TANGENC
TowArds Next GENeration Crops

OP JAK TANGENC

TOWARDS NEXT GENERATION CROPS: FROM DISCOVERY TO APPLICATIONS

28. - 29. 04. 2025

BOOK OF ABSTRACTS

IBP
Institute of Biophysics

TANGENC
TowArds Next GENeration Crops

OP JAK TANGENC

TOWARDS NEXT GENERATION CROPS: FROM DISCOVERY TO APPLICATIONS

28. - 29. 04. 2025

WITH THE SUPPORT OF THE PROGRAM:

SUSTAINABLE FOOD PRODUCTION AND CONSUMPTION
VP35

Czech Academy of Sciences

STRATEGY AV21
Top research in the public interest

IBP Institute of Biophysics

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

TANGENC
TowArds Next GENeration Crops

OP JAK TANGENC

TOWARDS NEXT GENERATION CROPS: FROM DISCOVERY TO APPLICATIONS

28. - 29. 04. 2025

MANY THANKS TO OUR CONFERENCE PARTNERS

SVEN BIOLABS S.R.O.
WWW.SVENBIOLABS.CZ

BioTech innovative

BIO-RAD

baria

IBP Institute of Biophysics

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

Monday, 28. 4. 2025			
Number:	Time:	Author:	Talk:
1.	10:30	Dmytro Abramov	Genetic engineering of Czech hop cultivars
2.	10:55	Veronika Jedličková	CRISPR-based genome editing in *Brassicaceae* using tissue culture systems
3.	11:20	Said Hafidh	From *Arabidopsis* to seaweed, investigating thermotolerance in a wider context
4.	11:45	Adéla Přibylová	Advanced bioengineering tools: News and Views
	12:20	SVEN BioLabs	Možnosti high-end zobrazovacích systémů z portfolia SVEN BioLabs
5.	14:30	Tomáš Vlčko	New trait for reduced plant height
6.	14:55	Kateřina Kaduchová	Transforming barley: Novel approaches and their application outcomes in the TANGENC project
7.	15:20	Mária Majeská Čudejková	Barley transformation and its application inthe frame of TANGENC

Tuesday, 29. 4. 2025			
Number:	Time:	Author:	Talk:
8.	9:00	Lev Levenets	Emerging evidence of rapid auxin response beyond *Arabidopsis*
9.	9:25	Markéta Pernisová	The impact of cytokinins and cytokinin metabolism in *in vitro* shoot regeneration
10.	9:50	Marie Komrsová	Differentially expressed genes in two distinct cytoplasmic male sterility (CMS) types of *Silene vulgaris*
11.	13:00	Ludmila Ohnoutková	History of barley cultivation
12.	13:25	Petr Smýkal	Domestication and harnessing plasticity in plant breeding for crop resilience

1.

GENETIC ENGINEERING OF CZECH HOP CULTIVARS

DMYTRO ABRAMOV

Hop (*Humulus lupulus* L.) is a perennial herbaceous plant belonging to the *Cannabaceae* family. In the Czech Republic, it holds an important place in agricultural production, with a long-standing tradition of cultivation and research. Best known for its essential role in brewing, the female flowers of hop contribute the characteristic bitterness, aroma, and flavor. Given the current global climate change, hop cultivation faces new challenges. High temperatures directly affect the growth, flowering and chemical composition of hop.

The main objective of this study is to develop hop lines with early flowering because hop has only one generation per year. It will greatly improve the efficiency of hop research and will enable us to apply functional genomics tools on the hop in the future, leading to the development of new hop varieties that are better able to cope with the challenges of climate change. To achieve this goal, it is necessary to improve the methods of transgene delivery using *Agrobacterium*-mediated transformation, as well as the conditions for *in vitro* regeneration of transgenic material.

In this project, we will combine state-of-the-art genetic engineering methods to provide a tool for functional genomics and the improvement of hop cultivars.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

2.

CRISPR-BASED GENOME EDITING IN *BRASSICACEAE* USING TISSUE CULTURE SYSTEMS

VERONIKA JEDLIČKOVÁ

CEITEC MU - CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY, MASARYK UNIVERSITY, BRNO, CZECH REPUBLIC

Hairy root cultures represent a versatile system applicable to a wide range of studies, including the functional characterization of genes and their regulatory elements. In our laboratory, we have established a CRISPR-based genome editing protocol for oilseed rape (*Brassica napus*) using hairy roots. This transformation system offers a straightforward and rapid approach to evaluate the functionality and efficiency of diverse CRISPR editing constructs. Using an optimized regeneration protocol, we are able to produce transgene-free, genome-edited plant lines. Our future objectives involve the application of CRISPR technologies for base editing, prime editing, and transcriptional regulation within the *Brassicaceae* family using hairy root cultures.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

3.

FROM ARABIDOPSIS TO SEAWEED, INVESTIGATING THERMOTOLERANCE IN A WIDER CONTEXT

SAID HAFIDH[1], ZUZANA GADIOU[1], PETRA ROZNOVSKA[1], STAVROS VRAGGALAS[2] AND SOTARIOUS FRAGOSTEFANAKIS[2]

[1]LABORATORY OF POLLEN BIOLOGY, INSTITUTE OF EXPERIMENTAL BOTANY OF THE CZECH ACADEMY OF SCIENCES, ROZVOJOVÁ 263, 165 00 PRAGUE 6, CZECH REPUBLIC

[2]INSTITUTE OF MOLECULAR BIOSCIENCES, MOLECULAR AND CELL BIOLOGY OF PLANTS, GOETHE UNIVERSITY FRANKFURT, FRANKFURT, GERMANY

Survival and recovery from heat stress (HS) rely on the synthesis of protective proteins, such as heat shock proteins (HSPs) and reactive oxygen species (ROS) scavengers, which help maintain cellular integrity and prevent irreversible damage. The expression of many HSPs and other HS-responsive genes is regulated by HS transcription factors (HSFs). In plants, members of the HSFA1 subfamily serve as key initiators of the HS response and are considered master regulators of thermotolerance. Their activity is further enhanced by interactions with other HSFs, such as HSFA2 and HSFA7.

Alternative splicing (AS) plays a crucial role in regulating stress-responsive HSFs, generating splice variants that encode protein isoforms with distinct properties and functions. Our recent studies demonstrate that alternative splicing of HSFA2 and HSFA7 mRNAs is critical for acquired thermotolerance (ATT) in tomato whereas upregulation of HSP70 miantain thermotolerance in pollen tubes of Arabidopsis thaliana. Here, we give an overview and a forward direction in implementing our studies into tomato and enviromental viable model seaweed in Zanzibar, Tanzania.

This study received financial support from the European Regional Development Fund (ERDF) Programme Johannes Amos Comenius project TowArds Next GENeration Crops (TANGENC) [reg. no. CZ.02.01.01/00/22_ 008/0004581]

4.

ADVANCED BIOENGINEERING TOOLS: NEWS AND VIEWS

ADÉLA PŘIBYLOVÁ[1*], SIMONA BÜRGELOVÁ[1], TEREZA UHLÍKOVÁ[1],LUKÁŠ FISCHER[1]

[1]CHARLES UNIVERSITY, FACULTY OF SCIENCE, DEPARTMENT OF EXPERIMENTAL PLANT BIOLOGY, VINIČNÁ 5, PRAGUE 4, 128 44, CZECH REPUBLIC

*Corresponding author: adela.pribylova@natur.cuni.cz

Bioengineering tools are constantly evolving, and many more are available when one learns how to use 'the old ones'. Is it worth looking into the new ones? Is it worth trying new technologies when putting them into practice in the lab is so challenging?

Currently, the most widely used systems in genetic engineering are programmable CRISPR/Cas-based systems. They are used for gene knock-outs, knock-ins, targeted mutagenesis, regulation of gene expression, and many others. Effector Cas proteins can be targeted to specific loci based on complementarity with the carried guide RNA (usually around 20 nt long); however, the targeted sequence must be adjacent to a so-called PAM sequence (protospacer-adjacent motif). There are a number of Cas variants that recognise different PAMs (NGG, TTV, TBN, TTN, TN,...), vary in size (from 422 to 1223 amino acids; aa), differ in cleavage activity, pre-crRNA processing, etc. The most widely used variants are *Sp*Cas9 and *Lb*Cas12a, with a size of around 1200 nt and recognising the PAM sequence NGG at the 3' end and TTTV at the 5' end of guide RNA, respectively.

Despite the enormous number of advantages, these systems have several limitations, the main ones being the need for a PAM sequence, limiting the selection of target sites, and the fact that their activity results in DNA breaks (except for catalytically inactive variants), most often double-stranded, which are subsequently repaired by the repair mechanisms of the target organism, introducing a significant element of, if not random, unpredictable outcomes into the whole system.

Yet, new technologies are emerging, such as **TIGR-Tas** (Tandem Interspaced Guide RNA (TIGR)/TIGR-associated (Tas) proteins) and **bridge-RNA.**

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

Tas proteins are around 300 aa in size, do not need a PAM sequence to recognise the target site, can process their own TIGR array carrying guide tigRNA, can cleave ss/dsDNA and are mismatch sensitive, which reduces the risk of unwanted mutations (off-targets). They could be a suitable alternative to CRISPR/Cas in many ways. In contrast, the bridge RNA systems use non-coding RNA to enable sequence-targeted recombination between two DNA molecules, a tool that has been lacking to date, allowing sequence-specific insertions, inversions and excision. The system is very small, with bridge RNA around 150-250 nt and effector recombinase 300-460 aa. The functionality of TIGR-Tas has already been demonstrated in human cell lines; the bridge RNA has only been demonstrated in bacteria so far. Will it be possible to adapt these systems to large plant genomes?

This work was supported by the project TowArds Next GENeration Crops [reg. no. CZ.02.01.01/00/22_008/0004581] of the ERDF Programme Johannes Amos Comenius.

5.

NEW TRAIT FOR REDUCED PLANT HEIGHT

TOMÁŠ VLČKO

The success of the Green Revolution depended on the introduction of semi-dwarfing alleles into major cereal crops. Plant height is critical because it determines the allocation of biomass to stem elongation versus other functions. It also controls node spacing and is a key indicator of lodging resistance. The semi-dwarfing genes, such as sdw1 in barley, act by attenuating the synthesis and signalling pathway of bioactive gibberellins. These semi-dwarfing genes have negative pleiotropic effects on plants. Therefore, there is a need to expand the pool of dwarfing genes by identifying and characterising additional dwarfing genes suitable for practical application in plant breeding for future varieties with high resistance to hostile environments. Phytochrome interacting factor like 1 (PIL1) is a member of the light perception signalling pathway that controls plant growth. It is a member of the bHLH family of transcription proteins and its gene expression is controlled by the circadian clock. Mutant pil1 lines showed a reduction in plant height of about 10 cm. This indicates the potential of this trait to reduce plant height.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

6.

TRANSFORMING BARLEY: NOVEL APPROACHES AND THEIR APPLICATION OUTCOMES IN THE TANGENC PROJECT

KATEŘINA KADUCHOVÁ

INSTITUTE OF EXPERIMENTAL BOTANY. CZECH ACADEMY OF SCIENCES. OLOMOUC. CZECH REPUBLIC

Recent advances in plant biotechnology have opened new avenues for improving transformation efficiency and precision in crop species. We present a set of strategies designed to improve and enhance the barley (Hordeum vulgare) transformation pipeline and make the selection of transgenic plant more efficient. Key advances include e.g. usage of Droplet Digital PCR (ddPCR) for rapid detection and selection of homozygous lines, and strategies for boosting regeneration efficiency. We also aim to speed barley life cycle by using “Speed breeding” approach.

In addition, we will share results from ongoing collaborative work in a frame of OP JAK – TANGENC project, with a focus on the most advanced projects.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

7.

BARLEY TRANSFORMATION AND ITS APPLICATION IN THE FRAME OF TANGENC

MÁRIA MAJESKÁ ČUDEJKOVÁ AND VERONIQUE BERGOUGNOUX FOJTÍK
(CROP ENGINEERING AND BIOTECHNOLOGY, CATRIN, UPOL, OLOMOUC)

The presentation outlines the barley transformation methods currently employed in our research. Key methodological steps of the barley transformation process, along with an overview of our results-including a summary of transformation efficiency from the past year-will be discussed. The presentation will conclude with our latest results within the TANGEC project.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

8.

EMERGING EVIDENCE OF RAPID AUXIN RESPONSE BEYOND *ARABIDOPSIS*

LEV LEVENETS

Recent years have brought ample amount of attention to the newly uncovered branch of non-transcriptional auxin signaling, now termed Cytoplasmic Auxin Pathway (CAP). With many aspects of its regulation still remaining enigmatic, one is clear — in model plant *Arabidopsis thaliana* CAP is governed by AFB1 protein. AFB1 protein from TIR1/AFB protein clade is, however, an unique evolutionary occurrence confined to *Brassicaceae* family. Here we demonstrate that model grass *Brachypodium distachyon*, lacking clear AFB1 orthologs, nevertheless demonstrates some hallmarks previously attributed to CAP, such as distinct root pH zonation, early gravitropic response and fast reaction to externally applied auxin.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

9.

THE IMPACT OF CYTOKININS AND CYTOKININ METABOLISM IN *IN VITRO* SHOOT REGENERATION

JÁN ŠMERINGAI, MARKÉTA PERNISOVÁ

The phytohormones cytokinins and auxin have been known as the principal regulators of plant development for a long time. In *in vitro* regeneration assays, the presence of high auxin-to-cytokinin concentration ratio in media induces root regeneration from various plant tissues. On the other hand, if the auxin-to-cytokinin ratio is low, shoots are formed.

The process of *in vitro* shoot regeneration is accompanied by strong activation of cytokinin signalling via AHK4 receptor and induction of shoot-specific homeodomain regulator WUSCHEL specifically in the regenerating organs. Exogenous but also endogenous cytokinins influence both the initiation of newly formed organs as well as the pace of organ developmental sequence. The role of cytokinins, importance of cytokinin biosynthesis and metabolism in the control of *in vitro* shoot regeneration will be discussed.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

10.

DIFFERENTIALLY EXPRESSED GENES IN TWO DISTINCT CYTOPLASMIC MALE STERILITY (CMS) TYPES OF *SILENE VULGARIS*

MARIE KOMRSOVÁ[1], FLORENCIA VERÓN SCHÖENFELD[1], HELENA ŠTORCHOVÁ[1]

[1]INSTITUTE OF EXPERIMENTAL BOTANY OF THE CZECH ACADEMY OF SCIENCES, ROZVOJOVÁ 263, 16502 PRAGUE 6, CZECH REPUBLIC

E-mail: komrsova.m@ueb.cas.cz

Silene vulgaris, a gynodioecious plant, has been used as a model for the study of cytoplasmic male sterility (CMS). Comparative transcriptomic analysis has previously revealed differentially expressed nuclear genes in female and hermaphrodite flower buds from two populations with distinct mitochondrial haplotypes, KOV and KRA.

Backcrossing of KOV and KRA was used to produce plants with the mitochondria of one population and the nuclear background of the other. Transcriptomes were constructed from a small set of females and hermaphrodites from each backcross to identify differentially expressed genes of interest.

This work aims to validate the expression of these genes of interest in more extensive sets of backcrossed plants. New backcrosses will be generated and plants of each gender will be investigated using RT-qPCR to determine whether the differences in gene expression are consistent.

Funding: project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581, of the ERDF Programme Johannes Amos Comenius.

11.

HISTORY OF BARLEY CULTIVATION

LUDMILA OHNOUTKOVÁ

Barley is one of the oldest agricultural crops and played a key role in the development of civilisation. It is one of the earliest domesticated crops in the world, beginning around 9,000 BC and ending around 7,000 BC. There is evidence for at least five different domestications: at least three sites in the Fertile Crescent, one in the Syrian desert, and one on the Tibetan plateau. According to archaeobotanical findings, barley was present in Europe in the Neolithic-Younger Stone Age (roughly 8,000 to 5,000 BC) in Cyprus, Turkey, and Greece. On the territory of the Czech Republic in the Older Stone Age, barley occurs in admixture with single-grain wheat at 80% of locations. In the early Middle Ages, naked two-row barley, which was more suitable for milling, began to be cultivated in Europe instead of four-row plough barley. The second half of the 19th century saw the development of the agricultural business, the establishment of sugar mills, breweries and the intensification of the cultivation of agricultural crops, including barley. Local landraces, a mixture of populations, were sown, and random simple selection was carried out. Especially in Haná, regional varieties were systematically selected, which formed the basis for further malting barley breeding throughout the world.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

12.

DOMESTICATION AND HARNESSING PLASTICITY IN PLANT BREEDING FOR CROP RESILIENCE

PETR SMÝKAL

DEPARTMENT OF BOTANY, FACULTY OF SCIENCE, PALACKY UNIVERSITY IN OLOMOUC
E-MAIL: PETR.SMYKAL@UPOL.CZ

Over the past 10,000 years, nearly all crops and vegetables consumed today have been domesticated from wild ancestors. This domestication process, often guided by a few key genetic changes, involved early humans selecting plants based on traits beneficial for survival and cultivation. These selections inadvertently led to the accumulation of advantageous mutations and recombinants, ultimately giving rise to crops that were easier to grow, harvest, and store. However, the environmental conditions in which modern crops are cultivated differ significantly from those of their wild progenitors. In natural ecosystems, plants are constantly exposed to a range of abiotic and biotic stresses, including heat, cold, drought, pests, and pathogens, and have evolved robust defense mechanisms to complete their life cycles under such pressures. These adaptive responses have been maintained through natural selection. In contrast, domestication shifted the selection pressure toward human preferences, while the development of agricultural practices reduced the need for plants to adapt to environmental variability. Modern breeding, with its emphasis on high-yielding cultivars optimized for high-input systems, has inadvertently increased crop sensitivity to environmental stress. As climate change intensifies and extreme weather events become more frequent, the threat posed by environmental stressors to agricultural productivity is growing. In this context, ***phenotypic plasticity***, the capacity of a single genotype to express different phenotypes in response to environmental variation, has emerged as a critical trait. For plants, which are immobile, plasticity is especially vital for coping with changing conditions. Genotypic variation in plasticity is reflected in genotype-by-environment (G×E) interactions, which are central to breeding crops that can thrive in diverse and unpredictable environments. While high plasticity may reduce yield in non-target environments, it is essential for developing cultivars that are well-adapted to specific conditions.

TowArds Next GENeration Crops (TANGENC), reg. N. CZ.02.01.01/00/22_008/0004581, Programme Johannes Amos Comenius

Conversely, the goal of modern breeding is to achieve stable yields across a broad range of environments, necessitating a careful balance between plasticity and performance. To leverage plasticity in breeding programs, it is essential to understand its genetic underpinnings and to design environmental testing frameworks that can reveal plastic genotypes. Recent studies have identified regulatory hub genes that mediate plastic responses across multiple traits and environments. However, most experimental approaches still rely on single-stressor analyses, which fail to capture the complexity of multiple simultaneous environmental cues encountered in real-world agricultural settings. A paradigm shift is underway in how we study plant responses to environmental stress and develop crop varieties suited for future climates. Identifying the genes that control plasticity will deepen our understanding of complex plant-environment interactions. This knowledge is pivotal for designing breeding strategies that enhance crop resilience and stability, cornerstones for sustainable agriculture and global food security in the face of climate change.

This contribution aims to stimulates discussion on following questions.

·What are the ecological and agronomic trade-offs associated with breeding for increased plasticity versus yield stability, and how can these be optimized in different agricultural systems?

·What is the genetic architecture of trait plasticity? Can insights from one species be transferred to others? What trade-offs are involved in enhancing yield stability?

·Which regulatory genes or networks serve as central hubs for plastic responses, and how can this knowledge be integrated into breeding programs to enhance crop resilience and adaptability?

References:

·Alseekh S, Klemmer A, Yan J et al. (2025) Embracing plant plasticity or robustness as a means of ensuring food security. Nat Commun 16, 461.

·Arnold PA, Kruuk LEB, Nicotra AB (2019) How to analyse plant phenotypic plasticity in response to a changing climate. New Phytol 222, 1235–1241.

·Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Advan Genetics 13, 115–155.

·Pigliucci M. (2005) Evolution of phenotypic plasticity: where are we going now? Trends Ecology & Evol 20, 481–486.

·Waddington CH (1942) Canalization of developmentand the inheritance of acquired characters. Nature 150, 563–565.

·Waters DL, van der Werf JHJ et al. (2023) Partitioning the forms of genotype-by-environment interaction in the reaction norm analysis of stability. Theor Appl Genetics 136, 99.