

Ideotype of high-yielding wheat plant based on genetic background of cytokinin-mediated regulation.

The main goal of the research is to establish the role of cytokinin and cytokinin regulating genes in different aspects of plant productivity of wheat. General hypothesis assumes that interaction of main genes affecting cytokinin-dependent development plays a key role in regulation of cytokinin homeostasis and determination of yield-related traits in wheat.

To achieve the goal, we chose four groups of factors which might strongly influence cytokinin action/homeostasis in developing generative organs, in seedling roots and in flag leaves of wheat. The general research plan will focus on getting knowledge on these factors and determination of their impact on wheat plant productivity. These are: 1) **Cytokinin metabolism** – site-specific regulation of cytokinin level by biosynthesis (*IPT*) and degradation genes (*CKX*), and their influence on development of seedling roots, flag leaves and generative organs. The goal of this task is to establish the most beneficial cytokinin level resulting from the balance between biosynthesis and catabolism in respective organs for plant productivity. Hypothesis – Grain productivity of wheat plants depends on the local balance between CKs' biosynthesis and catabolism in respective organs. 2) **Regulation of transcription** of metabolic cytokinin genes by NAC transcription factors (TFs) and their relationship with leaf senescence. The goal is to establish: 1) which *TaNAC* genes interact with cytokinin metabolism genes 2) which NAC TFs modulate cytokinin level in selected organs of wheat (seedling roots, generative organs, and flag leaves) to promote yield-related traits. Hypothesis: Selected TFs are secondary targets of cytokinin signalling affecting leaf senescence and grain yield. 3) **Participation of key genes**, which determine yield-related traits by cell division in co-regulation of CKs and determination of grain productivity. The goal is to establish whether other key genes determining productivity via increased cell division act in co-regulation of cytokinin content in roots or generative organs of wheat. Hypothesis: Key genes determining yield-related traits by increasing cell division cooperate with the genes, which determine cytokinin content in generative organs. 4) **Cytokinin, auxin, GAs and ABA crosstalk** in respective organs of developing wheat plants. **Designing of high-yielding wheat ideotype**. The goal is to investigate cross-regulation between the components of the cytokinin pathway and other plant hormone pathways in promoting plant productivity of wheat. Based on the detailed knowledge gained from the four WPs to design and to test the best ideotype of wheat plant. Hypothesis: Regulatory interactions among the cytokinins, auxin, GAs and ABA are complementary in promoting plant productivity. CKs are positive regulators of auxin and GAs, and negative of ABA in promoting yield-related traits of wheat.

Materials and research methods

Plant material will include: 1) two cultivars of bread wheat: Kontesa and Ostka, which have been applied in our previous research, 2) stable modified by RNAi technology lines with decreased expression of *TaCKX1* and *TaCKX2.1/2.2* genes promoting yield-related traits, which are the result of currently completed NSC project, 3) selected breeding lines, which significantly differ in expression. Methods will apply: 1) searches in publicly available wheat sequence databases and primer design, 2) analysis of expression by quantitative RT-qPCR, 3) gene editing by CRISPR/Cas9 for obtaining plants with lack-off-function homoeologues of *TaCKX5* and *TaCKX9* via *Agrobacterium*-mediated transformation in investigated cultivars, 4) enzyme activity – CKX, others, 5) hormone content (CKs, auxin, ABA, GAs) in selected tissues, 6) measurement of morphological traits, chlorophyll content, 7) transcriptome analysis by RNAseq, 8) statistical analysis [Statistica 13 (StatSoft)].

Most of the protocols have been developed or adapted in the lab, including CRISPR/Cas9 (described in patent application and publications). Hormone content will be performed by the specialist, Dr. A. Bajguz from University of Bialystok.

We provide comprehensive knowledge in all four subjects of research listed above, which show cross-regulation of various processes and mechanisms of cytokinin action in generative organs, flag leaves and roots of wheat and indicate the role of these factors in cytokinin signalling and determination of yield-related traits.