transcriptomes observed under different temperatures and at different time points were discovered. These differences were interpreted by GOSlim GOA annotation.

References:

1. B.D. Ondov, T.J. Treangen, P. Melsted et al. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol 17, 132; 2016.
2. K. Mikołajczak, A. Kuczyńska, P. Ogrodowicz et al. High-throughput sequencing data revealed genotype-specific changes evoked by heat stress in crown tissue of barley sdw1 near-isogenic lines. BMC Genomics 23, 177;2022.

Acknowledgments:

The research was supported by National Science Centre, Poland, projects: Harmonia 8 no. 2016/22/M/NZ9/00251 and Opus 12 no. 2016/23/B/NZ9/02677.

Keywords: distance, transcriptome, Mash, multifactorial experiments

1 Institute of Plant Genetics, Polish Academy of Sciences

P2.4

Title:

Identification and sequence diversity analysis of phosphate transporter families in Secale cereale L.

Author/Authors:

David Chan-Rodriguez[1], Sirine Werghi[1], Brian Wakimwayi Koboyi[1], Aleksandra Paradowska[1], Magdalena Święcicka[1], Anna Hawliczek[1], Maksymilian Królik[1], Hilderlith Abuya[1], Fatemeh Shoormij[2], Julia Maksymiuk[1], Hanna Bolibok-Brągoszewska[1]

Abstract:

Plants rely on specialized transporters to distribute phosphate to the various tissues and within cells. The PHOSPHATE TRANSPORTER 1 (PHT1) membrane-localized transporter family members are associated with the primary uptake, translocation, and allocation of inorganic phosphate (Pi) [1,2]. The other phosphate transporter families mobilize Pi to internal compartments such as the chloroplast (PHT2), mitochondria (PHT3), Golgi and plastids (PHT4), and vacuole (PHT5)[1,2]. Altogether, these phosphate transporters are fundamental to maintaining the phosphate homeostasis. However, the function and regulation of phosphate transporters in rye remain unexplored.

We surveyed the Secale cereale Lo7 and Weining reference genomes to identify putative members of the phosphate transporter family. We also performed phylogenetic, gene structure, and conserved motive comparative analyses to determine the evolutionary relationships of rye phosphate transporters to other grasses. We identified one Pht2, two Pht4, and four Pht5 putative transporters in both Lo7 and Weining rye genomes. Interestingly, the Lo7 contains 16 Pht1 and six Pht3, while Weining contains 19 Pht1 and nine Pht3 putative transporters. Some ScPht family members contain the P1BS cis-elements in the promoter region, suggesting phosphate deficiency as the expression driving force. We assessed the sequence diversity of the rye Pht genes using DArTreseq data from 94 diverse rye genotypes. We found a total of 820 polymorphic sites and 11 private variants. Expression profiling in the Lo7 inbred line shows that ScPht1;2 and ScPht3 were significantly upregulated in roots under low phosphorus conditions. ScPht3 also showed upregulation in shoots. Our results lay the groundwork for the functional characterization of PHT transporters and provide insight into the impact of different cultivation environments on the Pht families' gene sequence diversity.

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2 Department of Agronomy and Plant Breeding, Isfahan University of Technology, Iran

References:

1. Wang, D., Lv, S., Jiang, P., & Li, Y. (2017). Roles, regulation, and agricultural application of plant phosphate transporters. Frontiers in Plant Science, 8: 817.
2. Wang, Y,, Wang, F., Lu, H., Liu, Y., Mao, C. (2021) Phosphate uptake and transport in plants: an elaborate regulatory system, Plant and Cell Physiology, 62: 564–5721

Acknowledgments:

This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

Keywords:

Secale cereale, phosphate transporters, sequence diversity, DArTreseq, phosphate deficiency

Title:

Evaluation of total protein extraction methods for proteome profiling of rye (Secale cereale L.) inbred lines

Author/ Authors:

Brian Wakimwayi Koboyi[1], David Chan-Rodriguez[1], Sirine Werghi[1], Hanna Bolibok-Brągoszewska[1]

Abstract:

Plant quantitative proteomics is an excellent approach of understanding the plant's response to external stress through a comprehensive analysis of the abundant proteins. However, the high amount of interfering compounds such as polyphenols and pigments, and the presence of the insoluble membrane-bound proteins create a challenge for total protein extraction. Although utilizing ionic detergents like Sodium Dodecyl Sulphate (SDS) enables the solubilization of the low abundant membrane proteins, it interferes with the chemistry of downstream analyses such as trypsin digestions, liquid chromatography/tandem mass spectrometry (LC-MS/MS) and clogs the columns and spray needles. Therefore, an efficient method to get rid of SDS is required.

We aim to establish a total protein extraction protocol suitable for studying the phosphorus tolerance mechanism in rye (Secale cereale L.) shoots and roots. We compared two extraction protocols differing in SDS removal step: method 1 (SDS-containing extraction buffer + trichloroacetic acid clean-up step) and method 2 (SDS-containing extraction buffer + phenol clean-up step), and dissolved the pellets in 8M urea [1-3]. We evaluated the quality of the protein extracts through 10% SDS-PAGE (polyacrylamide) gels and visualized the proteins using silver staining.

The protein pellets from method 2 dissolved completely unlike the pellets from method 1. We found a considerably higher protein yield from the method 2 after quantification using the modified Lowry protein assay kit. Furthermore, for method 2 we observed clearer and sharper bands with a high uniformity between replications on SDS-PAGE gels.

Based on these findings, we will extract proteins using method 2 from two inbred lines with contrasting responses to phosphorus deficiency to observe the differentially expressed proteins (DEPs) using LC-MS/MS with TMT labeling and analyze their expression levels.

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References:

1. Wu, X., Xiong, E., Wang, W., Scali, M., & Cresti, M. (2014). Universal sample preparation method integrating trichloroacetic acid/acetone precipitation with phenol extraction for crop proteomic analysis. Nature Protocols, 9(2), 362-374.
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3. Niu, L., Zhang, H., Wu, Z., Wang, Y., Liu, H., Wu, X., & Wang, W. (2018). Modified TCA/acetone precipitation of plant proteins for proteomic analysis. PloS One, 13(12), e0202238.

Acknowledgments :

This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

Keywords:

Secale cereale, total protein, proteomics, LC-MS/MS, phosphate deficiency

Title:

Analysis of DNA methylation changes under low phosphorus conditions in roots and shoots of rye (Secale cereale L.) inbred lines with contrasting phosphorus deficiency tolerance

Author/Authors:

Sirine Werghi[1], Brian Wakimwayi Koboyi[1], David Chan-Rodriguez[1], Hanna Bolibok-Brągoszewska[1]

Abstract:

Phosphorus (P) deficiency invokes a wide range of adaptive responses at molecular, physiological, biochemical and phenotypic levels. Environmental stresses influence DNA methylation, and methylome changes in response to P starvation were reported, including modifications within genic regions, which often correlated with changes in gene expression [1]. Rye (Secale cereale L.) is a cereal closely related to wheat and barley, but exhibiting a much higher tolerance of biotic and abiotic stresses, including P deficiency. The aim of the study is to understand the molecular mechanism of rye's tolerance to P deficiency by, among others, characterization of changes in DNA methylation.

Based on P deficiency response screen in hydroponic conditions two inbred lines of rye L9 and K3, exhibiting a contrasting response, were chosen. DNA was isolated from roots and shoots of 14 and 21 day old plants grown in phosphorus deficient and control conditions. Methylation status was examined via Methyl Sensitive DArT sequencing [2] at Diversity Arrays Technology (Bruce, Australia).

Our result provide the first insight into methylome regulation in rye under nutrient stress and provide a starting point for comparative analyses od DNA methylation dynamics upon P starvation among rye, related crops, and model plant species.

References:

1. Yong-Villalobos L et al, Proc Natl Acad Sci USA, 112: E7293-E7302 (2015)
2. Pereira WJ et al. Plos One 15: e0233800 (2020)

Acknowledgments :

This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

Keywords: Secale cereale, methylation profiling, phosphate deficiency, Methyl Sensitive DArT sequencing

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Title:

The KL, SOD2 and SESN2 genes as potential genetic markers of endurance performance based on Arabian horses model

Author/Authors:

Grzegorz Myćka[1], Katarzyna Ropka-Molik[1], Anna Cywińska[2], Monika Stefaniuk-Szmukier[1]

Abstract:

Endurance riding is an equestrian sport based on controlled long-distance rides. The ride distance may reach up to 160km what is an extreme physical exertion for the competitors. The Arabian horses are characterized by extraordinary exercise phenotype, however the molecular basis of these unique features haven't been studied so far. The main purpose of the study was to describe the difference in the expression level of specific genes during the ride. A group of n=10 Arabian horses took part in the study in which the blood samples have been taken 1 h before and 1 h after the end of the 120 km endurance ride under the supervision of the president of the veterinary commission (the year of ride – 2018). After the RNA isolation, RNA-seq and NGS methods were performed to find the most up- and downregulated genes. In total, 6458 differentially expressed genes (DEGs) were used in the study. The David software with Kyoto Encyclopaedia of Genes and Genomes (KEGG) database were used for the gene-enrichment analysis with the obtained DEGs. The results described wider in Mycka et al., 2024 allowed us to propose 3 genetic markers – the KL (FC = - 4.54) gene as an indicator of overtraining and exhaustion of the organism, the SOD2 (FC = 3.30) gene as an indicator of a proper oxygenation and efficient cellular respiration, and the SESN2 (FC = 5.11) gene as an indicator of an efficient neutralization of harmful ROS (Reactive Oxygen Species). The FC (Fold Change) parameter describes an increased level of expression of SOD2 and SESN2 genes in response to physical exercise what allows to assume a crucial role in long distance rides metabolism. On the other hand, the decreased expression of KL after the ride allows to consider it can be an overtraining marker. The usage of the potential genetic markers may lead to the selection of horses with an appropriate riding potential at an early stage of breeding.

1 Department of Animal Molecular Biology, National Research Institute of Animal Production
2 b Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun

References:

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Acknowledgments :

Financing

This study was supported by a statutory grant from the National Research Institute of Animal Production (No. 501-180-811).

Keywords:

Arabian horse, ride, RNA-seq, genetic marker

P2.8

Title:

Carrot MITEs provide binding sites for a circadian clock transcription factor, LHY

Author/Authors:

Alicja Macko-Podgórni[1], Wojciech Wesołowski[1], Kinga Zygmuntowicz[1], Kornelia Kwolek[1], Natalia Piasecka[1], Marcelina Skrabucha[1], Dariusz Grzebelus[1]

Abstract:

The circadian clock in plants plays a pivotal role in orchestrating various growth and cellular processes. A key regulator, the Late Elongated Hypocotyl (LHY) transcription factor, not only controls the circadian clock by suppressing afternoon and evening genes but also influences genes related to growth and development. Recent research shows LHY binding sites are enriched in carrot Miniature Inverted-repeat Transposable Elements (MITEs).

In this study, we used DNA affinity purification sequencing (DAP-seq) to experimentally identify and characterize LHY binding sites across the genome. In addition, we sequenced the transcriptomes of carrot plant leaves exposed to four hours of heat, cold and salinity stress.

We identified 11,779 binding peaks, 20% in promoter regions. Gene Ontology (GO) analysis revealed genes involved in vegetative to reproductive phase transition of meristem, rhythmic processes, flower development, cell division, chromatin binding and signal transduction. Key KEGG pathways included circadian rhythm and carotenoid biosynthesis leading to ABA biosynthesis. Of the 2,346 genes with DAP-seq peaks in promoter regions, 74% were expressed, with 694 genes showing altered expression under abiotic stress. Notably, LHY expression decreased under heat stress, and 36% of the differentially expressed genes, including TOC1 and other circadian clock-related genes, were upregulated under heat stress.

We also compared the experimentally identified LHY binding sites with the locations of MITE transposons. We found that 1,428 (12%) of DAP-seq peaks overlapped with MITE copies, and 41% of these overlapped peaks were within elements of the Tourist_15 family, comprising 56% of all elements in this family. In promoter regions, 48% of MITEs were Tourist_15 copies. Thus, Tourist_15 MITEs can provide LHY binding sites and, if mobilized, may rewire the circadian clock network, potentially accelerating adaptation to abiotic stresses.

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Acknowledgments:

The research was financed by the Polish National Science Center (NCN) (2019/33/B/NZ9/ 00757).

Keywords:

transcription factors, DAPseq, expression, transposon

Session 3

COP GENOMICS

L3.O1

Title:

Unlocking plant structural genome variation with pangenomics

Author/Authors:

Agnieszka Golicz

Abstract:

Genomic technologies have become a cornerstone of modern plant research. Ongoing improvements in genome sequencing and assembly now allow for the generation of high quality genomes at a fraction of previous computational and monetary cost. The availability of multiple genomes of individuals from the same species facilitates detailed comparative analyses, more precise variant identification and improved marker development.

The term pangenome was introduced to describe a collection of genomic sequence found in the entire population rather than in a single individual. Compared with single reference genomes, pangenomes can represent the entire variation repertoire of a certain species or genus. By combining the genomic data of multiple accessions, pangenomes allow for the detection and annotation of complex DNA polymorphisms such as structural variations (SVs), one of the major determinants of genetic diversity within a species. From disease resistance to plant morphology and yield, combined with transcriptomic and epigenomic data, pangenomes provide a powerful framework for understanding of variation underlying key traits.

The talk will discuss the most recent developments in genomic data analysis including the concept of pangenome, highlighting its potential current applications and future directions.

Title:

Identification of candidate genes conditioning carrot vernalization requirement

Auhor/Authors:

Josefina Wohlfeiler[1], Andres Morales[2], María Soledad Alessandro[2], Douglas Senalik[3], Shelby Ellison[4], Alicja Macko-Podgórni[5], Dariusz Grzebelus[5], Claudio Galmarini[2], Philipp Simon[6], Pablo Cavagnaro[5]

Abstract:

Vernalization is the process by which a prolonged period of cold exposure, which length is genetically-conditioned and influenced by environmental factors, renders plants competent to flower. In carrot, vernalization requirement (VR) conditions flowering time and -thereby- the timing of sawing and harvesting, both for root and seed production crops. This trait is also used to classify carrot cultivars as annual (or early flowering) or biennial (or late flowering). Due to its direct influence on the management of the crop, it is of interest to breeders to understand the genetic basis of carrot VR. To this end, this study performed linkage mapping of VR in an F2 segregating population derived from a cross between an annual and a biennial carrot. Plants of the F2 population were phenotyped based on their flowering habit as either annual or biennial, and genotyped using genotyping-by-sequencing (GBS) markers. In addition, comparative transcriptome analysis in vernalized versus non-vernalized plants of the annual and biennial parental lines was performed to identify differentially expressed genes (DEGs) associated with VR. Results from segregation analysis in the F2 revealed a 3:1 ratio for the 'annual : biennial' flowering habits (χ^2=0.01, p=0.99), suggesting a single dominant gene for the genetic control of VR. Linkage mapping localized VR (as a phenotypic trait) in the most distal part of chromosome 2. Over one hundred annotated genes were found in the map region associated with VR. Transcriptome analysis revealed 3-55 DEGs in the VR region, depending on the comparison considered, and included several transcription factors, some of them homologous to flowering-time genes and

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key regulators of the vernalization pathway in other species. In-depth analysis of these genes, showing concomitant expression with other flowering genes, suggest at least two major candidate genes for carrot VR. Based on these results, a model for carrot VR is proposed.

Keywords:

Daucus carota, vernalization requirement, flowering, candidate genes

Title:

Acyltransferases Involved in Phospholipid Metabolism and Their Role in Camelina sativa Adaptation to Temperature Conditions

Author/Authors:

Sylwia Klińska-Bąchor[1], Joanna Mancewicz[1], Sara Kędzierska[1], Kamil Demski[2], Antoni Banaś[1]

Abstract:

Acyltransferases are enzymes that catalyze the transfer of acyl groups, contributing to both the synthesis of individual lipids and the editing their fatty acid composition. In plants, lipids serve as structural components of cell membrane (membrane lipids) and energy reservoir (storage lipids). While the biochemical function of many plant acyltransferases is well understood, their physiological roles have remained unclear.

This research focuses on acyltransferases directly associated with membrane lipid, particularly phospholipid. These enzymes play a crucial role in maintaining cell integrity and fluidity, molecular transport, and cell signaling. Therefore, any changes in their content and composition can alter membrane homeostasis and ultimately impact plant adaptation to the environment. Acyl-CoA:lysophospholipid acyltransferases (LPLAT) are enzymes directly involved in phospholipid metabolism. They possess bidirectional activity, utilizing lysophospholipids and acyl-CoAs to synthesize phospholipids, and vice versa. This study focused on LPEAT, an enzyme involved in the remodeling of phosphatidylethanolamine.

To investigate the role of LPEAT in planta, we used the CRISPR-Cas9 method to create a knockout line of oilseed Camelina sativa. We successfully obtained a line with a triple deletion of lpeat gene in each of the subgenomes. Further study focused on examining biochemical changes in lipids, as well as the physiological consequences of this mutation with particular emphasis on adaptation to temperature conditions (cold, standard, heat). Differences in the remodeling of membrane lipid turned out to be a vital aspect of plant adaptation. Significant changes were also noticeable in the phenotype. Mutant plants with increased lipid remodeling exhibited boosted growth and better fitness. These findings shed light on the role of these enzymes in plant physiology and adaptation to adverse conditions, offering agricultural potential for improved yields and vitality.

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Acknowledgments :

This work was partially supported by NCN grant No. UMO-2017/25/B/NZ3/00721 and UGrants 533-N000-GS0G-24.

Keywords:

temperature stress, abiotic stress, phospholipid metabolism, Camelina sativa, CRISPR-Cas9

Title:

Elucidating the molecular basis for monogermy in sugar beet

Author/Authors:

Marcelina Skrabucha[1], Monika Szewczyk[1], Alicja Macko-Podgorni[1], Dariusz Grzebelus[1]

Abstract:

Monogermy in sugar beet is an agriculturally desirable trait as it removes the requirement for manual thinning. According to classical genetics studies, it is conditioned by a single gene. A recessive homozygote mm governs the formation of a single flower while in multigerm beets (MM or Mm) inflorescences are placed in the axils of the leaf bracts. Here, we searched for a defined genomic region encompassing the M gene and identified a candidate gene for the trait. We used a strategy based on whole genome sequencing of bulk segregants representing 35 F2 populations derived from crosses between monogerm maternal lines and multigerm pollinators. We developed ten libraries representing five pairs of bulk segregants and used them for Illumina sequencing. The reads were used to call SNPs using EL10 sugar beet genome assembly as a reference. FST coefficients were calculated for approximately 5M filtered high quality SNPs. The highest FST values, exceeding 0.15, were observed for SNPs located at the distal region of the log arm of chromosome IV, spanning ca. 3 Mb. However, a single peak of SNPs with FST exceeding 0.4 pointed at a much more narrow region of ca. 200 Kb. Around 20 protein-coding genes were localized in that region, including an AP2-like gene which occurred to be a homolog of FRUITFULL - a gene previously reported to be involved in double fruit formation at high temperature in sweet cherry. Thus, bulk segregant whole genome sequencing provided effective means to identify a candidate gene for a simply inherited trait of agricultural importance.

The research was financed by the basic research for biological progress in agriculture programme of the Polish Ministry of Agriculture and Rural Development.

Keywords:

Beta vulgaris, seed production, AP2-like, BSA-seq

1University of Agriculture in Krakow

Title:

Xyloglucan endotransglucosylase/hydrolase (XTH)-mediated cell wall remodeling under hypoxia stress at Solanum lycopersicum L.

Author/Authors:

Małgorzata Czernicka[1], Kinga Kęska-Izworska[1], Emilia Wilmowicz[2], Małgorzata Kapusta[3]

Abstract:

The lack of knowledge on how crops respond and adapt to waterlogging or flooding imposes a major barrier to enhancing their productivity. Hence, understanding the stress responsive mechanisms of crops is indispensable for developing new waterlogging-tolerant cultivars. Differential expression profile of genes related to cell wall metabolism, cellulose synthesis, and cell wall degradation caused by waterlogging indicates that cell wall biosynthesis is inhibited by hypoxia stress. The xyloglucan endotransglucosylase/hydrolase (XTH) family is a multigene family, the function of which plays a significant role in cell-wall rebuilding and also hypoxia stress tolerance in plants. To reveal the effects of XTH on cell wall reconstruction under waterlogging in S. lycopersicon, we analyzed the expression levels of XTH genes according to RNA-seq data. We identified 34 XTHs as waterlogging-stress-induced DEGs.To visualize dynamic changes of XTHs in roots following waterlogging, we applied the immuno-dot-blot and immunostaining using three anti-XG monoclonal antibodies (LM15, LM24, LM25). Stronger fluorescence was detected in waterlogging-tolerant tomato accession (POL 7/15) in relation to waterlogging-sensitive accession (PZ 215). According to the results, we supposed hypoxia stress induced by waterlogging could stimulate XTH activity by regulating the expressions of XTH genes, which might further participate in the transformation of xyloglucan, affect the cell wall remodeling of tomato roots.

Acknowledgments :

Research at the University of Agriculture in Krakow was subvented by the Polish Ministry of Education and Science in 2023-2024.

Keywords: hypoxia, immunostaining, RNA-seq, tomato, waterlogging, XTH

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Title:

BSA-seq approach identifies genomic regions associated with altered plant growth in cucumber (Cucumis sativus L.)

Author/Authors:

Renata Słomnicka[1], Karolina Kaźmińska[1], Szymon Mużacz[1], Maria Grad[1], Kacper Zembrzucki[1], Dominika Stokowiec[1], Grzegorz Bartoszewski[1]

Abstract:

Plant architecture plays a crucial role in crop management and yield. Identification of genes that control plant growth habit is essential for modern plant breeding and can significantly accelerate breeding progress. In cucumber, only several genes responsible for altered plant growth at the molecular level have been identified (Liu et al. 2021).

The study aimed to use bulked segregant analysis coupled with whole-genome sequencing (BSA-seq) to identify genomic regions carrying cp-2, spc and sp-2 genes that control growth habit in cucumber.

We applied BSA-seq (Zhou et al. 2022) to three mapping populations (F2:3) derived from the cross between maternal line (L500) with normal growth type and paternal lines (L504, L505 and L511) characterized by altered growth due to cp-2, spc and sp-2 genes. For each population, two pooled samples were prepared, consisting of equimolar DNA isolated from dominant homozygotes (normal growth) and recessive homozygotes (altered growth). Paired-end libraries for each pool were sequenced using Illumina NovaSeq technology. The resequencing data were mapped on the cucumber reference genome (9930 v3, Li et al. 2019). Single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) were identified and annotated. The ?All-index parameter was used to define genomic regions associated with altered plant growth.

The resequencing of pooled samples resulted in high-quality reads that mapped to the reference genome with average mapping rate of 96% and genome coverage exceeding 30×. SNPs and INDELs analysis allowed to identify scp and cp-2 genes at the upper and lower arm of chromosome 4 respectively, and sp-2 on the chromosome 3. BSA-seq revealed also an uneven distribution of polymorphisms for two populations which could be related to the low level of genetic diversity between parental lines. This study provides new insights into the genomic regions controlling altered growth type in cucumber.

1 Warsaw University of Life Sciences – WULS-SGGW

References:

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2. Liu X et al. (2021) Genetic regulation of shoot architecture in cucumber. Hortic Res 8:143
3. Zhou et al. (2022) Conjunctive analyses of BSA-Seq and BSR-Seq unveil the Msβ-GAL and MsJMT as key candidate genes for cytoplasmic male sterility in alfalfa (Medicago sativa L.). Int J Mol Sci 23:7172

Acknowledgments:

The study was supported by a research program of the Polish Ministry of Agriculture and Rural Development ”Basic research for biological progress in crop production 2021-2027”, specifically task 33 “Identification of genes associated with altered growth type in cucumber plants (Cucumis sativus L.)”.

Keywords:

BSA-seq, Cucumis sativus L., mapping

Title:

Identifying genetic determinates of 'carota" and 'gummifer' morphotypes in the wild Daucus carota L.

Author/Authors:

Alicja Macko-Podgórni[1], Marcelina Skrabucha[1], Dariusz Grzebelus[1]

Abstract:

Wild Daucus carota can be classified into two distinct morphotypes: the coastal 'gummifer' and the inland 'carota'. Previous studies have revealed that individuals within these morphotypes tend to form clusters based on their geographical origin rather than solely on their morphological characteristics. This observation suggests the presence of genetic determinants for the 'gummifer' 'carota' transitions, particularly considering evidence of five independent selections of inland morphotypes (defined as geographically confined subspecies) in various restricted maritime environments.

The objective of the study was to investigate the genetic determinants underlying the transition between the 'carota' and 'gummifer' morphotypes in wild Daucus carota populations. To accomplish this, we utilized Genotyping by Sequencing (GBS) data available from previous studies (Martínez-Flores et al., 2020). We used the 29K SNP panel produced for 160 'carota' and 'gummifer' accessions to run a case-control Genome-Wide Association Study (GWAS) experiment, utilizing morphotype classification as a 'trait'.

Our analysis identified five genomic regions significantly associated with the morphotype, located on chromosomes 1 (two regions), 5 (one region), and 6 (two regions). Notably, two of these regions contained genes potentially involved in increased wax deposition on the leaf surface of the 'gummifer'. Specifically, a region on chromosome 1 comprised three tandemly duplicated copies of wax synthases, coinciding with significantly associated SNPs. Furthermore, on chromosome 5, we identified the ethylene-responsive transcription factor WIN1-like, known as a 'wax inducer'. Following analysis involved examining the expression levels of these candidate genes.

1 Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

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Acknowledgments:

This Research was financed by the Ministry of Education and Science of the Republic of Poland

Keywords:

GWAS, wild carrot, adaptation

Title:

Polymorphisms in resistance genes within breeding lines of cucumber (Cucumis sativus L.)

Author/Authors:

Magdalena Cieplak[1], Renata Słomnicka[1], Bartosz Biernacik[1], Dominika Stokowiec[1], Karolina Kaźmińska[1], Grzegorz Bartoszewski[1]

Abstract:

Cucumber is an important vegetable crop in the Cucurbitaceae family with high nutritional value and economic importance in many countries. However, its production is largely limited due to various infectious agents, including bacteria, viruses, fungi, and oomycetes (He et al, 2022). Among these, are oomycete, and fungal infections - such as downy mildew and bacterial infections caused by Pseudomonas amygdali pv. lachrymans pose considerable challenges (Call et al 2012, Słomnicka et al, 2015). Traditionally, chemical treatments have been employed to control diseases in cucumber production. However, this approach has several disadvantages, including potential effects on food safety and environmental pollution. In organic production, chemical control of diseases is limited, making disease management particularly challenging. To address this issue breeders aim to develop cucumber cultivars with genetically determined resistance, suited for organic production. Resistance breeding in plants relies on monogenic resistance and the introduction of resistance genes into elite breeding material. In the case of cucumber, there is not much data on resistance genes, and only a few candidate resistance genes have been identified so far. In this study, we analyzed the polymorphisms within major disease-resistance genes in cucumber breeding lines using genome resequencing data. Understanding the genetic variability of resistance genes is crucial for successful disease-resistance breeding. This study contributes to the broader goal of sustainable cucumber production.

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References:

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3. Slomnicka, R., Olczak-Woltman, H., Bartoszewski, G., & Niemirowicz-Szczytt, K. (2015). Genetic and pathogenic diversity of Pseudomonas syringae isolated from cucurbits. European J Plant Pathol 141:1–14

Acknowledgments:

This study was conducted as part of the InnoSeed PROW Współpraca project, financially supported by the Polish Agency for Restructuring and Modernisation of Agriculture.

Keywords:

Cucumis sativus, disease, resistance genes, cucumber

Title:

Phosphorus tolerance variation in rye (Secale cereale L.) inbred lines

Author/Authors:

David Chan-Rodriguez[1], Brian Wakimwayi Koboyi[1], Leszek Bolibok[2], Julia Maksymiuk[1], Hanna Bolibok-Brągoszewska[1]

Abstract:

Low-phosphorus availability soils, and especially acidic soils pose a significant challenge to agricultural production[1,2]. Rye (Secale cereale L.) is a cereal with a high tolerance to abiotic stresses, including phosphorus deficiency. Rye's resilience to low-nutrient stresses makes this grass a good model for studying the genetic basis of phosphorus-deficiency tolerance and discovering loci contributing to phosphorus-deficiency resilience. Our research aims to select rye genotypes tolerant and sensitive to phosphorus deficiency for further analyses at the transcriptome, proteome, and methylome levels.

We screened various rye inbred lines to identify genotypes with contrasting phenotypes under phosphate-deficient conditions in a hydroponic-growing system. We measured fresh and dry weight and inorganic phosphorus content in shoots and roots. In our screening, the total dry weight (shoot + root dry weight) of the rye inbred lines showed a wide range of variation in phosphate-sufficient conditions. However, the rye inbred lines did not show significant differences in the total dry weight under phosphorus deficiency suggesting that phosphate deficiency affects the total dry weight in every tested rye genotype similarly. Regarding inorganic phosphorus content in shoots, we observed that rye inbred lines fall into two groups - high and low inorganic phosphate content classes - under control conditions. Under phosphate deficiency, rye genotypes fell into four groups, with L9 and K3 inbred lines showing significant differences. These two inbred lines exhibited contrasting inorganic phosphate accumulation in shoots; the L9 line contains higher phosphate than the K3 line. We selected the L9 and K3 rye inbred lines for comparative transcriptomics. Our work will aid in identifying genes conferring high inorganic phosphate in shoots during low-phosphate regimes.

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References:

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Acknowledgments:

This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

Keywords:

Secale cereale, inbred lines, hydroponic-growing system, comparative transcriptomics, phosphorus-deficiency tolerance

Title:

The role of FLA7 (Bradi3g39740) in Brachypodium distachyon in salt stress response and plant development

Author/Authors:

Hilke Wittocx[1], Artur Pinski[1], Manfred Beckmann[2], Luis Mur[2], Bozena Skupien-Rabian[3], Urszula Jankowska[3], Robert Hasterok[1], Elzbieta Wolny[1]

Abstract:

The fasciclin-like arabinogalactans (FLAs) are cell wall glycoproteins that belong to the diverse family of hydroxyproline-rich glycoproteins that are expressed during different stages of plant development and are upregulated in response to stresses. In Arabidopsis thaliana, for instance, the disruption of FLA4 (also known as SOS5) leads to heightened sensitivity to salt stress, manifesting in swollen root cells, while fla1 mutants exhibit defects in shoot regeneration. However, the precise functions of FLAs, particularly in monocots, remain largely unexplored. In Brachypodium distachyon Bd21, a model species for temperate C3 grass crops, 23 genes encoding FLAs were identified. Within this research, we are focusing on FLA7 (Bradi3g39740) and its role in plant development and stress response.

The inactivation of the FLA7 gene led to decreased germination efficiency and root length compared to the wild-type while exposed to salt stress. The proteomics revealed similar behavior of fla7 and wild-type plant roots in response to the salt stress. Still, the upregulation of the stress-related genes, like late embryogenesis abundant protein (dehydrins) and cupins was more pronounced in the wild-type. The metabolomics showed perturbations in polyphenols, phytochromes and amino acids in response to salt stress. We also observed differences in the size of the seeds, with fla7 being shorter and slightly wider than wild-type seeds. The metabolomics of seeds revealed a decrease in polyphenols, jasmonic acid biosynthesis pathway and glutathione pool accompanied by an increase in fatty acids in fla7.

Our results show the vital role of the FLA7 in B. distachyon in seed and root development as well as the salt stress response. Our ongoing efforts aim to elucidate the molecular mechanisms underlying FLA7 activity.

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References:

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Keywords:

Brachypodium distachyon, Fasciclin-like arabinogalactan protein, plant development, metabolomics, proteomics

Title:

An attempt to use the GBS-t markers for mapping minor genes that restore male fertility in rye (Secale cereale L.) with the Pampa sterility-inducing cytoplasm

Author/Authors:

Wojciech Wesołowski[1], Anna Bienias[2], Beata Domnicz[1], Marek Szklarczyk[1], Stefan Stojałowski[2]

Abstract:

Restoration of male fertility of plants with CMS-Pampa stays under control of several genes. Major gene (or genes) has been mapped on the 4RL chromosome (Miedaner et al. 2000; Stracke et al. 2003; Hackauf et al. 2017) and the existence of some undefined number of minor restorers has been reported (Miedaner et al. 2000). The aim of our study was to apply the GBS-t method for mapping of minor restorer genes in hybrid rye with Pampa cytoplasm.

The plant material consisted of 165 individuals of the unregistered three-way F1 cultivar SMH604 (provided by I. Kolasińska) revealing high efficiency in pollen production. Among tested SMH604 population we identified only one fully sterile plant. One hundred randomly chosen individuals revealing phenotypic variation were used in further research. The mRNA was isolated from leaves, then obtained cDNA libraries were pair-end (PE150) sequenced at low coverage (app. 1Gb per sample) using Illumina platform. For identification of markers correlating with pollen production, two groups of individuals were selected: first contained 10 extremely fertile and the second one 10 almost sterile plants. The bioinformatic analysis of these combined two datasets was performed using the QTL-seq method. As an equivalent of maternal parent in mapping population, the only one male sterile plant was used, according to procedure of informatic analysis elaborated exclusively for this project by Wesołowski (unpublished). As a genomic reference the Gene Bank sequence coded GCA_016097815.1 was used. Statistically significant GBS-t markers were mapped on 6 chromosomes of rye. Only one chromosome did not contain minor restorer genes and it was 4R, where the major restorer (or restorers) is localized. The results are based on one mapping population which was phenotyped in only one environment, nevertheless, it shows that the GBS-t method combined with appropriate bioinformatics may be a valuable tool for genomic analyses in crop breeding.

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References:

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Acknowledgments:

This research was supported financially by Polish Ministry of Agriculture and Rural Development under the Programme of Basic Research for Biological Progress in Plant Production (task 10)

Keywords:

rye, GBS-t markers, cytoplasmic male sterility

Title:

Mitotypes of triticale materials (xTriticosecale Wittmack) with the male sterility-inducing cytoplasm of Aegilops sharonensis

Author/Authors:

Magdalena Simlat[1], Katarzyna Cetnarowska[1], Alicja Matera[1], Marek Szklarczyk[2]

Abstract:

Cytoplasmic male sterility (CMS) manifests in the lack of production of viable pollen. It often occurs as a result of interspecies crossing, when the cytoplasm of one species is combined with the nucleus of another species. CMS can be suppressed by the appropriate nuclear genes that restore male fertility (Rf). Rf genes in most cases encode RNA-binding pentatricopeptide repeat (PPR) proteins. These proteins are imported into the mitochondria where they prevent the accumulation of either transcripts or proteins encoded by the CMS-inducing ORFs (Open Reading Frames) (Melonek et al. 2021). However, there is no universal mechanism for maintaining sterility and restoring male fertility in different cytoplasms.

In this study we have analyzed the molecular background for different phenotypes of triticale lines with the Aegilops sharonensis cytoplasm (Warzecha and Góral 2009). Three tested lines expressed complete male sterility and another two lines expressed full male fertility. To analyze their mitotypes we used SCAR markers for mitochondrial genes cox1 and nad6 (Stojałowski et al. 2006). The obtained PCR products were sequenced, and the obtained sequences were subjected to bioinformatic analysis.

The size of the amplified PCR products co-segregated with the male fertility/sterility phenotype of the tested lines. In the CMS lines, the size of the PCR product for the cox1 gene was approximately 0.89 kb, while in the lines with restored fertility, it was 0.94 kb. Similarly, for the nad6 gene: in the CMS lines a single product with the size of 0.85 kb was observed, while in the lines with restored fertility three products were obtained with their size in the range of 0.78-1.0 kb. Similarity between sequences from the triticale CMS lines and the corresponding sequences from the rye Pampa cytoplasm was observed. Based on these results we can hypothesize that the cox1 and nad6 sequences of Ae. sharonensis were modified in response to the presence of specific restorer genes.

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2 University of Agriculture in Krakow, Department of Plant Biology and Biotechnology

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Acknowledgments:

The authors would like to thank mgr Krystyna Gołąb (Department of Plant Breeding, Physiology and Seed Science) for her excellent technical assistance during field experiments.

Keywords:

cytoplasmic male sterility (CMS), mitochondrial polymorphisms, triticale

Title:

Bioinformatic Prediction of the Localization of LHY Transcription Factor Binding Sites and Functional Analysis of its Target Genes in Carrot

Author/Authors:

Kinga Zygmuntowicz[1], Alicja Macko-Podgórni[1]

Abstract:

The Earth's rotational movement induces fluctuations in light, temperature, humidity, and herbivore activity. Plants have developed an endogenous circadian clock optimizing their metabolism and physiology in response. This clock governs processes like photosynthesis, growth, stomatal aperture regulation, starch accumulation, and flowering, operating through negative feedback involving clock and effector genes. Clock genes form feedback loops regulating the circadian rhythm, while effector genes transmit signals to physiological processes. LHY is a key component of the clock, peaking in expression early in the morning.

This analysis aimed to predict LHY binding sites, target genes, and their functional annotation in carrot.Two carrots genome assemblies (DH and Kuroda) underwent motif searches using FIMO from Meme Suite. Two motifs were used: the canonical LHY binding motif from Arabidopsis thaliana (MA1185.1) from the Jaspar database, and the carrot-specific LHY binding motif identified in previous studies using the DAPseq method. Genes with predicted TFBSs in their promoters (2 kb upstream CDS) were extracted and annotated using Kobas with the KEGG database.

The total number of predicted LHY binding sites using the canonical motif in both DH1 and Kuroda genomes was approximately 110,000. Slightly more sites with the carrot-specific motif were found in DH1 (108,000) than in Kuroda (88,000). The carrot-specific LHY motif appears more specific to DH. Approximately 55% of all identified motifs were common to DH and Kuroda. For each analysis, about 10% of motifs were located in promoters. Functional analysis revealed enrichment of genes responsible for circadian rhythm, biosynthesis of compounds (zeaxanthin, carotenoids, flavonoids), amino acid biosynthesis, photosynthesis, and homologous recombination.

Acknowledgments:

This Research was financed by the Ministry of Education and Science of the Republic of Poland.

Keywords: LHY, carrot, TFBS, circadian rhythm

1 Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Title:

Cytological and morphological evaluation of interspecific hybrids of highbush blueberry (Vaccinium corymbosum L.) and bilberry (Vaccinium myrtillus L.)

Author/Authors:

Małgorzata Podwyszyńska[1], Agnieszka Marasek-Ciołakowska[1], Monika Markiewicz[1], Katarzyna Mynett[1]

Abstract:

The aim of the research is to obtain interspecific hybrids of a tetraploid cultivated species, highbush blueberry, and a wild diploid species, bilberry, characterized by new valuable features (Podwyszyńska et al. 2021). Plants obtained from crosses of both species, with hybrid status confirmed using ISSR/SSR molecular markers, were assessed in terms of morphological and cytological characteristics. The hybrids were characterized by a much smaller number of hairs on the adaxial surface of the leaves compared to highbush blueberry leaves and an intermediate type of serration of the leaf edges (in bilberry the leaves are much more serrated and only single hairs are visible on their surface). The structure of the stomata was similar in both species and in the hybrids. The number of flowers in the inflorescence was smaller than in highbush blueberries, and the flowers in individual hybrids showed different morphology. The fruits of some hybrids had undeveloped seeds. In the following years, it is planned to evaluate interspecific hybrids in terms of their suitability for breeding new improved cultivars of highbush blueberry.

References:

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Acknowledgments:

The work was supported by the Ministry of Agriculture and Rural Development within the programme 'Basic research for the biological progress in plant production – task 45'

Keywords:

Vaccinium corymbosum, Vaccinium myrtillus, interspecific crossing, plant morphology

1 The National Institute of Horticultural Research

Title:

DEVELOPMENT OF WHEAT GENOTYPES WITH GENES CONFERRING RESISTANCE AGAINST HIGHLY VIRULENT RACES OF STEM RUST BY MARKER-ASSISTED SELECTION

Author/Authors:

Yaroslav Pirko[1], Nataliya Kozub[1], Anatoliy Karelov[1], Oksana Sozinova[1], Anastasiya Rabokon[1], Olena Shysha[1], Rostyslav Blume[1], Anna Kvasko[1], Ihor Sozinov[1], Alla Yemets[1]

Abstract:

Stem rust (pathogen - Puccinia graminis f. sp. tritici) is one of the most dangerous diseases of wheat. Until recently, due to the widespread introduction of stem rust genes (Sr genes), the threat to world wheat production by stem rust was not substantial. However, the emergence of new UG99 races in Africa, and TKKTP and TTRTF races in Europe, changed the situation. Own race-specific resistance genes of wheat are commonly not effective against modern stem rust races. Numerous introgressive genes are effective against most P. graminis races. Still, many have been transferred from distant relatives with chromosome fragments carrying genetic material that affects the agronomical traits of wheat. The Sr33, Sr39, and Sr40 genes are considered effective against stem rust races, including UG99 races. Therefore, the study aimed to develop the breeding wheat material with genes conferring resistance to highly pathogenic races of stem rust using molecular markers.

For this, we analyzed progeny from crosses of winter wheat cultivars with spring lines carrying Sr33, Sr39, or Sr40 genes, which derive from Aegilops tauschii. STS marker Sr33A (Ivashchuk et al, 2018) was used to detect Sr33 and Sr39#22 (Mago et al, 2009), and MS markers - for Sr39 and Sr40. Alleles at the gliadin loci Gli-A1, Gli-B1, Gli-D1, and high-molecular-weight glutenin subunit loci Glu-A1, Glu-B1, Glu-D1 were analyzed in the parental lines. The alleles Glu-B1al, Glu-D1d, Glu-B1b, and Glu-B1i, associated with high bread-making quality, were identified among the studied lines. In particular, we developed winter bread wheat F4 and F5 lines with the Sr33 gene in combination with resistance gene Sr57 (Lr34) from crossing the winter cultivar Myrkhad with the spring line DH31 (Sr33, Sr57). Among those F5 lines, there were carriers of high-quality alleles Glu-B1al and Glu-D1d. Such material is promising for developing stem rust-resistant cultivars with high grain quality.

1 Institute of Food Biotechnology and Genomics

Acknowledgments:

The research is carried out within the framework of the NRFU project 2021.01/0313 "Development of the wheat genotypes with the genes conferring resistance to highly pathogenic races of stem rust with use of molecular markers to ensure food security of Ukraine" (2023-2025, state registration number #0123U102941).

Keywords:

Wheat, Puccinia graminis, race Ug99, stem rust resistance genes

Title:

Two chromosomal locations of fertility restorers for petaloid CMS in carrots

Author/Authors:

Ajeeth Prakash Vairamuthu[1], Ewelina Ciepłak[1], Beata Domnicz[1], Marek Szklarczyk[1]

Abstract:

Cytoplasmic male sterility (CMS) is a maternally inherited condition in which a plant is unable to produce functional pollen. In carrots (Daucus carota L.), there are two main CMS types: petaloid where stamens are substituted by petals, and the brown anther type with degenerated anthers. In both cases the effect of sterilization can be suppressed by nuclear genes referred to as fertility restorers. The aim of this study was to determine the chromosomal location of fertility restores in four analyzed carrot populations: 168, 172, 510-14 and 511. Each population segregated into male-sterile and male-fertile (restored) plants. In all these populations the petaloid type of CMS was observed. Genotyping was performed with molecular markers from chromosomes 4 and 9. In population 168 co-segregation with the phenotype was observed for three markers from chromosome 4. Depending on the used marker co-segregation ranged from 72 to 84 %. In case of population 172 only one marker showed co-segregation (67 %) with the phenotype. That marker originated from chromosome 9. The highest co-segregation value – 98 % was observed for population 510-14 genotyped with another marker from chromosome 9. None of the analyzed markers showed co-segregation with the phenotype in population 511.

Acknowledgments:

The research was funded by the Ministry of Agriculture and Rural Development – decision no. DHR.hn.802.14.2023.

Keywords:

cytoplasmic male sterility, CMS, restorer genes

1 University of Agriculture in Krakow

P3.13

Title:

Genome-wide characterisation of miniature inverted-repeat transposable elements (MITEs) polymorphisms in sugar beet (Beta vulgaris L.)

Author/Authors:

Emilia Morańska[1], Gabriela Machaj[2], Alicja Macko-Podgórni[1], Dariusz Grzebelus[3]

Abstract:

Sugar beet (Beta vulgaris L.) is an important crop in temperate climate regions and the major substrate for global sugar production. In addition, it is used as a source of bioethanol and animal feed (Dohm et al. 2014). Miniature inverted-repeat transposable elements (MITEs) are short non-autonomous DNA transposons ubiquitous in plant genomes and mobilised by the related autonomous elements. They tend to insert into genes and genic regions and therefore can alter gene expression and influence genome evolution (Nandini 2020).

In the present study, 24 resequenced genomes from two segregating sugar beet populations (P1 and P4) were used for MITEs identification and distribution analysis with bioinformatic tools. The database of MITEs contained consensus sequences representing 170 MITEs families grouped into 5 superfamilies. The reference assembly genome of sugar beet was EL10_1.0.

The total number of MITEs insertions in the P1 population was 67517 whereas for the P4 population - 74074. The number of non-reference insertions was approximately twice as high as reference insertions for both populations. The largest representation of MITEs was Stowaway-like elements in both P1 and P4 populations. The highest number of MITEs insertions was identified on the 5th chromosome while on the remaining chromosomes, it was comparable.

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Keywords:

sugar beet, transposable elements

Title:

Analysis of parameters related to water management of winter wheat (Triticum aestivum L.) under soil drought

Author/Authors:

Edyta Skrzypek[1], Marzena Warchoł[1], Ilona Czyczyło-Mysza[1], Kinga Dziurka[1], Kamila Laskoś[1], Agnieszka Ostrowska[1], Katarzyna Juzoń-Sikora[1]

Abstract:

Water availability is one of the most important factor determining plants yield. Recently occuring small amounts of atmospheric precipitation contributes to a reduction of wheat yield by up to 21% due to soil drought on a global scale.

The aim of the study was to analyze the physiological parameters related to water management in winter wheat (Triticum aestivum L.) growing under soil drought (20% field water capacity (FWC)) for a period of 21 days and under optimal irrigation conditions (70% FWC). Plant material consisted of 30 wheat cultivars growing in a vegetation tunnel. Drought tolerance was assessed in plants subjected to soil drought in the tillering phase based on the analysis of relative water content in leaves (RWC), osmotic potential of leaf cell sap (Ψo) and electrolyte leakage (EL).

The analysis of RWC differentiated wheat cultivars under soil drought stress conditions compared to optimally irrigated plants. In all wheat cultivars, a statistically significant decrease in the Ψo value was found under the influence of soil drought and it was in the range from 0.84 MPa to 1.76 MPa. Under optimal hydration, EL ranged from 6.4% to 80.2%, while in drought the value of this parameter ranged from 13.9% to 64.1%. Based on the obtained results, soil drought stress sensitivity indices (SSI) were calculated and winter wheat cultivars that better tolerate water deficits were selected. The greatest differences in drought tolerance of wheat cultivars were recorded for the SSI index calculated on the basis of WUE and EL. The lower the SSI index for WUE and EL values, the greater the plants' tolerance to drought.

Acknowledgments:

The research was funded by the Ministry of Agriculture and Rural Development in 2023, project no. 3.

Keywords: wheat, drought tolerance, water relations

1 The Franciszek Górski Institute of Plant Physiology Polish Academy of Sciences

Title:

Changes of gas exchange parameters of winter wheat (Triticum aestivum L.) under soil drought

Author/Authors:

Marzena Warchoł[1], Edyta Skrzypek[1], Ilona Czyczyło-Mysza[1], Kinga Dziurka[1], Kamila Laskoś[1], Agnieszka Ostrowska[1], Katarzyna Juzoń-Sikora[1]

Abstract:

The susceptibility of plants to soil drought depends mainly on the intensity of stress and the phase of plant development. In order to overcome drought, plants show a number of defense reactions, including: the closing of stomata and a reduction in photosynthetic activity, which is manifested in changes of gas exchange parameters in the leaves, and in extreme cases, damage to the photosynthetic apparatus.

The aim of the study was to analyze the gas exchange parameters of winter wheat cultivars (*Triticum aestivum* L.) subjected to drought stress in the tillering phase.

The plant material consisted of 30 cultivars of winter wheat growing under soil drought conditions (20% field water capacity (FWC)) for a period of 21 days and under optimal irrigation conditions (70% FWC). Drought tolerance was assessed in plants subjected to soil drought stress based on the measurement of gas exchange parameters: net photosynthetic rate (Pn), stomatal conductance (gs) and transpiration (E).

In all wheat cultivars subjected to soil drought, a statistically significant decrease in Pn values from 15.8 to 7.1 µmol (CO_2) m^{-2} s^{-1}, gs from 0.220 to - 0.073 mol (H_2O) m^{-2} s^{-1} and E from 3.8 to 1.7 mmol (H_2O) m^{-2} s^{-1} was observed. The average values of intercellular CO_2 concentration (CO_{2int}) and water use efficiency (WUE) did not differ significantly between optimally irrigated plants and plants subjected to drought. In optimal hydration, CO_{2int} was 221 µmol (CO_2) mol (air)$^{-1}$, and WUE was 4.78 µmol (CO_2) mmol (H_2O)$^{-1}$, similarly in plants subjected to soil drought, CO_{2int} was 226 µmol (CO_2) mol (air)$^{-1}$, and WUE 4.78 µmol (CO_2) mmol (H_2O)$^{-1}$.

The analysis of soil drought stress sensitivity indices (SSI) in the wheat tillering phase for all measured gas exchange parameters showed that the lower the SSI index values for CO_{2int}, the greater the plants' tolerance to drought.

Acknowledgments:

The research was funded by the Ministry of Agriculture and Rural Development in 2023, project no. 3.

Keywords: wheat, drought tolerance, gas exchange

1 The Franciszek Górski Institute of Plant Physiology Polish Academy of Sciences

Session 4

COMPUTATIONAL SPECTROMETRY AND SPECTROSCOPY

L4.O1

Title:

Highly accurate discovery of terpene synthases powered by machine learning reveals functional terpene cyclization in Archaea

Author:

Raman Samusevich[1]

Abstract:

Terpene synthases (TPS) are enzymes responsible for the biosynthesis of the core scaffolds of the largest class of natural products, including widely used flavors, fragrances, and first-line medicines. Discovering new TPSs can accelerate the progress in bioprospecting, natural product biosynthesis, and synthetic biology. However, applying state-of-the-art ML models to an enzymatic family is challenging due to a small and sparse training dataset. We assembled a curated dataset of 2.5k characterized TPS reactions and developed highly accurate machine-learning (ML) models for functional annotation from the relatively small dataset. First, we segmented AlphaFold2-generated structures into TPS structural domains. By applying unsupervised ML to the segmented structural modules, we discovered previously unreported types of the TPS domains. Then, we devised self-supervised deep learning models for TPS detection and substrate classification, taking the recognized domain types into account. Our models significantly outperform existing methods, increasing the mean average precision of TPS substrate classification from 0.69 to 0.89. By applying the models to large protein sequence databases, we discovered and experimentally confirmed the activity of seven TPS enzymes previously undetected by state-of-the-art bioinformatic tools, including the first reported TPSs in the major domain of life Archaea. This work demonstrates the potential of ML to speed up the discovery and characterization of novel TPS.

1 Institute of Organic Chemistry And Biochemisty of the Czech Academy of Sciences

Title:

Raman Optical Activity in the study of chirality and structure of bio-compounds engaged in macro- and supramolecular arrangements

Author/Authors:

Monika Hałat[1], Magdalena Klimek-Chodacka[1], Rafał Barański[1], Grzegorz Zając[2], Valery Andrushchenko[3], Petr Bouř[3], Katarzyna Pajor[4], Małgorzata Barańska[5]

Abstract:

Among chiroptical spectroscopies, Raman Optical Activity (ROA) is a perfect tool to study 3D structure of chiral compounds in water environment. The possibility to easily measure aqueous solutions of biomolecules, such as proteins, nucleic acids, polysaccharides and carotenoids, to monitor structural changes in their natural surroundings, is a great advantage over other popular methods like X-ray crystallography. ROA also gives an opportunity to reflect more directly fast conformational changes than NMR spectroscopy. Here, we present the ROA study of the Cas9 protein and its ribonucleoprotein complex (RNP) belonging to the CRISPR/Cas system1 known as the "molecular scissors" technology intended for a precise genome editing and rewarded in 2020 by the Nobel Prize in Chemistry. We show that ROA together with CPL (Circularly Polarized Luminescence) can be used to monitor a structural change in the Cas9 geometry upon biding a specifically designed gRNA molecule that modifies the protein from an inactive into an active DNA-binding conformation. Thus, both methods supported by nucleolytic activity testing and capillary electrophoresis, are able to identify in a non-destructive manner the formed active RNP complex, stabilized by electrostatic interactions. The non-covalent forces indeed play a very important role in stabilizing macro- and supramolecular structures, as well as in associated processes like chirality transfer.2 To this end, we show that mixing chiral polysaccharides (heparin, hyaluronic acid) with the achiral carotenoid canthaxanthin (CAX) leads to a unique chirality induction, with strong optical activity visible in the electronic circular dichroism (ECD) and resonance Raman optical activity (RROA) spectra.3 Interpretation of the data using density functional theory (DFT) and molecular dynamics (MD) modelling suggests an intricate supramolecular structure with multiple carotenoid–carotenoid interactions.

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References:

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Acknowledgments:

This research was funded by the National Science Centre in Poland within grant no. 2021/40/C/ST4/00190.

Keywords:

ROA, CPL, chirality induction, carotenoids aggregates, Cas9 protein

Session 5

PLANT GENETICS RESOURCES AND CYTOGENETICS

L5.O1

Title:

TurboID-based identification of a novel synaptonemal complex-related protein critical for CO patterning in Arabidopsis thaliana

Author/Authors:

Chao Feng[1], Elisabeth Roitinger[2], Otto Hudecz[2], Maria Cuacos[1], Jana Lorenz[1], Veit Schubert[1], Baicui Wang[1], Karl Mechtler[2], Stefan Heckmann[1]

Abstract:

Meiosis assures genetic variation through homologous recombination (HR) in form of crossover (CO; reciprocal genetic exchange between parental chromosomes). During prophase I, sister chromatids are arranged in a loop-base array along a proteinaceous structure called meiotic chromosome axes. Progressive chromosome axes alignment culminates in the formation of a tripartite structure physically connecting homologous chromosomes along their length during pachytene called synaptonemal complex (SC). Formation of meiotic CO depends on the interplay between HR and programmed meiotic chromosome axis remodeling including SC formation.

To unravel the composition and regulation of meiotic chromosome axes or the SC in plants, the limited number of meiotic cells embedded in floral organs constrains proteomic approaches. Embarking on the meiotic chromosome axis-associated proteins ASYNAPTIC1 (ASY1) and ASYNAPTIC3 (ASY3), we applied TurboID (TbID)-based proximity labelling in meiotic cells of *Arabidopsis thaliana*. Among identified candidates, one novel candidate was found to be part of the SC and being conserved across plants. In its absence, SC formation is disrupted and chiasmata formation is reduced while CO levels are increased and CO interference is virtually abolished. The candidate dynamically localizes at the central region of the SC, physically interacts with ZYP1 (transverse filament protein of the SC) and is together with other SC-components inter-dependent for SC assembly and synapsis.

In a nutshell, TbID-based proximity labeling enables the identification of protein proximate proteins in rare cell types such as meiotic cells in *A. thaliana*.

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2 Research Institute of Molecular Pathology (IMP), Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), The Gregor Mendel Institute of Molecular Plant Biology of the Austrian Academy of Sciences (GMI)

Title:

Cellular reprogramming processes in buckwheat from the epigenetic point of view

Author/Authors:

Katarzyna Sala-Cholewa[1], Alicja Tomasiak[1], Lea Sophie Berg[2], Katarzyna Nowak[1], Artur Piński[1], Agnieszka Brąszewska[1], Alexander Betekhtin[1]

Abstract:

Buckwheat is an abundant source of beneficial compounds, which have a positive impact on human health. Two mainly cultivated buckwheat species are common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*F. tataricum*). Despite their close relation, common and Tartary buckwheat differ in morphology, flower development, as well as the structure and development of the callus tissue. Common buckwheat characterises by flower heterostyly, possessing two floral morphs, Pin and Thrum, requiring external pollinators. Tartary buckwheat is a self-pollinating species, resulting in a higher yield. *In vitro* culture of common buckwheat exhibits traits typical for an embryogenic callus, remains stable for approximately three years and regenerates via somatic embryogenesis. Tartary buckwheat, on the other hand, has a morphogenic callus, which is able to regenerate *via* organogenesis as well as somatic embryogenesis. It is highly stable and remains at low chromosome number for over ten years of culture. These differences provide an excellent system for analysing cell dedifferentiation and differentiation.

Under *in vitro* conditions, differentiated plant cells have the ability to return to a previous developmental state (dedifferentiate) and regain pluri- or totipotency, resulting in organogenesis and somatic embryogenesis. *In vivo*, proper flower development ensures plant reproduction. These processes rely on fine-tuned machinery of transcription factors, enzymes and epigenetic modifications to function correctly. DNA methylation, an universal and heritable epigenetic mechanism, regulates gene expression and the chromatin structure.

Changes in the epigenetic modifications were examined in both, in *in vitro* callus tissues as well as *in vivo* floral organs. Conducted research shed light on the fine-tuned machinery that coordinates the correct plant development and extended our knowledge whether biological barriers can be crossed to obtain the desirable qualities of buckwheat.

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Acknowledgments:

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Keywords:

Fagopyrum sp., DNA methylation, tissue culture, flower development

Title:

Freezing temperature effects on photosystem II in Antarctic lichens evaluated by chlorophyll fluorescence

Author/Authors:

Aleksandra Andrzejowska[1], Josef Hájek[2], Anton Puhovkin[3], Hubert Harańczyk[4], Miloš Barták[5]

Abstract:

Poikilohydric autotrophic organisms living in an environment as extreme as Antarctica face the abiotic stressors of cold and dehydration on a daily basis. Therefore, Antarctic lichens seem to be ideal candidates for exploring the limits of photosynthesis at subzero temperatures.

We compared cryoresistance in six Antarctic lichens: Sphaerophorus globosus, Caloplaca regalis, Umbilicaria antarctica, Pseudephebe minuscula, Parmelia saxatilis and Lecania brialmontii combining constant-rate cooling and chlorophyll fluorescence methods.

The results revealed triphasic S-curves in the temperature response of the maximum quantum yield (F_V/F_M) and effective quantum yield of photosystem II (Φ_{PSII}) for all species. All investigated lichens showed high level of cryoresistance with critical temperatures (T_c) below −20°C. However, record low Tc temperatures have been discovered for L. brialmotii (−54°C for F_V/F_M and −40°C for Φ_{PSII}) followed by C. regalis (−52°C for F_V/F_M and −38°C for Φ_{PSII}). Additionally, the yield differentials ($F_V/F_M - \Phi_{PSII}$) in function of temperature revealed one or two peaks, with the larger one occurring for temperatures below −20°C for the above-mentioned species.

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Finally, Kautsky kinetics were measured and compared at different temperatures (20°C, 10°C, 0°C and −10°C and then −10°C after 1 h of incubation).

These discoveries challenge our beliefs on what the limits of photosynthesis are and serve as a foundation for further developing investigations into the biophysical mechanisms by which photosynthesis is carried out at subzero temperatures.

Acknowledgments:

The authors thank the projects related to the CzechPolar infrastructure (VAN2022, VAN2023, ECOPOLARIS - CZ.02.1.01/0.0/0.0/16_013/0001708, the Czech Ministry of Education, Sports and Youth) for providing field and laboratory facilities for the research reported in this study. The research was supported by Research Support Module no. RSM/34/AA as a part of Excellence Initiative of the Jagiellonian University. Participation in the conference was co-funded by the Ph.D. Students' Association of JU.

Keywords:

Antarctic lichens, cryoresistance, ChlF, linear cooling

P5.1

Title:

Phenotypic assessment of tetraploids of sweet cherry (Prunus avium L.)

Author/Authors:

Aleksandra Trzewik[1], Monika Marat[1], Małgorzata Grzelak[1]

Abstract:

Polyploidization is a tool widely used in the breeding programs of many crop plants. After doubling the number of chromosomes, favorable traits are often enhanced as a result of increasing the genes that determine the desired traits. By crossing various valuable tetraploids, beneficial traits can be accumulated in hybrids. For cherry, desirable traits include resistance of trees to bacterial canker (*Pseudomonas syringae*) and differentiated fruit ripening time. Treating axillary shoots in in vitro cultures with antimitotics, we obtained tetraploids for two sweet cherry cultivars 'Merton Premier' and 'Tamara'. The aim of this study was to phenotypically assessment of tetraploid of sweet cherry compared to their diploid counterparts under greenhouse conditions. Observations and measurements were carried out on two tetraploid lines of sweet cherries in the 'Tamara' cultivar (B3/6-4x, H13-4x) and three tetraploid lines in the 'Merton Premier' cultivar (C5-4x, C7-4x, D4-4x). Plant height, number of internodes, shoot diameter at the base, chlorophyll content index were determined. Microscopic observations of the number and length of stomata were also made.

Control plants of 'Tamara' and 'Merton Premier' cultivars had the highest growth rate, number of internodes and shoot diameter compared to tetraploids. The chlorophyll content index in the tetraploid genotypes B36-4x and H13-4x after two months of growth in the greenhouse was 20.4 and 21.3, respectively, and was higher compared to the diploid counterpart (15.9).

In tetraploid genotypes of the 'Tamara' and 'Merton Premier' cultivar, the length of the stomata was greater compared to the control. In contrast, the density of stomata in tetraploids was lower compared to the control.

Acknowledgments:

This research was supported by the Ministry of Agriculture and Rural Development as grant for Biological Progress in Crop Production (No 46)

Keywords: Polyploidization, antimitotics, tetraploid, Prunus avium

1 The National Institute of Horticultural Research

Title:

Efficient transfer of Rht semi-dwarf genes from wheat (Triticum aestivum L.) to triticale (xTriticosecale) in current breeding materials.

Author/Authors:

Małgorzata Niewińska[1], Monika Hanek[1], Piotr Kaźmierczak[1], Danuta Kurleto[1], Aleksandra Fornalczyk[1], Mirosław Pojmaj[1], Bogusława Ługowska[1], Jerzy Bogacki[1], Waldemar Brukwiński[1], Katarzyna Banaszak[1], Renata Krysztofik[1], Agnieszka Katańska-Kaczmarek[1], Eugeniusz Paszkowski[1], Hanna Bielerzewska-Kaźmierczak[1], Jacek Kaczmarek[1], Piotr Urbańczyk[1], Jerzy Nawracała[2], Michał T. Kwiatek[2]

Abstract:

Winter triticale (*xTriticosecale*) is one of the most widely grown cereals in Poland. An important factor determining the high yield of this cereal is resistance to lodging. Longer straw varieties may have a tendency to lodging. To prevent this, Danko Hodowla Roślin Sp. z o.o. in his triticale breeding program introduces *Rht* semi-dwarf genes by crossing with winter wheat (*Triticum aestivum L.*). These genes are designed to shorten the straw length and eliminate lodging problems. In winter wheat cultivars selected for crossing, the *Rht-B1b (Rht1)*, *Rht-D1b (Rht2)* and *Rht 8* genes were identified.

In 2020, using molecular markers, 152 genotypes of triticale were tested for the presence of *Rht* genes. The *Rht-B1b (Rht1)* allele was identified in 142 studied breeding lines. The products characteristic for the *Rht8* gene were identified in 45 genotypes, and among these 45 genotypes, 5 had the allele (192bp) determining the semi-dwarf trait. The use of the marker *Xgwm312* to identify the *Rht21* gene resulted in high polymorphism. 13 types of bandings with different sizes of the PCR product (bp) were characterized. The indicated genotypes were evaluated in terms of height. It was 15-25 cm lower than the paternal variety Borowik and 5 cm lower than the paternal variety Kasyno. In relation to the standards cultivars in F5 trials, the height of selected breeding lines was lower from 5 cm to 15 cm. A 10 genotypes are continued in further trials (F6). All of them have the presence of the *Rht-B1b (Rht1)* allele, confirmed by molecular test. One breeding line have additionally the *Rht8 allele (192bp)*.

The genotypes with identifies breeding lines were sown in plots. Currently in all generations 64 genotypes with Rht genes are continued in triticale breeding program.

1 Danko Hodowla Roślin Sp. z o.o.
2 Poznan University of Life Sciences

Acknowledgments:

Project under the title „New plant variety breeding - modern varieties of selected species of cereals and pea, based on innovative biotechnological methods" co-financed by the European Regional Development Fund under the Smart Growth Operational Programme 2014-2020, Action 1.1 R&D projects of enterprises, Agreement No.POIR.01.01.01.-00-1363 / 15, years of research 2016-2022.

Keywords:

Rht genes, Triticale, breeding, cereals

P5.3

Title:

Evaluation of grass drought tolerance by means of multi-trait analysis

Author/Authors:

Grzegorz Żurek[1], Danuta Martyniak[1], Gabriela Skowron[1], Krystyna Rybka[1], Agnieszka Niedziela1, Monika Żurek1

Figure captions:

Fig. 1. Results of the best-performing varieties after 3 years of observations.

Abstract:

The threat to crop production from droughts is now a primary concern. At the same time, it is essential to maintain the competitiveness of domestic plant breeding. The solution to both of these issues is, among other things, to reduce production inputs through the introduction of genotypes with increased tolerance to periodic droughts. Therefore, an attempt was made to develop and verify methods for selecting breeding materials with increased drought tolerance using the example of ryegrasses (Lolium sp.), among others. In 2021- 2023, 60 varieties within the ryegrass genus were studied, taking into account the following traits: electrical capacity of the root system (RC, Cseresnyés et al. 2018), chlorophyll fluorescence parameters (Fv, Fm, Fv/Fm, Tfm), relative chlorophyll content (CCI), plant condition under simulated drought and regrowth after drought. The results of the average values of each trait in each year for each variety within 5 variety groups (Lolium multiflorum, L. hybridum, L. perenne, fodder varieties, L. perenne, turf varieties), obtained in successive years, were transformed into ranks, with the assumption that a value of 1 is the closest to the expected (desired) value and 0 is the lowest value within a given variety group. Based on the average ranks from each year of the study, the mean overall ranks (MR) were calculated for individual objects within each group. Objects were distinguished based on (a) MR values above 0.50; (b) the number of years in which the form obtained high rank values, i.e. above 0.70 (L). It was assumed that for the best varieties, the value of the MR x L should be in the range of 2.0 to 3.0. Varieties with a value of this product in the range of 1.0 - 1.9 can be considered good. Objects with a value of the product of 0.0 can be considered completely unresponsive to periodic water shortages.

A total of 13 varieties were selected out of 60 tested, including 5 domestic varieties (Fig.1).

1 Plant Breeding & Acclimatization Institute, National Research Institute

References:

1. Cseresnyés I, Szitár K, Rajkai K, Füzy A, Mikó P, Kovács R, Takács T (2018) Application of Electrical Capacitance Method for Prediction of Plant Root Mass and Activity in Field-Grown Crops. Frontiers in Plant Science 9. DOI: 10.3389/fpls.2018.00093.

Acknowledgments:

experiments were financially supported by the Polish Ministry of Agriculture and Rural Areas, under the Target grant No 2-3-00-0-05, Task 3.5 “Selection of forms of cultivated plants of increased resistance for seasonal water deficits”. Our great thanks to Kamil Prokopiuk, PhD, our former colleague, for his generous help during project performance in 2021.

Keywords:

drought, grasses, breeding, Lolium perenne, roots

Session 6

STRUCTURAL BIOINFORMATICS

L6.O1

Title:

The Encyclopedia of Domains'

Author:

Nicola Bordin[1]

Abstract:

The AlphaFold Protein Structure Database (AFDB) contains full-length predictions of the three-dimensional structures of almost every protein in UniProt. Because protein function is closely linked to structure, the AFDB is poised to revolutionise our understanding of biology, evolution and more. Protein structures are composed of domains, independently folding units that can be found in multiple structural contexts and functional roles. The AFDB's potential remains untapped due to the difficulty of characterising 200 million structures. Here we present The Encyclopedia of Domains or TED, which combines state-of-the-art deep learning-based domain parsing and structure comparison algorithms to segment and classify domains across the whole AFDB. TED describes over 370 million domains, over 100 million more than detectable by sequence-based methods. Nearly 80% of TED domains share similarities to known superfamilies in CATH, greatly expanding the set of known protein structural domains. We uncover over 10,000 previously unseen structural interactions between superfamilies, expand domain coverage to over 1 million taxa, and unveil thousands of architectures and folds across the unexplored continuum of protein fold space. We expect TED to be a valuable resource that provides a functional interface to the AFDB, empowering it to be useful for a multitude of downstream analyses.

1 University College London

Title:

Structural and functional diversity across large protein databases

Author/Authors:

Paweł Szczerbiak[1], Łukasz Szydłowski[1], Witold Wydmański[2], Tomasz Kosciolek[1]

Abstract:

Since the advent of AlphaFold and ESMFold, we have gained access to huge databases of protein structure predictions (over 800 million) that have surpassed the capabilities of traditional tools used till now to analyze a relatively small set of models (approx. 200,000) deposited in the Protein Data Bank. This catalyzed the development of highly scalable tools such as Foldseek, Foldcomp, and ProteStAr, enabling us to explore the full potential of the AlpahFold database (AF-DB) and ESMAtlas. Our work comprehensively examines the structural clusters obtained from the AlphaFold-Database (AF-DB), a high-quality subset of ESMAtlas (hclust30), and the Microbiome Immunity Project (MIP). While AF-DB, based on Uniprot, consists of protein structure predictions extensively studied over the years (with a significant eukaryotic component), the latter two databases contain only bacterial and archaeal proteins derived mainly from metagenomic studies. This provides a unique opportunity to understand the differences between these realms regarding their structural diversity. In addition, we elucidate the functional coverage of these databases by showing divergences and finding functional blind spots. Finally, we identify regions in the structural space that still need to be explored (annotated) because they are not well represented in Uniprot (AF-DB). Our findings lay the groundwork for more in-depth studies concerning protein sequence-structure-function relationships, where various biological questions can be asked concerning taxonomic assignments, environmental factors, or functional specificity, to name a few.

Acknowledgments:

This work has been funded by the National Science Centre, Poland grant 2023/05/Y/NZ2/00080.

Keywords:

Protein structure, protein function, AlphaFold, ESMAtlas, Microbiome Immunity Project

1 Sano Centre for Computational Medicine
2 Małopolska Centre of Biotechnology, Jagiellonian University

Title:

Sequence-Based Thermostability Prediction for Proteins

Author/Authors:

Adam Sułek[1], Jakub Jończyk[2], Tomasz Kościółek[1]

Abstract:

Protein thermostability is a critical feature for bioengineered proteins, with significant scientific and industrial applications. However, achieving thermostable proteins can be both costly and complex. In this study, we explore sequence alignment analysis to identify key sequence changes that contribute to protein thermostability. Furthermore, recent advances in Protein Language Models (pLM) provide a promising framework for sequence-to-sequence analysis in this domain.

We compare different protein language model embeddings and examine their interpretability to identify significant features associated with protein stability. To accomplish this, we used a dataset containing 30,000 protein sequences with known melting temperatures. Our findings demonstrate the potential of pLM to enhance our understanding of protein thermostability, paving the way for the rational design of enzymes for various applications.

Figure captions:

Using a Pre-Trained pLM Autoencoder's Latent Space in a Downstream Task

References:

1. Jarzab, Anna, et al. "Meltome atlas—thermal proteome stability across the tree of life." *Nature methods* 17.5 (2020): 495-503.

2. Jung, Felix, et al. "DeepSTABp: a deep learning approach for the prediction of thermal protein stability." *International Journal of Molecular Sciences* 24.8 (2023): 7444.

3. Ku, Tienhsiung, et al. "Predicting melting temperature directly from protein sequences." *Computational biology and chemistry* 33.6 (2009): 445-450.

1 Sano – Centre for Computational Personalised Medicine
2 Jagiellonian University Medical College

Acknowledgments:

The work was created within the project of the Minister of Science and Higher Education ”Support for the activity of Centers of Excellence established in Poland under Horizon 2020” on the basis of the contract number MEiN/2023/DIR/3796. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 857533.

Keywords:

protein thermostability , protein language models, transfer learning , ProtTrans

Title:

Three longitudinal regimes of the human gut microbiome

Author/Authors:

Zuzanna Karwowska[1], Paweł Szczerbiak[2], Tomasz Kościółek[2]

Abstract:

Despite the majority of microbiome studies being cross-sectional, it is widely acknowledged that the microbiome is a dynamic ecosystem.

Here, we analyse how the gut microbiome changes over time as a community, how different bacterial species behave over time, and whether there are clusters of bacteria that exhibit similar fluctuations?

We show that a healthy human gut microbiome is stationary, seasonal, and non-random. Moreover, we demonstrate that it is self-explanatory to some extent, and its behavior can be predicted.

The analysis of individual bacterial species uncovered the existence of three distinct longitudinal regimes in the healthy human gut microbiome. These regimes consist of 1) stationary and highly prevalent bacteria that exhibit resistance to environmental changes; 2) volatile bacteria that exhibit dynamic reactions to external stimuli, causing their presence to fluctuate over time; and 3) white noise. Clustering analysis revealed the presence of taxonomically diverse bacterial groups that exhibit similar fluctuations over time.

In conclusion, our study highlights the importance of longitudinal data and provides new insights into the dynamics of the healthy human gut microbiome. We offer clear guidelines for clinicians and statisticians who conduct longitudinal studies and develop models to predict the behavior of the gut microbiome over time.

Keywords:

Microbiome, computational biology, time series

1 Małopolska Center of Biotechnology
2 SANO

Title:

GO2COG: Mapping Gene Ontology (GO) IDs to Clusters of Orthologous Genes (COG) categories.

Author/Authors:

Lukasz Szydlowski[1], Pawel Szczerbiak[1], Tomasz Kosciolek[1]

Abstract:

The rapid expansion of genomic databases necessitates robust tools for functional annotation interoperability. This work presents a novel tool designed to map Gene Ontology (GO) terms to one of the 26 Clusters of Orthologous Genes (COG) categories, as maintained in the NCBI database. The COG categories have been further aggregated into three SuperCOG groups: Cellular Processing and Signaling, Metabolism, and Information Storage and Processing. This hierarchical aggregation facilitates the analysis of large datasets, enhancing the efficiency and accuracy of functional annotations.

The integration of GO terms with COG categories addresses the critical need for interoperability within the computational biology community, particularly as genomic databases continue to grow in size and complexity. Furthermore, aggregating COG categories into SuperCOG groups offers several benefits for large-scale genomic analysis, such as simplified data interpretation, which helps in identifying overarching functional trends and patterns across different genomes. Both COG and SuperCOG groups aid in comparative genomics by enabling more straightforward comparisons between different organisms. High-throughput genomic analyses benefit from the aggregation of metagenomic data into COG/SuperCOG groups, as it allows for the automation of data processing and analysis. This is crucial for handling the vast amounts of data generated by modern sequencing technologies, enabling researchers to perform large-scale studies more effectively. Overall, the GO2COG enhances the efficiency, clarity, and scalability of genomic analyses, making it a valuable approach for researchers working with extensive genomic datasets.

Keywords:

Gene Ontology, Clusters of Orthologous Genes, Functional Annotation, Metagenomics

1 Sano Science

Title:

link between amyloid-related diseases and functional amyloids

Author/Authors:

Jakub Wojciechowski[1], Alicja Wojciechowska[2], Kinga Zielińska[3], Johannes Soeding[4], Tomasz Kościółek[1], Małgorzta Kotulska[2]

Abstract:

Amyloids are insoluble protein aggregates with a cross-beta structure which are traditionally associated with disorders such as Alzheimer's or Parkinson's disease. In the recent two decades discovery of functional amyloids revolutionized our perception of protein aggregation. Functional amyloids are present across different kingdoms of life and execute a grand variety of roles from biofilm stabilization and construction, through signaling, and even support of melanin production. Multiple examples of amyloid-producing bacteria inhabit the human microbiome. The by now undisputed link between health and gut microbiota has focussed attention on the potential role of functional amyloid in diseases, especially neurodegenerative ones. In this work, for the first time, we computationally analyze the human microbiome in the context of functional amyloids. We show their grand diversity in gut microbiota across different taxonomic units and frequent presence in the extracellular space. We predict interactions between gut microbiome functional amyloids and human proteins and observe their potential to trigger inflammation and affect transport and signaling processes, similarly to pathological amyloids. Finally, we perform a metagenomic study and find a greater abundance of functional amyloids in diseased patients than in healthy controls. Our results provide a rationale for the suspected existing link between amyloid-related diseases and functional amyloids. The exact underlying molecular pathways may include inflammation triggering or amyloid interactions.

Keywords:

MIcrobiome, Proteins, Amyloids

1 SANO
2 Politechnika Wrocławska
3 Małopolskie Centrum Biotechnologii
4 Max Planck Institute for Multidisciplinary Sciences

Session 7

SOIL AND PLANT METAGENOMICS

L7.O1

Title:

Exploration of the wheat endosphere mycobiome crucial for the functioning of plant genome

Temat:

Soil and plant metagenomics

Author/Authors:

Lidia Błaszczyk[1]

Abstract:

Understanding the entire species composition of fungi inhabiting the inner tissues of wheat and understanding the complex interactions of endophytes with wheat plants could contribute to the identification of symbiotic, plant-beneficial strains that could be used to improve the resistance of wheat plants to biotic and abiotic stresses or as bio-stimulants of growth and yielding of this cereal in modern plant cultivation management systems. Based on the classical approach and high-throughput sequencing, the structure of fungal communities inhabiting the endosphere of 10 Polish wheat cultivars was assessed in four types of plant organs (grain, stem, leaf, root) and in 3 growing conditions - greenhouse / controlled and field with two management systems. In total, from 119 trials, 726 endogenous fungal isolates were obtained, which were identified at the genus or species level (barkoding DNA), and 220 operational taxonomic units (OTU). Metadata analysis showed that the wheat microbiome is shaped mainly by the type of plant organs and plant growth conditions, and tillage affects the number of fungi to a greater extent than the structure of fungal communities, while the influence of host genotype and wheat form on the wheat endosphere is undetectable. It was observed that the wheat endosphere is dominated by representatives of the classes Dothidiomycetes, Sordariomycetes and Eurotiomycetes, and the core microbiome in wheat is composed of fungi from the genera *Cladosporium*, *Penicillium*, *Sarocladium* and *Fusarium*. It has also been confirmed, that fungi known from the literature as beneficial and pathogenic co-occur in the wheat endosphere. Metabolome and transcriptome analyzes proved that the reaction of wheat to inoculation with endogenous fungi depends on the wheat variety and fungal species. Most importantly, wheat plants have been authenticated as a valuable source of potential biological control and growth stimulants.

1 INSTITUTE OF PLANT GENETICS, POLISH ACADEMY OF SCIENCES

Acknowledgments:

NSC, OPUS 14, no. 2017/27/B/NZ9/01591 and OPUS 24, no 2022/47/B/NZ9/01282

Keywords:

fungi, endophytes, MiSeq Illumina, DNA barcoding, ITS

Title:

Exploring the role of soil microbiota in potato blackleg and soft rot incidences

Author:

Weronika Babińska-Wensierska[123]

Abstract:

Potato crops worldwide face threats from various pathogens, with *Pectobacterium* and *Dickeya* species being among these. These infections lead to rapid tissue decay and significant yield losses, with annual global crop losses exceeding $420 million, significantly impacting food security and agricultural productivity. Since 1982, scientists have speculated that certain soil bacteria may inhibit the development of these diseases. Therefore, this study investigates the physicochemical properties and bacterial microbiota of soils from fields with long-term differences in the disease incidences triggered by *Dickeya* spp. and *Pectobacterium* spp.-related infections.

Analysis of the soil samples from potato fields, which experience varying levels of blackleg and soft rot diseases, showed that most of the physicochemical properties we examined, 13 out of the total we tested, were the same regardless of whether the disease occurrence was high or low. Only four deviations were observed in relation to the contents of magnesium (Mg), manganese (Mn), organic carbon (C), and organic substances. Through methods involving both microbial culture techniques and molecular diagnostics, we were able to detect 20 different strains of the bacterium *Pectobacterium* spp. in the field where there was a high diseases incidence. In addition, our research utilized 16S rRNA gene amplicon sequencing, identifying significant taxonomic differences in the microbiota of the studied fields. We noticed that bacteria from the genera *Bacillus*, *Rumeliibacillus*, *Acidobacterium*, and *Gaiella* were more prevalent in soil samples from the field with a lower occurrence of pectinolytic bacterial infections compared to the field with a higher frequency of such infections.

1 Laboratory of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk

2 Research and Development Laboratory, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk

3 Laboratory of Physical Biochemistry, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk

This comprehensive analysis, supported by long-term monitoring and metagenomic data, confirms that the microbial composition of soil is a crucial factor in the prevalence and management of potato blackleg and soft rot diseases. Ongoing research is necessary to further understand the dynamics of these microbial communities and their interaction with crop pathogens, aiming to enhance sustainable agricultural practices and reduce global crop losses.

Funding:

National Science Centre, Preludium 21 2022/45/N/NZ9/01923

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Title:

Innovative microbiological fertilizers in horticultural crops. Presentation of the results of the EcoNutri and ResBerry projects

Author/Authors:

Lidia Sas-Paszt[1], Anna Lisek[1], Paweł Trzciński[1], Krzysztof Górnik[1], Edyta Derkowska[1], Beata Sumorok[1], Sławomir Głuszek[1], Mateusz Frąc[1]

Abstract:

Within the activities of the research projects: EcoNutri and ResBerry, the National Institute of Horticultural Research developed microbial consortia with a biostimulating effect on the growth and yielding of horticultural crops, limiting the losses of mineral nutrients to the soil, water and air. The mechanisms of action of beneficial microorganisms, as components of microbial consortia, are based on: making mineral ions available to plants, producing hormones, revealing antagonism towards pathogens attacking horticultural crops as well as biodegradation of organic matter residues. The aim of the EcoNutri project is to develop and implement innovative nutrient management strategies and cultivation technologies for horticultural and agricultural crops in order to reduce soil, water and air pollution, resulting from excessive use of mineral fertilizers and chemical plant protection products and to avoid problems related to inappropriate use of organic wastes. Microbiological consortia have been developed to improve the growth and yield of horticultural crops and to compost agricultural wastes. The components of the developed consortia are Bacillus, Priestia, Klebsiella, Streptomyces and Pseudomonas bacteria. The aim of the ResBerry project is to demonstrate the effectiveness of biological factors that increase biodiversity in organic orchards, in order to increase the resistance of raspberry and strawberry plants to pests and plant pathogens. Microbial consortia containing strains of Paenibacillus sp. and Bacillus sp. bacteria with biostimulating and plant protective properties have been developed, limiting the occurrence of the diseases, caused by plant and soil pathogens (Botrytis cinerea, Verticillium sp., Phytophtora sp.).

The EcoNutri project no. 101081858 is co-financed by the European Commission, under the Horizon Europe 2020 program. The ResBerry project no. CORE Organic/III/55/ResBerry/2022 is co-financed by the National Center for Research and Development.

1 The National Institute of Horticultural Research

Title:

The study of the interactions between Pectobacterium zantedeschiae and plants rhizobiome.

Author/Authors:

Daria Horoszkiewicz[1], Małgorzata Waleron[1], Michał Waleron[1], Krzysztof Waleron[2], Jan Gawor[3]

Abstract:

The phytopathogen, to colonise and efficiently infect plants, must defeat the host's defence systems, including the plant's microbiome. To study the microbiome's influence on the interaction between the pathogen and the plant, we examined the changes in the composition of the rhizobiome of monocotyledonous and dicotyledonous plants during the interaction with Pectobacterium. Therefore, P. zantedeschiae species capable of causing disease symptoms in both types of plants was chosen. Interestingly, disease symptoms after inoculating the soil with the bacterial suspension were only observed for Zantedeschiae aethiopica, which is the host plant for the P. zantedeschiae. Using the 16S rRNA amplicon sequencing method, we determined the composition of the soil and rhizobiomes of Arabidopsis thaliana, Brassica rapa, Zantedeschiae aethiopica and Curcuma longa before and after Pectobacterium inoculation. The number of cultivable bacteria was counted, pure cultures were isolated, and their interaction with P. zantedeschiae was investigated. Sequencing of 16S rRNA showed a decrease in biodiversity and a different species composition of the rhizosphere of a given plant species. The presence of P. zantedeschiae was observed to reduce the number of identified taxa in case of all tested plants. Moreover, the presence of Pectobacterium zantedeschiae in the rhizosphere of Z. aethiopica resulted in a reduction in the diversity of microbiont species below the level of the diversity of the soil itself. The antibiosis assay showed that Pectobacterium inhibits the growth of rhizobionts by bacteriocin production, as well as by prophage induction. Obtained results indicate that P. zantedeschiae has a negative impact on the composition of the plant rhizosphere, which may lead to ineffective functioning of the protective barrier and, subsequently, to the development of disease symptoms.

Funding OPUS18-2019/35/B/NZ9/01973

Keywords: Pectobacterium, metagenomics, rhizobiome, SRP, antibiosis

1 Department of Plant Protection and Biotechnology Intercollegiate Faculty of Biotechnology UG&MUG

2 Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Medical University of Gdansk

3 DNA Sequencing and Oligonucleotide Synthesis Laboratory IBB PAS

Title:

Biostimulation of the common ice plant (Mesembryanthemum crystallinum) by plant growth-promoting rhizospheric bacteria: implications for Cd phytoremediation

Author/Authors:

Paweł Kaszycki[1], Sileola Olatunji[1], Katarzyna Starzec[1], Zbigniew Miszalski[2], Paulina Supel[1]

Abstract:

Cadmium (Cd) is a heavy metal extremely toxic to soil microbiota, plants and humans. When released into the environment, it causes plant wilting, chlorosis and necrosis. The common ice plant (Mesembryanthemum crystallinum L.) has been considered a suitable model for Cd phytoremediation since it can tolerate high levels of cadmium in soil. The aim of the study was to establish whether the high Cd-tolerance of the common ice plant results from a synergistic action of the plant with the Cd/salt resistant bacteria as well as to test if these strains reveal growth-promoting activity towards M. crystallinum. The microorganisms were earlier isolated from the rhizosphere of M. crystallinum grown in the presence of Cd and were applied as a consortium consisting of five strains. During the experiment, plant growth and soil microbiota development was tested in the presence of NaCl and two applied cadmium concentrations. The plants inoculated with the microbial consortium developed more leaves than the uninoculated control objects. Also, for the inoculated plants treated with salt and 10mM cadmium, a lower Cd concentration in soil together with a higher content in shoots were detected compared to the control samples. The results suggest that the common ice plant grown in the presence of the tested microbial consortium can be used for cadmium phytoextraction from contaminated soils.

Kewyords:

cadmium, salinity, the common ice plant, root-zone microbiota, phytoextraction

1 University of Agriculture in Kraków
2 W. Szafer Institute of Botany; Polish Academy of Sciences

Title:

Response of Plantago coronopus L. to drought and salinity stress after plant growth medium inoculation with sulfur-oxidizing bacteria.

Author/Authors:

Aleksandra Koźmińska[1], Cezary Kruszyna[1], Ewa Hanus-Fajerska[1]

Abstract:

The aim of study was to investigate the impact of introducing halophilic sulfur-oxidizing bacteria (SOB), particularly *Halothiobacillus halophilus*, to the growth substrate on the physiological and biochemical responses of the halophyte *Plantago coronopus* L. under drought and salt stress conditions. This study evaluated the plant's response to these stressors and bacterial inoculation by analyzing various factors, including the accumulation of elements (Na, Cl, S); growth parameters; levels of photosynthetic pigments, proline, and phenolic compounds; the formation of malondialdehyde (MDA); and the plant's potential to scavenge DPPH. An increase in sulfur content in the plants was observed after adding bacteria to the substrate in all treatments except for plants treated with high salinity (600 mM), which maintained a constant level of phenolic compounds (including phenylpropanoids, flavonols, and anthocyanins). The water content in the plants decreased dramatically under drought conditions compared to other treatments, but an increase in water content percentage (WC%) was observed only in drought-treated plants after adding SOB. Inoculation with SOB resulted in a 30% increase in leaf growth tolerance index (GTI) in control plants and a 55% increase in plants subjected to moderate salt stress (300 mM). The level of photosynthetic pigments (chlorophylls and carotenoids) decreased by 18% after bacterial application only in plants treated with moderate salinity. The addition of bacterial inoculum also resulted in a decrease in the accumulation of toxic chlorine and sodium ions in the leaves of plants treated with salinity (both 300 and 600 mM). These findings provide novel insights into how halophytes respond to abiotic stress following inoculation of the growth medium with sulfur-oxidizing bacteria. The data suggest that inoculating the substrate with SOB has a beneficial effect on *P. coronopus* tolerance to drought and moderate salt stress.

1 Department of Botany, Physiology and Plant Protection, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Al. Mickiewicza 21, 31-120 Cracow, Poland

References:

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Keywords:

salt stress, drought, halophytes, sulfur-oxidizing bacteria, stress markers

Title:

Influence of Biosurfactant extracted from Kocuria rosea and Pseudomonas aeruginosa on Germination of Glycine max and Pisum sativum seedling

Author/Authors:

Latika Shendre[1], Neelu Nawani[1], Dnyaneshwar Rathod[2]

Abstract:

Biosurfactant is a structurally diverse group of surface-active molecules, synthesized by microorganisms. *Kocuria rosea* and *Pseudomonas aeruginosa* strains isolated from pesticide contaminated soil, which produces biosurfactant. Curd whey was used as a cheap source of growth medium for biosurfactant production. The formation of stable emulsions of biosurfactant containing broth with vegetable oil and kerosene was observed. These strains produced a clear zone in oil spreading test, which indicates the good biosurfactant activity. Both the strains produced extra cellular biosurfactant in the culture media and showed good foam stability in the culture medium. Biosurfactant was efficiently extracted from the culture broth by acetone-HCl precipitation. The biosurfactants from the two species, namely *Kocuria rosea* and *Pseudomonas aeruginosa* were found to have no effects on germinating seedlings of *Glycine max* and *Pisum sativum*, when treated with 25%, 50%, 75% and 100% with the combination of curd whey in the making of 100ml volume. Curd whey was considered as a control with no surfactant. From the above results we concluded that an efficient use of surfactant aided in bioremediation in agricultural land.

Figure captions:

Isolation, characterization and screening of Biosurfactant extracted from Kocuria rosea and Pseudomonas aeruginosa from contaminated soils.

References:

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2. Ocampo, G.Y. (2016) Role of biosurfactants in nature and biotechnological bacteriology and mycology. Bacteriol Mycol; 2(4): 2469-2786.

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2 Department of Biotechnology, Sant Gadge Baba Amravati University Amravati

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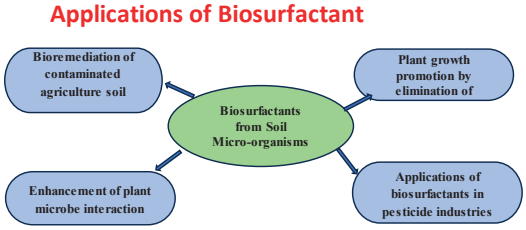
Keywords:

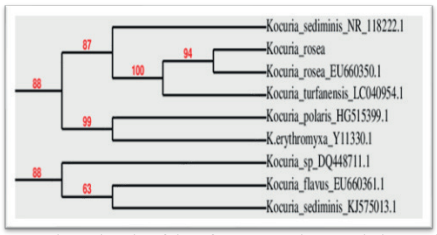
Biosurfactant, Kocuria rosea, Pseudomonas aeruginosa, Glycine max, Pisum sativum

Influence of Biosurfactant extracted from *Kocuria rosea* and *Pseudomonas aeruginosa* on Germination of *Glycine max* and *Pisum sativum* seedlings

Surfactants and emulsifiers are indispensable components of daily life. They are widely used in the in the food and health care industries, industrial cleaning of oil coated surfaces and in agricultural chemicals.



Kocuria rosea *Pseudomonas aeruginosa*



Phylogenetic relationships of biosurfactant producing bacterial isolate BS-04 derived from 16S rRNA gene sequence homology

Industrial waste (curd whey waste)

Processed curd whey

Glycine max

Pisum sativum

	Kocuria rosea					
	Glycine max			*Pisum sativum*		
	Weight of plant (g)	Shoot Length (cm)	Root Length (cm)	Weight of plant (g)	Shoot Length (cm)	Root Length (cm)
Control	28	13	11	37	17	9
Experiment	32	18	14	43	20	10
	Pseudomonas aeruginosa					
Control	28	13	11	37	17	9
Experiment	26	10	9	41	16	7

P7.3

Title:

Plant growth promoting properties of bacterial strains isolated from the rhizosphere of Mesembryanthemum crystallinum grown in saline and cadmium-contaminated soil

Author/Authors:

Paulina Supel[1], Anna Faruga[1], Paweł Kaszycki[1]

Abstract:

Microorganisms occurring in the rhizosphere may have beneficial influence on plant growth and development through different mechanisms including increase of the pool of bioavailable nutrients (N, P, K, Fe), synthesis of phytohormones (gibberellins, auxins, cytokinin) or reducing the level of ethylene through the production of ACC deaminase. Their additional role is related to limiting the growth of soil pathogens through the expression of enzymes responsible for cell wall lysis, productions of antibiotics and siderophores, as well as through the induction of plant systemic immunity (ISR - Induced Systemic Response). The study aimed at evaluation of plant growth promoting (PGP) properties of five bacterial strains isolated from the rhizosphere of a semi-halophyte *Mesembryanthemum crystallinum*, cultivated in the presence of cadmium. Three strains, identified as *Paenibacillus glucanolyticus* S7, *Rhodococcus erythropolis* S4 and Rh. erythropolis S10 were isolated from the plants performing CAM (*crassulacean acid metabolism*) which was induced with high soil salinity. The other two: *Providencia rettgeri* W6 and *P. rettgeri* W7 were obtained from the root zones of C3 plants . Six *in vitro* tests of selected biochemical PGP traits were conducted: indolyl-3-acetic acid (IAA) production, atmospheric nitrogen fixation, phosphate solubilization, protease, ammonia and siderophore production. The results showed that the isolated bacteria were characterized by diverse PGP traits. Each strain revealed at least two properties that may be considered beneficial for plant growth and development. None of the strains produced protease. Taken the individual differences in PGP characteristics it is suggested that the best effect on plants can be achieved applying a microbial consortium consisting of all five bacterial isolates.

Keywords:

salinity stress, Mesembryanthemum crystallinum, plant growth-promoting bacteria, phytoremediation

1 University of Agriculture in Kraków

Title:

Copper-tolerant semi-halophyte Mesembryanthemum crystallinum L. (the common ice plant) grown in normal and saline soils treated with a commercial Cu-based fungicide

Author/Authors:

Marta Śliwa-Cebula[1], Zbigniew Miszalski[2], Johannes Jehle[3], Annegret Schmitt[3], Paweł Kaszycki[1]

Abstract:

Copper is a micronutrient essential for plant growth but at soil concentrations above 20 mg/kg d.w. is considered potentially toxic, both to the soil microbiota and plants. Due to anthropogenic emissions, Cu often exceeds 100 mg/kg, bringing risk of severe ecotoxicity [1]. A considerable impact on soil contamination is due to the use of Cu-based crop protection preparations, still allowed in agricultural practice, including organic farming.

Cu-tolerant plants evolved the resistance mechanisms mainly via induction of specific genes and production of antioxidants. The preferred strategy is to limit the Cu-ion uptake and thus reduce its accumulation by chelation or precipitation with root exudates.

The study was aimed at testing M. crystallinum tolerance to elevated Cu levels. Normal and saline soils were artificially doped with Cu(II) using a CUPROZIN® progress fungicide. The applied Cu doses (600, 1760 and 3520 mg/kg d.w.) far exceeded the permissible soil contamination standards.

Earlier, we showed that the plant could grow on bottom sediments polluted with heavy metals (Cd, Cr, Ni, Cu, Zn) and revealed extreme tolerance to Cd and Cr(VI) [2,3]. Here, high Cu-resistance is evidenced by undisturbed growth at all Cu concentrations, both in normal and saline soils. Plant dry mass and biometric evaluation indicated lack of toxicity effects. Photosynthetic performance was not impaired, either: no oxidative stress and no differences in chloroplastic electron transport chain were detected.

In all variants, plant rhizospheres were highly populated with bacteria. The changed microbial biodiversities indicated microbiota adaptation to salinity and Cu exposure.

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2 W. Szafer Institute of Botany; Polish Academy of Sciences

3 Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Biological Control

Plant Cu-accumulation indices revealed poor phytoextraction potential (max Cu content was 294 mg/kg in roots after 11-day exposure to 3520 mg Cu/kg). The values of bioaccumulation factors were low and ranged 0.03–0.17. These results suggest the common ice plant has adopted an excluder's strategy against copper stress.

References:

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2. Śliwa-Cebula M., Kaszycki P., Kaczmarczyk A., Nosek M., Lis-Krzyścin A., Miszalski Z. The common ice plant (*Mesembryanthemum crystallinum* L.) – Phytoremediation potential for cadmium and chromate-contaminated Soils. Plants **2020**, 9, 1230. doi.org/10.3390/plants9091230
3. Śliwa-Cebula M., Koniarz T., Szara-Bąk M., Baran A., Miszalski Z., Kaszycki P. Phytoremediation of metal-contaminated bottom sediments by the common ice plant (*Mesembryanthemum crystallinum* L.) in Poland. J Soils Sediments **2023**, 23, 1065–1082. doi.org/10.1007/s11368-022-03401-x

Keywords:

heavy metals, salinity, copper stress, root-zone microbiota, plant excluders

P7.5

Title:

The influence of organic fertilizers on the soil microbial community structure and diversity

Author/Authors:

Monika Michalecka[1], Canfora Loredana[2], Andrea Manfredini[2], Ewa Furmańczyk[1], Małgorzata Tartanus[1], Eligio Malusa[1]

Abstract:

The main objective of this study was to investigate the impact of organic fertilizers on soil microbial community structure and diversity, as evaluated by T-RFLP (terminal restriction fragment length polymorphism).

In our study, we applied five different organic fertilizers once in May by watering the apple trees in various combinations, with three technical replications. We sampled the soil at three time points: 1st in May, before treatments (T0), 2nd in July (T1), and 3rd in October (T2), resulting in a total of 6 soil samples per combination. We extracted total DNA from bulk soil and conducted PCRs with fluorescently – labelled primers to amplify DNA of all Bacteria, Archaea, and filamentous fungi. The PCR – products were then digested with restriction enzymes and the fragments were analysed through capillary electrophoresis. The obtained products were compared for different combinations and time points.

On the basis of obtained fragments in T-RFLP assay, it was possible to estimate changes in number of OTU (Operational Taxonomic Units) and Shannon H' index diversity of Archaea, Bacteria and fungi. In the Archaea populations, there was an increase of OTU average values, corresponding with the rise of diversity index average values, observed in T1, while both parameters decreased during T2. In Bacteria populations, there was a decrease in OTU average values, corresponding with a slight decrease in diversity index, observed in T1 and T2. In populations of fungi, both decreases and increases were observed in the parameters, in Time 1 and 2, depending on the blocks.

Conclusions: Organic fertilization caused changes in the microbial community structure of the population of Bacteria, fungi and Archaea, expressed as changes in OTU and Shannon H'-index, observed 2 and 5 months after application. The most significant changes of these parameters were observed for the Archaea and Bacteria populations, while the lowest for fungi.

1 National Institute of Horticultural Research
2 Council for Agricultural Research and Agricultural Economy Analysis

Acknowledgments:

DOMINO project, co-financed by European Union in the frame of the program Core Organics Cofund (Dynamic soil mulching and use of recycled amendments to increase biodiversity, resilience and sustainability of intensive organic apple orchards and vineyards).

Keywords:

soil microorganisms, organic compounds, T-RFLP

Session 8

CIVILIZATION DIESIESES

L8.O1

Title:

TARGETING GENOTOXIC AND PROTEOTOXIC CELLULAR STRESS PATHWAYS IN CARCINOGENESIS AND CANCER TREATMENT

Author:

Martin Mistrik[1]1

Abstract:

Carcinogenesis, a complex process driven by genetic mutations and cellular dysregulations, is profoundly influenced by genotoxic and proteotoxic stresses. Our research aims to elucidate these stresses, which damage DNA and proteins, and their interconnected roles in cancer development and treatment. Genotoxic stresses, such as DNA strand breaks and base damage, arise from external sources like chemicals and radiation, as well as internal metabolic byproducts. These stresses often act as catalysts for carcinogenesis, initiating a cascade of events that lead to cancer. As malignancies progress, DNA repair mechanisms frequently become faulty, accelerating the accumulation of DNA lesions and mutations. Increased levels of DNA lesions and repair defects are hallmark features of cancer cells, rendering them highly sensitive to DNA-damaging agents known as first-generation chemotherapeutics. These agents induce irreparable lesions or overwhelm repair capacities, leading to cancer cell death.

Simultaneously, accumulating mutations in cancer cells impact multiple protein products, resulting in elevated levels of misfolded, aggregated, and malfunctioning proteins. This defective protein overload, typical of advanced cancer stages and certain degenerative diseases, is termed proteotoxic stress. Proteotoxic stress disrupts cellular homeostasis by straining energy-intensive degradation processes and the tightly regulated protein degradation system.

Our research is dedicated to the dual targeting of genotoxic and proteotoxic stresses, a novel approach that holds great promise for more effective cancer therapies. By combining DNA-damaging agents with strategies that exacerbate proteotoxic stress, we aim to enhance treatment efficacy and overcome the limitations of current approaches. This synergistic approach leverages the vulnerabilities of cancer cells, promoting their death while potentially reducing the emergence of drug resistance.

Acknowledgment:

Research was supported by the project SALVAGE (OP JAC; reg. no. CZ.02.01.01/00/22_008/0004644) – co-funded by the European Union and by the State Budget of the Czech Republic.

1 Placky University Olomuc

L8.O3

Title:

Advances in diabetes therapy: Using DYRK1A inhibitors for therapeutic innovation

Author:

Anna Czarna[1]

Abstract:

Diabetes is a widespread health problem worldwide, with the number of cases expected to rise from 422 million to 700 million by 2045 due to modern lifestyles, stress and genetic predisposition. The disease is well managed by medicatoin and restrictive diet, but to provide a cure, an ideal approach would involve reversing the pathological changes associated with these conditions.

Understanding the molecular mechanisms of this disease is essential to developing effective treatments. Our research focuses on creating inhibitors for DYRK1A kinase, which has demonstrated potential in promoting β-cell proliferation, crucial for diabetes therapy. We utilized a hiPSC line derived from blood cells using Sendai non-integrating vectors to develop pancreatic islet organoids. These organoids, which express insulin and exhibit a strong glucose-stimulated insulin secretion (GSIS) response, serve as models to test the efficacy of our inhibitors.

Our comprehensive cellular studies using advanced 3D organoid models, including confocal microscopy, flow cytometry, RT-qPCR, ELISA, and animal studies, indicate the strong translational potential of DYRK1A inhibitors.

This research could pave the way for new, safe therapies for diabetes, significantly advancing diabetes treatment.

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L8.O4

Title:

FIRST-IN-CLASS MODULATOR OF CHOLINERGIC AND GABA-ERGIC NEUROTRANSMISSION: A NOVEL DIRECTION IN ALZHEIMER'S DISEASE TREATMENT

Authors:

Anna Pasieka[1]*, Dawid Panek[1], Anna Więckowska[1]

*annamarta.pasieka@uj.edu.pl

Abstract:

Alzheimer's disease (AD) is one of the civilization diseases associated with the ageing of society. AD is a neurodegenerative disorder characterized not only by memory loss but also by Behavioral and Psychological Symptoms of Dementia (BPSD), such as agitation, aggression, depression, and anxiety.[1] For many years, the loss of cholinergic neurons was believed to be the primary cause of AD, resulting in most current treatments focusing on enhancing cholinergic neurotransmission.[2] However, imbalances in other neurotransmitters, such as GABA, are also observed in AD pathogenesis and lead to cognitive disturbances and BPSD symptoms.[3] Balancing both compromised cholinergic and GABAergic neurotransmission simultaneously could be an effective approach to reducing cognitive and BPSD symptoms. The observed impairment of cholinergic neurotransmission may be compensated by inhibiting enzymes that hydrolyse acetylcholine, such as acetylcholinesterase (AChE) and/or butyrylcholinesterase (BuChE).[2] On the other hand, the overexpression of γ-aminobutyric acid transporter subtype 3 (GAT-3) on the astrocytes and microglial cells may be addressed by inhibiting GAT-3.[3] Therefore, in our research, we focused on **the development of dual inhibitor of BuChE and GAT-3 as a first-in-class modulator of cholinergic and GABA-ergic neurotransmission** with the potential to treat Alzheimer's disease and BPSD. Screening of our in-house library led us to identify a "hit" compound that we optimized in terms of biological activity and drug-likeness using crystallography and computational methods. Finally, we developed compound **6** that inhibits BuChE (IC_{50} = 0.21 μM) and GABA transporter 3 (IC_{50} = 7.41 μM) with a favourable drug-likeness profile, high metabolic stability and low cytotoxicity. Moreover, compound **6** demonstrated effectiveness in enhancing memory retention and alleviating anxiety and depression symptoms in animal models, while also proving safe and bioavailable for oral administration. The promising data suggest that this first-in-class dual modulator of cholinergic and GABA-ergic neurotransmission **6** could be a game changer in treating cognitive deficits and BPSD in AD.

1 Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna St. 9, 30-688, Kraków, Poland

References:

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2. Hampel H. et. al. Brain A J. Neurol. 141 (2018) 1917-1933.
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Acknowledgements:

The work was supported by the National Science Center of Poland (UMO-2021/41/B/NZ7/02825).

Keywords:

Alzheimer's disease, multifunctional ligands, inhibitors, butyrylcholinesterase, GABA transporters

Title:

Conjugation of platinum(II) complexes to peptide nucleic acids for the development of antibacterials

Author/Authors:

Rosa Maria Dell'Acqua[1], Monika Wojciechowska[2], Francesco Fagnani[1], Daniele Marinotto[3], Joanna Trylska[2], Alessia Colombo[1], Silvia Cauteruccio[1]

Abstract:

Infectious diseases caused by bacteria represent one of the biggest threats to global health. Therefore, novel and effective strategies to develop antibacterial agents are continuously searched. Recently, the use of antisense oligonucleotides, such as peptide nucleic acids (PNAs)[1], has emerged for its potential to treat bacterial infections. PNAs represent excellent candidates for inhibiting the synthesis of proteins essential for bacteria to sustain life, being helpful in the fight against bacterial infections[2].

In our research on PNAs, we are developing novel bioorganometallic platinum(II)-PNA conjugates that can be used as dual-activity antibacterial agents. These systems combine the antisense properties of PNAs with the photodynamic properties of a Pt(II) complex, which serves as the photosensitizer capable of generating cytotoxic singlet oxygen under light excitation[3].

The innovative Pt(II)-PNA conjugates (*Figure 1*) were synthesized on solid phase and their photophysical properties were characterized, revealing that the luminescent features of the Pt(II) complexes were enhanced by the conjugation with PNAs. The melting temperatures of the PNA and PNA conjugates with complementary DNA and RNA were recorded to evaluate how the Pt(II) complexes affect the recognition properties of the PNA. Circular dichroism (CD) was also used to analyze the influence of the Pt(II) complexes on the helical structure formed by PNA with DNA and RNA.

The most promising Pt(II) complex was also conjugated to the antibacterial PNA sequence to target mRNA of the acpP gene encoding the essential acyl carrier protein in *E. coli*. The antibacterial properties of these unique compounds have been tested to evaluate their potential in acting as antibacterial agent.

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2 Centrum Nowych Technologii Uniwersytetu Warszawskiego
3 Istituto di Scienze e Tecnologie Chimiche (SCITEC) “Giulio Natta”, Consiglio Nazionale delle Ricerche (CNR)

Figure captions:

Figure 1: Study of luminescent features, hybridization properties and antibacterial effects of Pt(II)-PNA conjugates.

References:

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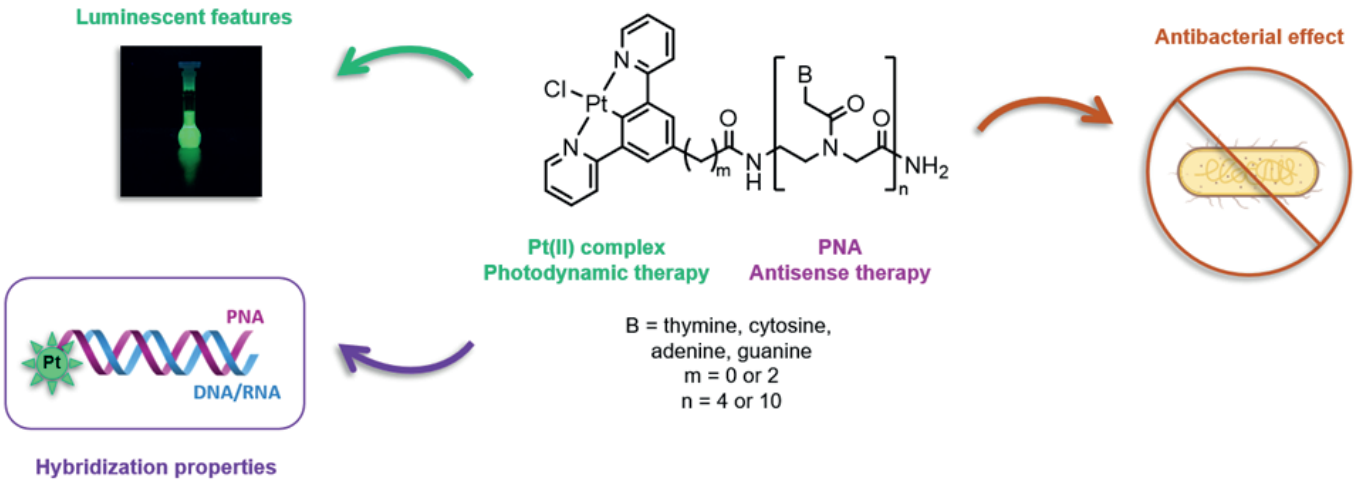
2. Wojciechowska, M. et al. Molecules, **2020**, 25, 559.
3. Wu, Y. et al. Chem. Sci., **2022**, 13, 5085-5106.

Keywords:

peptide nucleic acids, platinum complex, antibacterial agents



Title:

Healthy microbiome - moving towards functional interpretation

Author/Authors:

Kinga Zielinska[1], Klas Udekwu[2], Witold Rudnicki[3], Alina Frolova[4], Tomasz Kościółek[5], Paweł P Łabaj[1]

Abstract:

Stool-based disease prediction has a significant potential as an early, non-invasive marker of multiple public health conditions such as dysbiosis of the human gut microbiota, inflammatory bowel disease, diabetes and even cancer. Existing tools, or microbiome health indexes, are often based solely on a microbiome's species richness and are heavily dependent on taxonomic classification. More recently, an increased understanding of microbiome metabolic and phenotypic complexity revealed substantial restrictions of such approaches[1]. In this study, we introduce a new health microbiome index created as an answer to updated microbiome definitions. The novelty of our approach is a more holistic consideration of metabolic functions including ecological interactions between species in the effort to distinguish between healthy and diseased states. We compare our method to not only the taxonomy-based Gut Microbiome Health Index (GMHI) and the high dimensional principal component analysis (hiPCA) method, the most comprehensive indices to date, but also to taxon- and function-based Shannon entropy and demonstrate a significant improvement to these approaches. We validate our index's performance using a variety of complementary benchmarking methods on datasets representing a range of gut health conditions and showcase the robustness of its superiority over the GMHI and the hiPCA. Overall, we emphasize the potential of this approach to assess health from stool and to make the from-home personalized diagnoses a not-too-distant future. More information about our method, q2-predict-dysbiosis, can be found in our pre-print[2].

Figure captions:

Shannon entropy, GMHI, hiPCA and q2-predict-dysbiosis scores for HMP2 and AGP healthy, iinflammantory bowel disease (IBD) and Obese individuals.

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2 Department of Biological Sciences, University of Idaho
3 Faculty of Computer Science, University of Białystok
4 Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine
5 Department of Data Science and Engineering, Silesian University of Technology

References:

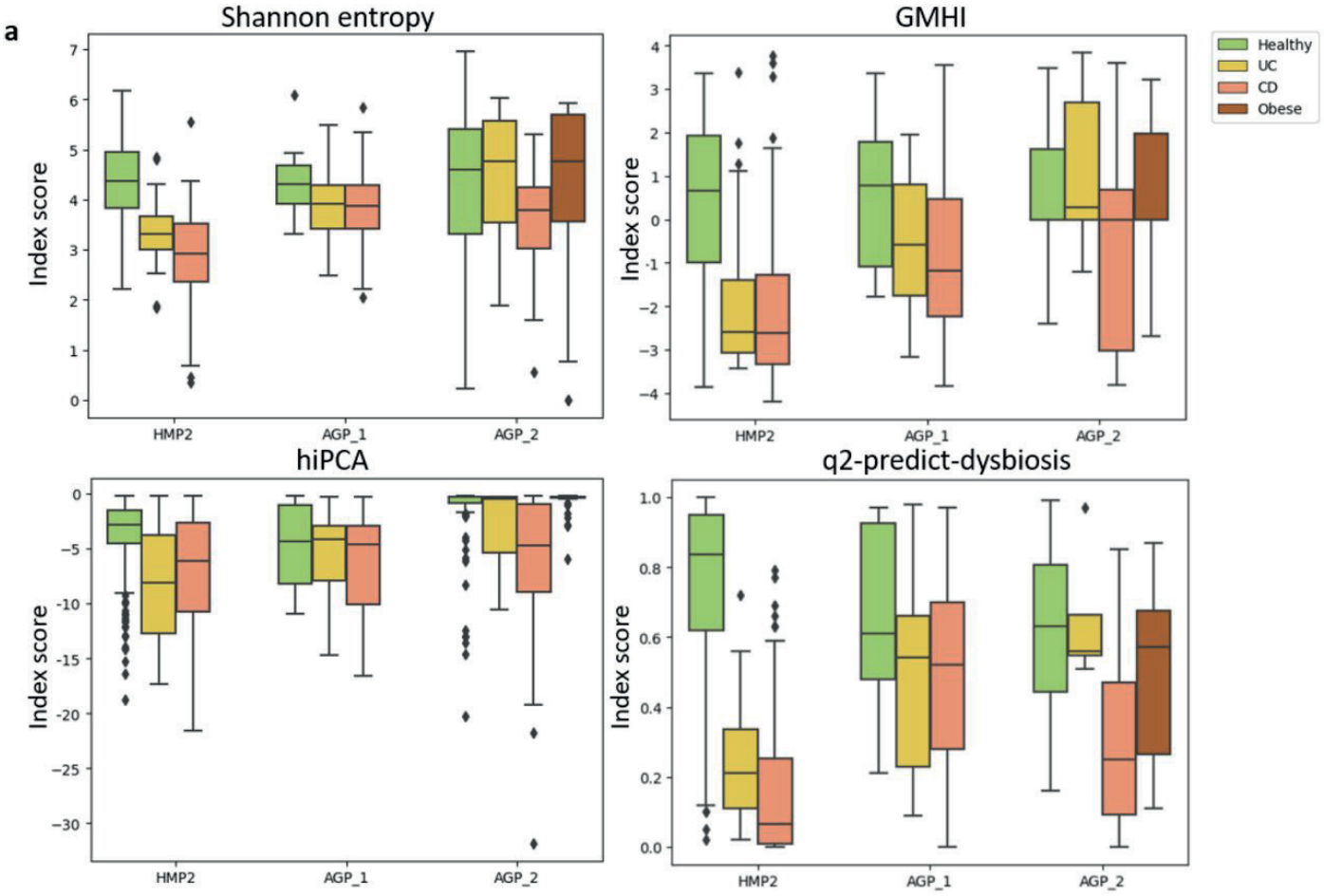
1. Berg, G. et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome 8, 103 (2020)
2. Zielińska, K. et al., Healthy microbiome - moving towards functional interpretation, bioRxiv 2023.12.04.569909

Acknowledgments:

This research was conducted as a part of the NCN Sonata BIS grant number 2020/38/E/NZ2/00598. We gratefully acknowledge Poland's high-performance Infrastructure PLGrid (HPC Centers: ACK Cyfronet AGH, PCSS, CI TASK, WCSS) for providing computer facilities.

Keywords:

microbiome, health, diagnosis, dysbiosis



Title:

Synthesis and investigation of properties of conductive nanocomposites for nerve regeneration applications

Author/Authors:

Aleksandra Sierakowska-Byczek[1], Julia Radwan-Pragłowska[1], Łukasz Junus[1]

Abstract:

The nervous system is considered the most important in the human body, because it controls the other systems. The basic division distinguishes the central nervous system and the peripheral nervous system. The peripheral nervous system, unlike the central nervous system, can regenerate[1]. In the case of injuries requiring surgical intervention, autologous transplantation is considered the "gold standard", but it is associated with many limitations, among which one can distinguish insufficient tissue availability and additional burden on the body associated with the procedure at the place of collection. Another solution may be the use of nerve canals, but products available on the market have limitations such as supporting the regeneration of only short sections of nerves and lower efficiency compared to autografts[2].

In order to increase the effectiveness of nerve regeneration, nerve channels based on collagen and chitosan were obtained, which were additionally functionalized with Ag, Au, Pt nanoparticles coated with conductive polymers such as polypyrrole, polyaniline and polyvinylpyrrolidone[3,4]. The nerve canals were obtained using electrospinning and 3D printing technology. The morphology of the nanoparticles was investigated using TEM, while the conductive properties were determined by the conductivity method. The physicochemical properties of the obtained nanocomposites, such as chemical structure, swelling capacity, porosity, mechanical strength, were investigated. Biomaterials have also been tested for degradation, biodegradation, antibacterial properties and cytotoxicity.

Figure captions:

Figure 1: (a) TEM image of Ag/PP nanoparticles; (b) qualitative assessment of cytotoxicity on MG-63 cells after 72 h cell culture; (c) the nerve canal obtained

1 Cracow University of Technology

References:

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2. Silva, J. et al. (2017) Nerve conduits for treating peripheral nerve injuries: A systematic literature review, Hand Surg Rehabil. 36, 71-85
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Acknowledgments:

This research was funded by Ministry of Education and Science, Diamentowy Grant, grant number 0023/DIA/2020/49.

Keywords:

biomaterials, nanofibers, nerve guidance conduits, tissue engineering

(a) (b) (c)

Title:

AMOTL2 protein as an example of the cell fate commitment determinant

Author/Authors:

Małgorzata Grabowska[1], Marta Orlicka-Płocka[1], Anna Jędrzejak[1], Małgorzata Borowiak[1]

Abstract:

β-cells are crucial as they produce insulin maintaining a healthy balance of blood sugar levels. In diabetes, β-cells are either depleted or dysfunctional. A promising solution for regenerative medicine to investigate diabetes mechanisms and treatments is a differentiation of human pluripotent stem cells. While there is comprehensive knowledge of transcriptional factors regulation the stemness[1], less is known about other mechanisms that impact stem cell biology and differentiation potential.

In accordance with data obtained from single-cell RNA sequencing, we have identified angiomotin-like 2 (AMOTL2) as a gene with elevated expression in a newly identified subpopulation of delaminating endocrine progenitors (EPs) at e16.5 (embryonic day 16.5) but not at e14.5[2]. EPs at e16.5 were found to be more prone to β-cell formation, suggesting the importance of AMOTL2 in diabetes therapy. AMOTL2 is known for modulating the Hippo pathway, governs various cellular processes such as organ size regulation and the determination of cell fate[3].The knockout of the AMOTL2 gene in hPSCs leads to changes in cell and colony morphology, as well as excessive confluence, resulting from increased proliferation. The opposite effect is seen in case of AMOTL2 overexpression. We hypothesize that AMOTL2 plays a crucial role in maintaining stem cell properties and influencing their potential for differentiation not only through Hippo pathway, but also via cytoskeleton organization and cellular energy metabolism. These predictions are supported by experimental data indicating alterations in the organization and structure of F-actin cytoskeleton and in the expression of intracellular junctions markers, as well as altered mitochondrial network and energy metabolism. Based on our preliminary data that AMOTL2 regulates mitochondria homeostasis and cytoskeleton rearrangements, we speculate that β-cell formation and function will be impaired by AMOTL2 loss.

1 Adam Mickiewicz University

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Keywords:

stem cells, diabetes, Hippo pathway, cytoskeleton, mitochondria

Title:

The viability of sentinel cells orchestrates inflammation in periodontitis – human gingival fibroblast and macrophage study

Author/Authors:

Alicja Plonczynska[1], Maja Sochalska[1], Jan Potempa[1]

Abstract:

Purpose: Periodontitis is a bacterial chronic inflammatory disease caused by ex. *Porphyromonas gingivalis (Pg)*. The disease progresses due to aberrantly activated immune cells: macrophages (MDMs) and neutrophils, and their disturbed interaction with dominating the oral cavity gingival fibroblasts (GFs).

Results: MDM survival was altered upon Pg administration due to NLRP3 inflammasome activation, followed by gasdermin D cleavage. The expression level of Bcl-2 family proteins remained unaffected upon *Pg* challenge, indicating mainly participation of pyroptotic cell death mode by this periodontopathogen. Pg strongly activated the secretion of pro-inflammatory agents by MDMs: TNF-α, IL-6, IL-8, and IL-1β, further proving the engagement of pyroptosis. In opposite, the viability of GFs was not influenced by Pg, however, GFs participated in the robust inflammation through the release of IL-6 and IL-8, but not IL-1β, and TNF-α.

Importantly, pharmacological inhibition of BTK (Ibrutinib) and SYK (R406) signaling pathways resulted in restraint pro-inflammatory agent secretion in both MDMs and GFs, as well as in coculture assay. Importantly, inhibition of BTK limited the occurrence of MDM pyroptosis, restraining the cleavage of Gasdermin D by caspase-1. Furthermore, flow cytometric analysis revealed that SYK inhibition redirected the infected MDMs as well as GFs toward apoptosis.

Conclusion: This study finally uncovered the role of survival and inflammatory pathways' crosstalk in orchestrating the response of macrophages and gingival fibroblasts, acting as sentinel cells during bacterial infection in periodontal disease. In summary, our results discovered novel molecular targets subverted by *Pg*, which can serve as potential candidates for the treatment of periodontal patients.

Acknowledgments:

Project founded by NCN SONATA: 2020/39/D/NZ5/02075 and NCN PRELUDIUM: 2022/45/N/NZ5/02779.

Keywords: periodontitis, inflammation, macrophage, fibroblast, cell death

1 Jagiellonian University

P8.2

Title:

Phylogenetic analysis of clinical Lacticaseibacillus rhamnosus strains using core genome MLST method.

Author/Authors:

Piotr Jarocki[1], Jan Sadurski[1], Martyna Siuda[1], Mateusz Romanowicz[1]

Abstract:

Lactobacilus rhamnosus, in the new nomenclature *Lacticaseibacillus rhamnosus*, is one of the bacterial species intensively used in the pharmaceutical industry and food production. Research conducted with *L. rhamnosus* strains has mainly focused on their potential health-promoting properties. However, numerous reports have reported clinical cases of L. rhamnosus-induced bacteremia. There have also been documented cases of liver abscess, endocarditis, pleural empyema and retropharyngeal abscess which were caused by bacteria belonging to the *L. casei* group.

It should be emphasized that both probiotic characteristics and pathogenicity are attributed not to the bacterial species themselves, but are characteristic of specific strains. Accurate identification of specific strains seems particularly important in clinical cases when potentially health-promoting bacteria cause life-threatening pathological conditions in patients. In earlier work, a number of procedures have been presented to differentiate and identify bacteria belonging to the *L. casei* group, based on PCR reaction and analysis of protein profiles. Nevertheless, taking into account the increasingly lower costs of next-generation sequencing and also the increasing availability of bioinformatics tools, it seems that analysis of complete bacterial genomes is likely to be the optimal tool providing enough information for precise identification and accurate characterization of specific strains. Therefore, in this paper we present the use of cgMLST method in genotyping of L. rhamnosus strains isolated from clinical samples. Analysis of 622 L. rhamnosus genomes showed that the strains studied showed similarity to bacteria isolated from both clinical samples, as well as such sources as intestine, vagina, mouth, dairy products and commercial probiotic supplements. Simultaneously, it was difficult to unequivocally determine the association of the tested strains with a specific geographic location.

1 University of Life Sciences in Lublin

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Acknowledgments:

The research was financially supported by grant UMO-2016/23/D/NZ9/02661 from the National Science Centre, Poland

P8.3

Title:

A proprietory application of senotherapeutics in periodontitis – human gingival fibroblasts study

Author/Authors:

Alicja Płonczyńska[1], Maja Sochalska[2], Aleksander M. Grabiec[2], Jan Potempa[3]

Abstract:

Periodontitis (PD) is a chronic inflammatory disease caused by e.g. *Porphyromonas gingivalis (Pg)*. The disruption of homeostasis between gingival fibroblasts (GFs) and immune cells fuels the inflammatory state and disease progression. Based on recent studies we hypothesized that senotherapeutic agents can provide a novel solution for limiting the hyper-immune activity in PD.

GFs obtained from young healthy (yhGF), aged healthy (ahGF), and aged periodontitis (apGF) donors were treated with vital Pg for 24h (acute inflammation) or 7 and 14 days (chronic inflammation), and analyzed for survival by AnnexinV/PI staining. The cell death, cell cycle, and inflammatory response were further characterized by Western Blot (proteins from the Bcl-2 family, p21, p16), and ELISA assays. The potential use of senotheraputics as anti-inflammatory drugs in PD was assessed by GFs treatment with SYK (R406), BTK (Ibrutinib) inhibitors, or pan Bcl-2 inhibitor (ABT-737).

After 7 days of treatment, *Pg* rescued yhGFs from cell death leading to cell cycle arrest (measured as p21 expression). Upon infection, a robust secretion of IL-6 and IL-8 was observed that escalated over time. Interestingly, in samples from acute PD ahGFs exhibited the strongest inflammatory response. Importantly, senolytic agent SYKi decreased the viability of GFs in all tested groups after 24h of *Pg* challenge, attenuating Pg-driven inflammation. Conversely, in SYKi application in chronic PD, GFs partially restored survival rate, without losing anti-inflammatory properties. Senomorphic agent BTKi did not alter GFs survival, but limited inflammation in chronic disease with ahGFs being the least sensitive for the treatment. ABT-737, a classic senolythic drug, induced cell death in all donor groups and manifested strong anti-inflammatory properties only in apGFs in the chronic disease model.

This study has shown the potential application and various modes of action of senotherapeutics as a novel treatment for PD.

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2 Jagiellonian University
3 Jagiellonian University/University of Louisville School of Dentistry

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Keywords:

periodontitis, gingival fibroblast, senolytics, senescence

P8.4

Title:

Potential Health-Beneficial Compounds in Wine: Testing grape maceration time

Author/Authors:

Anna Kostecka-Gugała[1], Jacek Stanula[1], Paweł Kaszycki[1]

Abstract:

Wine is a rich source of polyphenols which are the primary antioxidants and are known to have numerous health benefits, such as protecting the cardiovascular system, reducing the risk of type II diabetes and macular degeneration, and delaying the onset of neurodegenerative diseases [1,2]. Many epidemiologic studies have documented a relatively low incidence of coronary heart disease (CHD) in individuals who consume moderate amounts of red wine and follow a diet rich in saturated fat, which is considered a risk factor for CHD [3,4.]

The content of phytochemicals in wine depends on the grape variety, cultivation manner and vinification methods, as well as on the use of pre- and post-fermentation maceration, which is typically used in the standard red wine production. Here, white grape var. 'Johanniter' maceration was applied to enrich the wine with bioactive compounds. Antioxidant (FRAP assay) and antiradical (DPPH and ORAC-Fl assays) properties as well as the content of several polyphenols (HPLC) in the white wine 'Johanniter' was then compared with the red wine var. 'Regent' from the same vineyard, as a function of maceration time.

Both wines demonstrated a substantial increase in antioxidant and antiradical capacities, and the total phenolic content (TPC, Folin assay) after 4 days of maceration. Further treatment did not significantly enrich the wines with bioactive compounds. At the start of the experiment, all parameter values were higher for the red wine, as expected. It is noteworthy that upon 4-day maceration the content of DPPH nitroxyl radical scavengers was markedly higher in the white wine and remained elevated during maceration. In contrast, the peroxyl radical scavenger content (measured by ORAC-Fl), antioxidant capacity and TPC were higher in all the red wine samples. The antioxidant and antiradical properties of the tested Polish wines were comparable or even exceeded those determined for selected wines produced in Western European vineyards.

1 University of Agriculture in Krakow, Faculty of Biotechnology and Horticulture, Department of Plant Biology and Biotechnology

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Keywords:

wine, maceration, antioxidants, polyphenols, ORAC

Session 10

PLANT TISSUE CUTURES

L10.O1

Title:

Leveraging Nanoparticles for Enhanced In Vitro Culture Systems of Ornamental Perennials

Author/Authos:

Dariusz Kulus[1], Alicja Tymoszuk[1], Alicja Kulpińska[1]

Abstract:

In vitro culture systems are a fundamental tool in the propagation, breeding, and preservation of ornamental perennials. However, challenges, such as low regeneration rates, genetic instability, and cryo-injuries persist. Nanoparticles (NPs) have emerged as useful agents to support these techniques due to their unique physicochemical properties and biocompatibility. Our study aimed to explore the multifaceted role of NPs in enhancing various aspects of in vitro culture systems for ornamental perennials, including micropropagation, mutation breeding, and cryopreservation. Micropropagation, a pivotal technique for mass clonal propagation, benefits from NPs by promoting shoot proliferation, enhancing rooting, and mitigating physiological disorders. NPs, acting as carriers for growth regulators, facilitate efficient nutrient uptake and stimulate growth in tissue cultures. Moreover, their antimicrobial properties prevent contamination. In mutation breeding, NPs can be successfully used as mutagen agents if used at high concentrations, thereby accelerating the generation of novel variants with desirable traits. This approach expedites the breeding process, leading to the development of improved cultivars with enhanced ornamental characteristics. Furthermore, in cryopreservation, NPs act as cryoprotectants and ice nucleation inhibitors, safeguarding cellular integrity during freezing and thawing. Due to their unique properties and high thermal conductivity, incorporating NPs into cryopreservation protocols enhances post-thaw survival rates and preserves the genetic diversity of ornamental perennials. Even though, their final effect depends on biotic (cultivar) and abiotic parameters (such NPs type, concentration and size), NPs offer a holistic approach towards advancing the field of ornamental horticulture.

Acknowledgments:

This research was funded in part by National Science Centre, Poland (Grant number: 2020/39/D/NZ9/01592)

Keywords:

bleeding heart, chrysanthemum, cryopreservation, micropropagation, mutation breeding

1 Bydgoszcz University of Science and Technology

Title:

Molecular regulation of somatic embryogenesis in plants – genetic and epigenetics aspects

Autthor/Authors:

KATARZYNA NOWAK[1], MAŁGORZATA D. GAJ[1]

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katarzyna.nowak@us.edu.pl

Abstract:

Since plant regeneration techniques are widely applied in plant biotechnology, the studies of developmental processes engaged in plant morphogenesis in vitro are of interest to modern biology, especially genetics and genomics of plants. Two alternative morphogenic processes, including shoot organogenesis and somatic embryogenesis (SE), are involved in plant regeneration in vitro. In particular, a plant-specific process of SE attracted the most attention from researchers. In SE, a somatic embryo is formed from somatic cell/cells without a fertilization event. Thus, SE manifests a unique phenomenon of toti-/pluripotency of somatic plant cells. The somatic cells that enter the embryogenic transition must change cell identity through the extensive genetic reprogramming of the cell transcriptome. The transcriptomic reprogramming of the cell is controlled by transcription factors (TFs). Several auxin-related TFs, including LEAFY COTYLEDON (LEC2, LEC2), PLETHORA (BBM), AGL15, and WUS were identified to play a central role in the SE-regulatory network (Gaj et al. 2005; Harding et al. 2003; Boutilier et al. 2002; Nowak et al. 2015). In turn, the SE-involved TFs are controlled by different epigenetic factors, including microRNA molecules (miRNAs) that post-transcriptionally regulate gene expression. Within SE-miRNAs, miR393, miR160, and miR166/67 via targeting TIR1 and ARF transcripts were indicated to control the auxin-related processes of central function in the embryogenic induction (Wójcik and Gaj, 2016; Wójcik et al. 2017). Recently, epigenetic factors affecting chromatin structure, including DNA methylation, and also acetylation, and methylation of histones have been demonstrated to play a key role in adjusting gene expression patterns enabling SE induction (Wójcikowska et al. 2018; Grzybkowska et al. 2020; Morończyk et al. 2022).

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The recent insights into the genetic and epigenetic regulators of SE which have been gained mostly in a model plant of Arabidopsis resulted in the establishment of new genetic and epigenetic approaches improving plant regeneration efficiency of in vitro recalcitrant cereals such as maize and barley (Lowe et al. 2018; Nowak et al. 2024).

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Title:

Buckwheat in Tissue Culture Research: Current Status and Future Perspectives

Author/Authors:

Alexander Betekhtin[1], Ewa Grzebelus[2], Ewa Kurczyńska[1], Anna Milewska-Hendel[1], Artur Piński[1], Renee Perez-Perez[1], Elwira Śliwińska[3], Katarzyna Sala-Cholewa[1], Alicja Tomasiak[1], Magdalena Zaranek[1]

Abstract:

Buckwheat belongs to a genus that includes 22 species, the two most commonly cultivated of which are Fagopyrum esculentum (common buckwheat) and Fagopyrum tataricum (Tartary buckwheat). Buckwheat is an essential source of vitamins, minerals, fatty acids, and dietary fibre. It has a well-balanced amino acid profile and does not contain gluten. Due to its health-promoting properties, buckwheat is becoming increasingly popular among researchers and consumers. In vitro cultures of buckwheat are crucial for research on this species because they enable studying plant regeneration processes, callus induction, organogenesis, somatic embryogenesis and synthesising phenolic compounds.

Our research focuses on both buckwheat species (F. esculentum and F. tataricum) and includes, among others, developing effective protoplast breeding techniques and obtaining somatic hybrids by protoplast electrofusion. For the first time, we obtained stable F. tataricum transformants by expressing the green fluorescent protein GFP and GUS (beta-glucuronidase). Moreover, the development of the transformation technique allowed us to inactivate the phytoene desaturase gene using the CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeats-associated protein 9), allowing us to obtain albino plants. The conducted research opens new possibilities in basic and applied research, allowing the full potential of buckwheat to be used.

Acknowledgments:

Research financed by the National Science Center, project OPUS 19 (2020/37/B/NZ9/01499) and Sonata Bis (2020/38/E/NZ9/00033).

Keywords: common buckwheat, in vitro callus induction, in vitro plantlet regeneration, Tartary buckwheat, tissue culture

1 University of Silesia in Katowice
2 University of Agriculture in Krakow
3 Bydgoszcz University of Science and Technology

Title:

Cell wall reconstruction in hybrid protoplasts derived from Fagopyrum calli

Author/Authors:

Renee Perez-Perez[1], Anna Milewska-Hendel[1], Katarzyna Sala-Cholewa[1], Ewa Grzebelus[2], Alexander Betekhtin[1]

Abstract:

Buckwheat is an excellent nutritional source containing important phenolic compounds such as rutin and quercetin. To date, 22 species have been identified, among which common buckwheat (*F. esculentum*, *Fe*) and Tartary buckwheat (*F. tataricum*, *Ft*) are the most cultivated. Common buckwheat has a wider distribution and better ability to grow in poor soils; however, it forms dimorphic plants, resulting in self-incompatibility. On the other hand, Tartary buckwheat has better nutritional value and antioxidant activity and is a self-compatible species. Attempts to combine *F. esculentum* with *F. tataricum* or another wild species to improve crop yield by traditional methods have been unsuccessful due to the strong pre- and post-zygotic barriers that prevent cross-pollination between different species. The protoplast fusion technique offers the opportunity to transfer desired features from one species to another regardless of the interspecific crossing barriers. During protoplast culture, the cell wall is rebuilt de novo to ensure cell division, aggregates, and microcalli formation. The cell wall reconstruction pattern and timing differ between species or cell types. In this work, the differences between the detection of antibodies that recognise the components of the cell wall (cellulose, extensins, pectins, AGPs, xyloglucans) of parental protoplasts (*F. esculentum*, *F. tataricum*), and their hybrids (*Fe + Fe*, *Ft + Ft*, *Fe + Ft*) in different time points of the culture, were analysed. The results show a high similarity between all reconstruction patterns with specific differences in the spatio-temporal appearance of individual epitopes during the 72 h of the cell culture.

Acknowledgments:

Research funded by the National Science Centre Poland-Project Sonata Bis no: 2020/38/E/NZ9/00033.

Keywords:

homokaryon, heterokaryon, antibodies, epitopes, cell wall rebuilding

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2 University of Agriculture in Krakow

Title:

Efficient Micropropagation Method for Elite Miscanthus × giganteus Genotypes: Towards Sustainable Bioenergy Production.

Author/Authors:

Karolina Sobańska[1], Piotr Jedryszek[2], Stephen P. Long[3]

Abstract:

Miscanthus × giganteus, a perennial grass, emerges as a promising feedstock candidate for future low carbon emission Bioenergy production, owing to its exceptional biomass and biofuel yields, particularly in marginal lands. However, widespread adoption has been impeded by the dependence on sterile clones of triploid forms, necessitating labor-intensive rhizome propagation methods. In this study, we introduce an innovative micropropagation technique tailored for two elite *M. × giganteus genotypes*, Illinois and Ogi80, sourced from field-grown specimens. By implementing optimized surface disinfection and in vitro culture protocols, we achieved efficient somatic embryogenesis from shoot apical meristem (SAM) explants. Our findings showcase successful SAM differentiation into somatic embryos on specific growth medium formulations, resulting in substantial regeneration efficiencies for both genotypes after a 15-week culture period. Remarkably, plants regenerated via this approach exhibited biometric traits comparable to rhizome-propagated counterparts, with genotype Illinois demonstrating superior performance in select key traits. This pioneering endeavor represents the first successful somatic embryogenesis from vegetative tissues of field-harvested *M. × giganteus*, presenting a competitive alternative to rhizome propagation for augmenting yield potential in bioenergy production systems (Fig. 1.).

Figure captions:

Fig. 1. Graphic abstract

References:

1. Sobańska, K., Jedryszek, P., Kern, C., Basińska-Barczak, A., Pniewski, T., Long, S.P., 2022. An efficient indirect plant regeneration from shoot apical meristem (SAM) derived embryogenic callus of Miscanthus × giganteus. Biocatal. Agric. Biotechnol.

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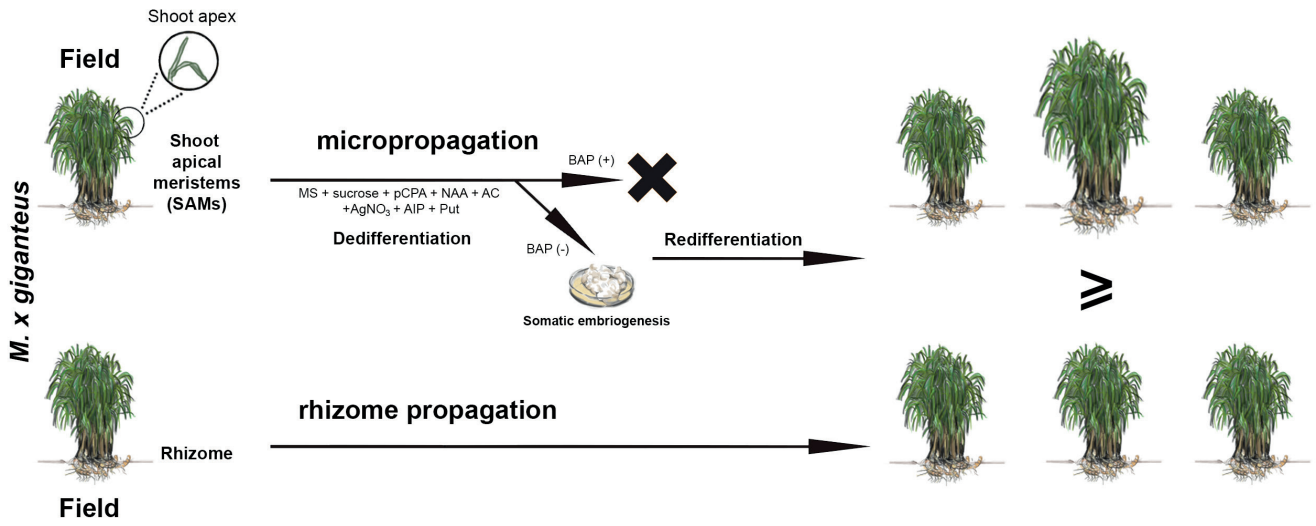
Acknowledgments :

Authors are thankful to Prof. Erik Sacks for providing the Miscanthus germplasm from The Energy Farm at the University of Illinois and to Kaleb Herman for his help in running in vitro cultures and plant care. Authors are thankful to Zuzanna Grochowska for preparing a graphic abstract. This work was supported by the U.S. Department of Energy, Office of Biological and Environmental Research

[Grant No. DE-SC-0018254] within project Renewable Oil Generated with Ultra-productive Energycane (ROGUE).

Keywords:

Callus induction, micropropagation, Miscanthus × giganteus, plant regeneration, shoot apical meristem, somatic embryogenesis



P10.1

Title:

Distillation residue-liquor from bioethanol production as a component of the substrate for the cultivation of Chlorella vulgaris microalgae

Author/Authors:

Katarzyna Górska[1], Joanna Bodakowska- Boczniewicz[1], Zbigniew Garncarek[1]

Abstract:

Distillation residue-liquor from bioethanol production can be a carbon source for heterotrophs. Microalgae Chlorella *vulgaris* can combine autotrophic and heterotrophic techniques under easy access to a carbon source.

The work aimed to determine the optimal addition of the distillation residue-liquor to the synthetic B11 medium used to cultivate *Chlorella vulgaris* 256. The optimization criterion was the maximization of algal biomass concentration.

Shake cultures of microalgae were carried out by adding distillation residue-liquor ranging from 0 to 70 mL per 150 mL of medium. Cultures were carried out at a temperature of 28°C, periodic irradiation to blue and red light: 12 h - light phase and 12 h - dark phase. The relationship between the biomass concentration and the amount of distillation residue-liquor added to the B11 medium and the optimal amount of distillation residue-liquor in the B11 medium (38 mL per 150 mL of medium) was determined using MATLAB. It allowed the obtainment of 1.19 g of biomass per 1 L. The optimal medium was used in bioreactor culture, obtaining, after 212 hours, 1.15 g of biomass in 1 L of cultivation medium. The COD reduction was 90%.

In the next stage, the B11 medium with distillation residue liquor was supplemented with glucose. The optimal medium was determined in a planned optimization experiment. The optimal medium was used for bioreactor culture. After 212 hours of cultivation, above 12 g of algal biomass per 1L of culture medium was obtained.

The concentrations of ammonium nitrogen and phosphorus were determined using Hach Lange cuvette tests, nitrate nitrogen by HPLC, protein using the Lowry method, and glucose using the enzymatic method. Glucose and nitrate nitrogen were wholly removed from the reaction mixture, and the phosphorus reduction was 85% and ammonium nitrogen 64%.

The synthetic B11 medium supplemented with distillation residue-liquor and glucose is suitable for producing biomass *Chlorella vulgaris* 256 microalgae.

Keywords: Chlorella vulgaris, distillation residue-liquor, batch cultures

1 Department of Biotechnology and Food Analysis, University of Economics and Business

P10.2

Title:

Application of the embryo rescue technique in breeding of polyploid hybrids in genus Ribes.

Author/Authors:

Aleksandra Machlańska[1], Agnieszka Marasek-Ciołakowska[1], Stanisław Pluta[1], Łukasz Seliga[1], Małgorzata Podwyszyńska[1]

Abstract:

Blackcurrant (*Ribes nigrum* L.) is economically important fruit crop in Poland. New varieties of black currant with increased resistance/tolerance to biotic and abiotic stresses and with improved quality of fruit, with larger, attractive fruits, better taste, delicate flesh and fewer seeds are in great demand. The sources of genetic variability in crop plants are distant crossbreeding and polyploidization. Black currant and almost all of them varieties are diploids. Homogeneous tetraploids of cultivars 'Gofert' and 'Polares' were obtained in The National Institute of Horticultural Research in Skierniewice, Poland (Podwyszyńska and Pluta, 2019). Crosses were made between selected clones derived from the 'Gofert' and 'Polares' cultivars involving diploid and tetraploid plants 2x × 4x, 4x × 2x and 4x × 4x. Embryo abortion was observed due to the failure of endosperm development in the interploid crosses. The aim of this study was to use embryo rescue (ER) techniques to overcome post-zygotic barriers and to prevent the embryos abortion. Immature embryos were isolated 40 and 55 days after pollination and placed directly on White's solid medium (1943) with the addition of 20 g/l of sucrose and 1 mg/l of kinetin. Embryos were subcultured to induce germination, and subsequently, to develop plants in a 16 h day/night photoperiod with 30-40 $\mu mol.m^{-2}.s^{-1}$ during the day. The highest percentage of embryos starting to grow was observed in the group of embryos isolated at the torpedo stage 55 days after pollination. Completely regenerated and adapted to *in vivo* conditions plants were obtained from crosses at tetraploid level (4x × 4x). The obtained seedlings were multiplied in *in vitro* cultures, acclimatized and planted *ex vitro*.

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1 The National Institute of Horticultural Research

Acknowledgments:

The research was carried out as part of the special-purpose subsidy of the Ministry of Agriculture and Rural Development - Task 3.7: "Production of initial materials of black-currant with dessert-quality fruit, useful for trellising system cultivation and resistant to gall mite, leaf and shoot diseases".

Keywords:

Ribes nigrum L., embryo rescue, breeding, ploidy levels, post-zygotic barriers

Title:

Plant regeneration from mesophyll-derived protoplasts of selected Brassica crops

Author/Authors:

Kamil Szymonik[1], Adrianna Putowska[1], Katarzyna Stelmach-Wityk[1], Ewa Grzebelus[1], Agnieszka Kiełkowska[1]

Abstract:

Plant protoplasts are cells with removed cell wall, which in certain conditions, have the ability to rebuild a cell wall and undergo cell divisions, allowing whole plant regeneration. Optimizing an efficient and reproducible protocol for protoplast culture opens the door to other applications, such as cell electrofusion or vector-free transfection. These techniques allow to increase variability more rapidly compare to conventional methods. Unfortunately, in the Brassica genus, the efficiency of protoplast isolation and regeneration is highly genotype-specific.

The aim of the study was to optimize the factors involved in the regeneration of Brassica protoplast cultures. Different varieties of red cabbage (*B. oleracea var. capitata f. rubra*), Brussels sprout (*B. oleracea var. gemmifera*) and kale (*B. oleracea var. sabellica*) were used.

Spherical protoplasts were released from mesophyll of 4-week-old seedlings after 14-16 h incubation in the maceration mixture. The yield of protoplasts ranged from 0.9 to 6.1 × 106 per gram of fresh tissue. Five days after isolation intense cell divisions of protoplast-derived cells were observed. Fifteen days after isolation, the division rate varied from 45 to 78%. Observed cell aggregates formed visible microcalli in three weeks-old cultures. Microcalluses released from alginate were transferred to different regeneration media. Light stimulated callus greening and indirect organogenesis. First shoots developed on callus after about three months of culturing on solid medium. The highest efficiency was observed for kale (33%), but other accessions also formed shoots (2-13%). Developed shoots were transferred to growth-regulator-free medium for further development, and after rooting were acclimatized to ex-vitro conditions.

The study showed that culture conditions at different stages of protoplast culture, from single cells to microcalli, affect the efficiency of plant regeneration and can vary even within the same variety.

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Acknowledgments:

The research was financed by Polish Ministry of Agriculture and Rural Development (Biological advancement in plant production 2021-2027).

Keywords:

red cabbage, kale, indirect organogenesis

Title:

Inhibitors of DNA methylation and histone deacetylation impact the regenerative capacity of parsnip protoplasts

Author/Authors:

Katarzyna Stelmach-Wityk[1], Ewa Grzebelus[1]

Abstract:

Epigenetic silencing is a natural phenomenon in which the expression of genes is regulated through modifications of DNA, RNA, or histone proteins. DNA methylation and histone deacetylation are considered major determinants of epigenetic silencing, as they lead to chromatin modifications resulting in a decreased transcriptional availability of genes. In vitro plant cell and tissue culture techniques serve as the foundation for numerous micropropagation and breeding programs. Epigenetic changes play a crucial role in determining cell differentiation, organogenesis, and somatic embryogenesis in in vitro cultures.

Parsnip is a monocarpic biennial crop of growing economic significance. Therefore, it is important to develop an efficient method for regenerating plants from protoplasts of parsnip.

The aim of this study was to evaluate the effect of inhibitors of DNA methylation and histone deacetylation on the regenerative ability of protoplasts derived from two parsnip cultivars. For this purpose, mesophyll-derived protoplasts were treated with either azacitidine (azaC) or vorinostat (SAHA).

The yield of protoplasts released from leaves ranged from 2.3 to 7.4 × 10^6 per gram of fresh tissue, depending on the accession. Their viability ranged from 68% to 85%. The survival rate depended on the genotype and the specific inhibitor used. Ten days after isolation, the protoplasts displayed changes characteristic for properly developing cultures, i.e., reorganization of the cytoplasm, increased cell volume, and changes of cell shape. Approximately 30 days after isolation, the protoplast-derived cell colonies were formed. After 90 days they developed into microcalli overgrowing alginate layers. AzaC significantly reduced the frequency of callus formation, while SAHA did not reduce callus formation. When transferred to a solid medium, callus proliferated and formed a proembryogenic mass within two to five months in azaC treated cultures and after five months in SAHA treated cultures.

Keywords:

protoplasts, parsnip, DNA methylation, histone deacetylation

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Title:

Ovule cultures in Vicia faba L. after foreign pollination

Author/Authors:

Agnieszka Kiełkowska[1], Natalia Gumulak Wołoszyn[1], Wiktor Skrzypkowski[1]

Abstract:

Doubled haploids (DH) plants have high potential for shortening the breeding of new varieties in plants by producing true homozygous lines in one generation. This technology was successfully applied in breeding of many important crops i.e. rape, wheat, barley, rice, tobacco or triticale (Forster et al. 2007). *V. faba* is a popular vegetable consumed worldwide, however a protocols for its haploidization have not been developed yet. The very few available trials on haploidization in *V. faba* concerned androgenesis and resulted with callus development, but no plant regeneration (Paratasilpin 1978, Hesemann 1980, Shlahi et al. 2012).

The study aimed at the stimulation of the development of haploid cells of the female gametophyte of V. faba after distant pollination with *Lathyrus odoratus*. The germination of foreign pollen grains on stigmas under a fluorescence microscope with aniline blue was analyzed. The results showed, that pollen of *L. odoratus germinates* on the stigma of *V. faba*, however in any of the tested samples it did not entered transmission track in the style nor reached the ovules, what make it suitable for stimulation of parthenogenesis. After 5-7 days after pollination pistils of *V. faba* (from pollinated flowers and unpollinated controls) were collected and surface disinfected. Then culture of pistil and isolated ovules was established. The development of the explants during *in vitro* culture was monitored. Majority of the cultured pistils (both pollinated and control) browned and died, while survived explants produced callus. Microscopic analyses of callus samples showed its somatic origin. In the cultures of isolated ovules 14-80% of cultured explants died, but approximately 40% of survived ovules produced callus tissue. The callus was observed mainly at the micropylar end of the developing ovules.

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Acknowledgments:

The research was financed by Polish Ministry of Agriculture and Rural Development (Biological advancement in plant production 2021-2026).

Keywords:

faba bean, gamethophte, haploid, pollen

P10.6

Title:

From single cell to plants: protoplasts of Fagopyrum as an efficient system for acquiring totipotency. Insight from the proteomics point of view

Author/Authors:

Magdalena Zaranek[1], Artur Piński[1], Kamila Godel-Jędrychowska[1], Bożena Skupień-Rabian[2], Urszula Jankowska[2], Ewa Kurczyńska[1], Ewa Grzebelus[3], Alexander Betekhtin[1]

Abstract:

The genus buckwheat (*Fagopyrum*) is a multipurpose crop with a high nutritional value, mainly high-quality proteins, and various phenolic compounds, especially rutin, quercetin, and C-glycosyl flavones, which have a positive therapeutic or dietary effect for promoting human health. *Fagopyrum esculentum* (common buckwheat) and *F. tataricum* (Tartary buckwheat) are the most commonly cultivated species.

Recently, our group demonstrated the ability to reprogram buckwheat cells from a differentiated state to a de-differentiated state, regaining totipotency. Effective regeneration of buckwheat cells derived from morphogenic callus in protoplast culture was reported for the first time. As a result, buckwheat protoplast technology made it possible to track the changes in the cell wall architecture and total proteome.

Immunocytochemistry allowed the detection of the occurrence and distribution of important cell wall components, i.e., polysaccharides and hydroxyproline-rich proteins. In turn, "shotgun" proteomics was used to identify proteins present during important time points of protoplast culture when the following were observed: first cell divisions, formation of multicellular aggregates, and microcallus.

Acknowledgments:

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Keywords:

buckwheat, dedifferentiation, differentiation, proteome, cell wall

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3 University of Agriculture in Krakow

Title:

Comparative analysis of chitosan and auxin concentrations on in vitro rooting of highbush blueberry (Vaccinium corymbosum L.) cv. Liberty

Author/Authors:

Marcelina Krupa-Małkiewicz[1], Ireneusz Ochmian[2]

Abstract:

Blueberries, belonging to the genus *Vaccinium* L. and family Ericaceae Juss., are witnessing an increase in global popularity. Poland has emerged as a major player, ranking as the second-largest producer of highbush blueberries in Europe and seventh worldwide (Brazelton, 2013). According to Cüce and Sökmen (2017), the genus *Vaccinium* is more variable than other members of the Ericaceae family. These genetic variations manifest in different growth habits, fruiting times, and responses to environmental conditions.

This study assessed the impact of auxin and chitosan on *in vitro* rhizogenesis. Following the multiplication stage, explants were placed on Woody Plant Medium (WPM, Lloyd and McCown 1980) supplemented with 0.1 mg dm^{-3} zeatin, and indole-3-butyric acid (IBA) at the concentration of 1.0 and 2.0 mg dm^{-3}, or chitosan of molecular weight 800 kDa at the concentrations of 20 and 40 ppm. Our findings revealed that the combination of 2.0 mg dm^{-3} IBA and 0.1 mg dm^{-3} zeatin yielded optimal shoot and root lengths of 6.28 cm and 1.73 cm, respectively. Higher concentrations of chitosan (40 ppm) were associated with increased shoot and root growth. Remarkably, the medium with the addition of 0.1 mg dm^{-3} zeatin and 40 ppm chitosan achieved a 100% acclimatization rate. Additionally, leaves from blueberries cultivated in this medium showed enhanced brightness by 22 to 36% and greater greenness (CIE a* values -2.70 to -4.56) compared to those grown in media supplemented with IBA.

The findings from this study contribute to the understanding of *in vitro* rooting processes for V. corymbosum L. cv. Liberty. The experimental results demonstrated that adding chitosan with a molecular weight of 800 kDa to the WPM medium achieved a rooting rate comparable to that obtained with the auxin IBA. Further research to improve the rooting protocol of V. corymbosum L. is expected to increase plant survival during subsequent phases of adaptation to ex vitro conditions.

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Acknowledgments:

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Keywords:

plant acclimatization, biopolymer, rhizogenesis, WPM medium, highbush blueberry, Cie L*a*b*

P10.8

Title:

Haploidization in male sterile tomatoes (Solanum lycopersicum L.) via ovule culture

Author/Authors:

Wiktor Skrzypkowski[1], Agnieszka Kiełkowska[1]

Abstract:

Plant haploidization is a method that allow for the acceleration of development of plant breeding programs. Its undeniable advantage is the significant reduction in the time required to produce homozygous plants by doubling the genome of haploid forms, resulting in the generation of so-called doubled haploids (DH). Effective haploidization procedures have been implemented for many commercially important plant species but for tomato, effective methods are still lacking. Attempts at haploidization in tomatoes began in the 1970s and 1980s and initially relied on attempts to induce androgenesis in anther culture (Gulshan et al. 1981) and microspores (Sharp et al. 1972; Bal & Abak 2005). As a result, only a few callus structures were obtained, which did not undergo regeneration and further conversion into plants. Attempts at inducing gynogenesis also did not yield the expected results; while they led to plant regeneration, they originated from the development of somatic tissues (Zhao et al. 2014).

These studies aimed on inducing the development of haploid cells within the ovule sac of tomato. For this purpose, ovaries were excised from flower buds of two male sterile breeding lines (II-PS and I-MS10) at the stage just before anthesis. After surface sterilization, ovules were isolated by crushing the ovary and releasing the ovules separated from the placental tissue.. They were then cultured on media inducing callus development (B5 medium supplemented with TDZ, NAA, and 2,4-D). Callus production was observed in both genotypes during the culture period. Efficient callus production allowed for ploidy analysis, which revealed variable ploidy levels in the calli, including diploid and mixoploid ones; however, haploid calluses were also identified.

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Acknowledgments:

Acknowledgements: The research was financed by Polish Ministry of Agriculture and Rural Development (Biological advancement in plant production 2021-2026).

Keywords:

haploidization, gynogenesis, tomato, in vitro

Title:

Salicylic acid enhances viability and mitotic divisions of Nigella damascena protoplasts

Author/Authiors:

Magdalena Klimek-Chodacka[1], Magdalena Pieczara[1], Rafał Barański[1]

Abstract:

Nigella damascena L. (*Ranunculaceae*), commonly known as love-in-a-mist, has been used in traditional medicine for centuries due to its diverse pharmacological properties. Its seeds contain a wide range of bioactive compounds, including β-elemene and the alkaloid damascenine, which exhibits antibacterial, antifungal, antitumour, antioxidant, anti-inflammatory, antipyretic and antiviral activities.

In this study, we investigated the effects of 25, 50 and 75 µM salicylic acid (SA) on the viability and mitotic divisions of *Nigella* damascena protoplasts.

The viability of protoplasts in the control treatment (without SA) was approximately 91% after 24 hours of culture. Supplementation with SA did not significantly affect protoplast viability, which remained around 90%. Protoplast viability decreased with time in all treatments. In the control group, viability decreased to 88%, while the addition of SA did not result in a significant decrease. Mitogenic activity was also affected by SA treatment. In the control, the average mitotic activity was 34% and 43% after 7 and 14 days of culture, respectively. Supplementation with 25 µM SA stimulated mitotic divisions, so the mitotic activity increased to 62% after 14 days. Higher concentrations of SA (50 and 75 µM) had a suppressive effect on mitotic activity, with values ranging from 26% to 42% after 14 days.

In conclusion, our results show that salicylic acid at a concentration of 25 µM enhances the viability and mitotic divisions of *Nigella* damascena protoplasts. This suggests that SA may have potential applications in optimising protoplast culture systems for *Nigella* damascena and other plant species, potentially leading to increased production of secondary metabolites with therapeutic value.

Acknowledgments:

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Keywords: Nigella damascena, tissue culture, Salicylic acid, protoplast

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P10.10

Title:

Influence of titanium oxide nanoparticles on Nigella damascena L. in vitro cultures

Author/Authors:

Dominik Huber[1], Magdalena Klimek-Chodacka[1], Agnieszka Szewczyk[2], Rafał Barański[1]

Abstract:

Plant tissue cultures are a potent source of a wide range of secondary metabolites with various medicinal properties. Among many other techniques, elicitation can significantly increase the content of bioactive compounds in tissue cultures. Nanoparticle elicitation has been shown to increase the levels of phenolics, flavonoids, stilbenes, alkaloids and antioxidant activity in various plant species. *Nigella* damascena, an annual plant species is an important member of the buttercup family (*Ranunculaceae*). It is capable of synthesizing various secondary metabolites with medicinal applications, as evidenced by scientific research. Seed extracts and essential oils have anti-cancer, anti-inflammatory, antipyretic, analgesic, diuretic and mild antimicrobial properties. The main constituents of the essential oil extracted from the seeds are β-elemene and damascenine. β-elemene has antibacterial, anticancer, and anti-inflammatory properties, while damascenine has anti-inflammatory, antipyretic, analgesic, and diuretic effects.

The aim of the research was to investigate the effect of titanium oxide nanoparticles supplemented growth media on the content of secondary metabolites and dry biomass of *Nigella* damascena *in vitro* cultures. The response of *in vitro* cultures to the titanium oxide nanoparticles supplementation in the range of 5 to 400 mg/L was determined. The research showed the effect of nanoparticles on the dry mass content and the content of substances determined using high-performance liquid chromatography (HPLC). The highest increase in dry mass (120.8% compared to the control) was observed after application of nanoparticles at a concentration of 100 mg/l, further increase in nanoparticles concentration resulted in dry mass decline. Nanoparticles supplementation showed an inhibitory effect on the synthesis of chlorogenic and neochlorogenic acid in concentration-dependent manner

Acknowledgments:

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Keywords: Nigella damascena, secondary metabolites, nanoparticles, in vitro cultures

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Dyskusja panelowa:

NOWE TECHNIKI GENOMOWE – WPROWADZENIE

Title:

NGT – New Genomic Techniques – Why are they new ?

Author:

Rafał Barański[1]

Abstract:

According to EU nomenclature, New Genomic Techniques (NGT) are a group of diverse techniques used to alter the genetic material of an organism that have been developed after the adoption of EU legislation on genetically modified organisms (GMOs) in 2001. Consequently, some of NGTs are more than 20 years old and their use in plant breeding has led to the development of new cultivars that have already been commercialised in some countries, but outside the EU. The most powerful NGTs, such as those based on the CRISPR/Cas system, allow for planned changes to target genes or non-coding regions of the genome with high precision and efficiency. Usually the changes are small mutations causing gene knockouts, but the latest tools work with better specificity and predictability, enabling also precise gene correction. The use of NGTs may also led to the development of cells and subsequently regenerated plants having mutations at target sites that are free of foreign DNA or, in particular cases, mutations can be induced even without the use of foreign DNA. All these aspects give plants obtained using NGTs a significant advantage over GM plants and force the implementation of new regulations. Although NGTs are cost effective and have been introduced to plant breeding, their use may be slowed down if knowledge of the genetics of the plant target trait, genome structure or gene expression is limited. There may be also biological or technical constraints associated with the use of other biotechnologies that accompany NGT application, including cell or tissue culture and plant regeneration in vitro, and therefore need to be verified and optimised on a case-by-case basis before NGT tools are design and used.

Keywords:

plant genome editing, targeted mutagenesis, CRISPR/Cas tools

1 University of Agriculture in Krakow

Title:

The EU regulatory proposal for NGT plants – where are we headed?

Author:

Tomasz Zimny[1]

Abstract:

In the presentation I discuss the EU Commission's proposal for a regulation on plants obtained through new genomic techniques (NGT)[1], along with its several variants suggested throughout the adoption process. I present possible consequences of the adoption of the discussed variants for the R&D sector, as well as possible consequences of the project failing altogether.

The proposal presented by the EU Commission in July 2023 envisages a new regime for performing research with, and marketing of plants obtained through the application of NGTs (currently falling under the rigid GMO regime[2–4]). In broad terms, such plants shall be divided into two categories – NGT-1, deemed equivalent with conventional plants and NGT-2 – plants to which the current GMO regime will generally apply.

The project was met with mixed responses both by various stakeholders[5, 6] and the decision makers in the EU[7], hence its final shape and fate are, as of the time of writing of this abstract, uncertain. The issues raised throughout the adoption process include:

- the criteria of equivalence between NGT-1 and conventional plants,
- the justification of removal of NGT-1 plants from the organic sector and the practical possibility to separate the conventional and organic production chains,
- member states' ability to opt-out from the cultivation of NGT-2 plants,
- issues regarding risk assessment and detectability[8],
- issues regarding the patentability of NGT plants and their possible removal from patentability.

Some of the proposed amendments address evident drawbacks of the proposal (e.g. considering the ploidy of a plant when counting the number of modifications), while other touch controversial issues through adding new matter (e.g. the issues of patentability). I shall present the consequences of the adoption of the various amendments, as well as possible failure to adopt the proposal during the current presidency, in light of similar regulations adopted in third countries[9]

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Acknowledgments:

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Keywords:

NGT, GMO, CRISPR, EU Law

Title:

Placing on the market of plants obtained with new genomic techniques – challenges in the detection

Author:

Sławomir Sowa[1]

Abstract:

Authorisation of Genetically Modified Organisms (GMOs) for food an feed use or for cultivation in the European Union (EU) requires developing and validating a reliable method for detection and identification of each GMO event. In 2018 the European Court of Justice (Judgment in Case C-528/16) ruled that plants obtained by new genomic techniques (NGT) are GMOs which are, subject to the obligations laid down by the GMO Directive 2001/18. NGTs are various techniques of genetic modification that have been developed after 2001. They allow for precise genome editing of the plant genetic material (e.g. CRISPR,). The application of NGT can result in different types of DNA mutations and even insertion of a large DNA fragments. In July 2023 the European Commission published a proposal of Regulation on plants obtained by NGTs in which two categories of NGT plants have been discriminated. The category 1 NGT plants that are equivalent to the conventional plants to which the GMO regulations does not apply. These plants in general cannot differ from the parental plant by no more than 20 genetic modifications (substitution or insertion of no more than 20 nucleotides) or any other targeted modification of any size, on the condition that the resulting DNA sequences already occur in a species from the breeders' gene pool. The category 2 NGT plants for which environmental risk assessment shall be carried out in accordance with the principles set out in the Directive 2001/18/EC. Detection of short mutations and even the single nucleotide variants is possible with currently used analytical methods however identification of such GMOs might be impossible as the same type of modification can occur naturally or can be induced by conventional mutagenesis. Therefore new GMO detection methods and control strategies must be developed to ensure proper control of the EU market.

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Keywords: GMO, New Genomic Techniques, detection

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