Appendix No. 4 to the application for commencement of the procedure for the conferment of the post-doctoral degree of doctor habilitated to Dr. Agata Tyczewska

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SUMMARY OF PROFESSIONAL ACCOPMPLISHMENTS

Summary of Professional Accomplishments

1. **Name**: Agata Tyczewska

2. **Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation:**

2008 the degree of doctor of chemical sciences in the field of biochemistry obtained at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań. Doctoral thesis was carried out at the Laboratory of tRNA Biochemistry, Protein Biosynthesis Team, at the Institute of Bioorganic Chemistry Polish Academy of Sciences in Poznań.
The subject of the doctoral dissertation: "**Selection of RNA aptamers and their application in the study of the biological functions of HIV-1 RT and Dicer - proteins that specifically degrade RNA**", *supervisor* - prof. dr hab. Marek Figlerowicz, r*eviewers*: prof. dr ab. Mirosława Naskręt-Barciszewska and prof. dr hab. Artur Jarmołowski

2002 obtaining a master's degree in biotechnology. Master's thesis was prepared at the Faculty of Biology, Department of Genetics, Adam Mickiewicz University in Poznań.
Thesis topic: "**Research on chloroplast and mitochondrial DNA polymorphism in *Pinus sylvestris* and *Pinus mugo* and determination of F1 haplotypes in *Pinus uliginosa* in terms of hybridization**", *supervisor* - prof. dr hab. Wiesław Prus-Głowacki

3. **Information on employment in research institutes or faculties/departments or school of arts**.

01/09/2017 - until now - head of the Laboratory of Model Animal Organisms at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań

15/05/2017 - August 15, 2017 - postdoctoral fellowship at the Institute of Biomedical Research Friedrich Miescher, Basel, Switzerland, the team of prof. Ciosk, (Friedrich Miescher Institute for Biomedical Research (FMI), Ciosk Lab)

11.01.2011 - until now - assistant professor at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań

01/02/2009 - 31/10/2010 - postdoctoral fellowship at the Institute of Plant Molecular Biology Grzegorz Mendel, the Austrian Academy of Sciences, the team of prof. Matzke (Gregor Mendel Institute of Molecular Plant Biology (GMI), Matzke Lab)

4. **Description of the achievements, set out in art. 219 para 1 point 2 of the Act**

The title of the scientific achievement - a monothematic series of publications under a common title:

Crop molecular responses to environmental stress conditions in a temperate climate, as exemplified by corn (*Zea mays*) and herbicidal stress as well as soybean (*Glycine max* L.) and cold stress.

P1. **Tyczewska A**, Woźniak E, Gracz J, Kuczyński J, Twardowski T* (2018) Towards Food Security: Current State and Future Prospects of Agrobiotechnology. Trends in Biotechnology, 36(12):1219-1229, doi: https://doi.org/10.1016/j.tibtech.2018.07.008
MNiSW (2018): **45** IF (2018): **13.747** IF (5- year): **15.219**

P2. **Tyczewska A*,** Bąkowska-Żywicka B, Gracz J, Twardowski T (2016) Stress Responsive Non-protein Coding RNAs. Rozdział 7: 153-181, w: Abiotic and biotic stress - Recent Advances and Future Perspectives, InTech, doi: 10.5772/60477, ISBN 978-953-51-4590-5.

P3. Mahmoud M, Gracz-Bernaciak J, Żywicki M, Karłowski W, Twardowski T, **Tyczewska A*** (2020) Identification of Structural Variants in Two Novel Genomes of Maize Inbred Lines Possibly Related to Glyphosate Tolerance. Plants (Basel) 9(4):523. doi: 10.3390/plants9040523
MNiSW (2020): **70** IF (2020): **3.935** IF (5-year): **4.827** (2021)

P4. **Tyczewska A***, Gracz-Bernaciak, Szymkowiak J, Twardowski T (2021) Herbicide stress induced DNA methylation changes in two *Zea mays* inbred lines differing in Roundup® resistance. Journal of Applied Genetics 62(2):235-248. doi: 10.1007/s13353-021-00609-4
MNiSW (2021): **140** IF (2021): **3.240** IF (5-year): **2.756**

P5. Żywicki M, Gracz J, Karłowski W, Twardowski T, **Tyczewska A*** (2015) Expression of miRNAs involved in phosphate homeostasis and senescence is altered in glyphosate treated maize. Acta Physiologiae Plantarum 37:265. doi: 10.1007/s11738-015-2022-5
MNiSW (2015): **25** IF (2015): **1.563** IF (5- year): **1.692**

P6. **Tyczewska A***, Gracz J, Kuczyński J, Twardowski T (2016) Deciphering the soybean molecular stress response via high-throughput approaches. Acta Biochimica Polonica (63)4/2016, 631-643. doi: 10.18388/abp.2016_1340
MNiSW (2016): **15** IF (2016): **1.159** IF (5- year): **1.491**

P7. Kuczyński J, Twardowski T, Nawracała J, Gracz-Bernaciak J, **Tyczewska A*** (2020) Chilling stress tolerance of two soya bean cultivars: phenotypic and molecular responses. Journal of Agronomy and Crop Science, DOI: 10.1111/JAC.12431
MNiSW (2020): **140** IF (2020): **3.473** IF (5- year): **3.332**

P8. Kuczyński J, Gracz-Bernaciak J, Twardowski T, Karłowski W, **Tyczewska A*** (2021) Cold stress induced miRNA and degradome changes in four soybean varieties differing in chilling resistance. Journal of Agronomy and Crop Science, doi: 10.1111/jac.12557
MNiSW (2021): **140** IF (2021): **4.153** IF (5- year): **4.859**

The asterisk - * - indicates the corresponding author
The sum of the MNiSW points *for publications included in the scientific achievement*, calculated for the publication in accordance with the year of publication of a given item: **575**.
The sum of IF points of *publications included in the scientific achievement* was calculated on the basis of data from the Web of Science (WoS), in accordance with the year of publication of a given item: **30.701** (5-year IF: **34.37**).
Number of citations of articles included in the scienific achievement: **55** (without self-citations: **44**)

Regardless of the above list, the list and copies of the monothematic publication cycle constituting a scientific achievement and the statements of the co-authors specifying the individual contribution of each of them to the creation of these publications are included in Annexes 6 and 8.

A detailed description of the scientific achievement described in the above-mentioned works.

4.A. Agrobiotechnology - current state and development prospects in the era of modern biotechnology

As a result of thousands of years of agriculture, man has acquired many crop varieties, which have become the basis of the daily diet. The most important food plants in the world are grains. Of these, the highest production value/amount is achieved by wheat (basic bread grain), maize (mainly feed and industrial grain) and rice. The most important arable crops are also soybeans, for which the demand is constantly growing.

The publication **P1** discusses the broad background and context of the research included in this habilitation dissertation. It presents the need to intensify agricultural production in the context of food security, on the example of 4 main crops (wheat, maize, rice and soybean). Food security is a situation in which all people have physical, social and economic access at all times to sufficient, safe and nutritious food that meets their needs and allows them to lead an active and healthy life [1]. It has been estimated that in order to meet the needs of a growing human population, world food production needs to double (an increase of 2.4% per year over the next 30 years), a value difficult to achieve as currently the production growth rate for the 4 above-mentioned cereals is only between 0.9% and 1.6% [2]. The first chapter of the **P1** publication presents the challenges faced by mankind in the context of ensuring food security. These included the lack of additional land for agricultural production, land degradation/erosion, biotic and abiotic stresses. The next section presents the importance of genetically modified (GM) crops in agricultural production, briefly presenting the history of GM crops, as well as examples of genetic modification of soybeans, maize, wheat and rice. Attention was also paid to the directions of development of plant biotechnology on the example of attempts to introduce the photosynthetic C4 system to plants with the C3 system (soybean, wheat or potatoes), as well as the use of plants for the production of pharmaceuticals and compounds with high potential for industrial use (e.g. drugs, plastics, enzymes, cosmetics or epoxies). The work **P1** also considered whether GM plants can be a solution for ensuring food safety, by discussing the benefits of using GM plants in cultivation - no undesirable side effects of consuming GM food, lower degree of infection of GM plants with fungi, reduction of the amount of used herbicides and pesticides, and the high profits achieved by farmers from the cultivation of GM crops. The role of social acceptance of new biotechnology techniques and their use in agricultural production was also emphasized. With the advent of NBTs (New Breeding Techniques), it is extremely important to engage the scientific community in the biotechnology discourse and to prevent the dissemination of inaccurate or false information. Society mistakenly perceives that most of the benefits of GM crops come to multinationals (technology and new variety makers) and farmers, dismissing scientific evidence of GM food safety, increased yields (without increasing crop area), reduced use of herbicides, pesticides and fertilizers, reduced greenhouse (GHG) gas emissions and enabling sustainable agricultural practices. This is largely due to the lack of honest and reliable information about GM crops, and the dissemination of false information by GM opponents.

Thus, the need to feed the growing world population and adequately respond to the effects of climate change has become a necessity. However, in order to be able to modify crops, we first need to understand the basics of resistance by studying varieties better suited to surviving adverse environmental conditions. Advances in biotechnology and molecular biology in recent years have radically changed our understanding of the regulation of gene expression processes and plant responses to abiotic stress, providing us with new tools to help combat various stress conditions and improve the growth and yield of crops and species with industrial importance. Our journey in the world of small RNAs began in the 1990s with the discovery of the RNA interference pathway, followed by siRNA, and their role in DNA methylation and chromatin modifications. In the following years, further groups of small RNAs (small RNAs derived from tRNA, small RNAs derived from snoRNA, small RNAs derived from mRNAs) were discovered and their roles in cells and in adaptation to various environmental conditions began to be explored. With the advent of new high-throughput techniques, collecting vast amounts of different types of molecular data has become easier and much faster. Analyzing so much data and understanding the interrelationship between genes, their roles and small RNAs is now a

challenge scientists must face. The work **P2** describes the results of the analysis of literature data on the role of non-coding RNAs in response to abiotic stresses of organisms of industrial importance, such as crops and yeasts. To ensure precise control of gene expression under normal and stressful conditions, organisms have developed well-defined molecular regulatory mechanisms at the transcriptional and post-transcriptional levels. The work focused on the so-called canonical small RNAs (miRNA and siRNA) and also non-canonical ones (such as mRNA-derived RNA, tRNA-derived small RNA, snoRNA-derived small RNA (sdRNA), mRNA-derived small RNA and long non-coding RNA (lncRNA)). While there is evidence of the involvement of these different classes of non-coding RNAs in plant responses to stressful conditions, basic information about their role and the interaction between the various regulatory pathways is still lacking in most cases.

4.B. Research hypothesis and research goal

Agricultural production is the most important element of agrobiotechnology, representing a significant sector of the global economy and going far beyond food production. Over the past century, mankind has witnessed several landmark events that have significantly increased agricultural productivity. Initially, the improvement of arable crops was based on the judgment of farmers who selected good quality plants with high yields for cultivation and breeding purposes. The introduction of chemical fertilizers (already in the nineteenth century) as well as pesticides and herbicides (after World War II) resulted in another, rapid increase in yields. The "Green Revolution", initiated in the 1940s in Mexico by Dr. Borlaug, led to another significant increase in yields. New varieties obtained from traditional breeding were not only resistant to diseases, but also better adapted to various growing conditions, having an increased yielding potential. The multiplication of the yield is attributed to the use of synthetic fertilizers, the protection of plants with pesticides and herbicides, and the development and widespread use of improved varieties of crops and agricultural machinery. Another milestone in agricultural production was made possible by the use of a wide range of biotechnology tools. The manipulation of genetic information and the creation of genetically modified plants became a reality at the end of the 20th century, when the first GMOs were introduced to the market. Despite the great achievements of the past centuries, the urgent need remains to improve the existing varieties and increase agricultural production, dictated by the continuous growth of the human population and global climate change, accompanied by the exposure of organisms to greater biotic and abiotic stresses.

The intricate but well-defined molecular mechanisms activated in plants in response to biotic and abiotic stresses, despite the intense work of scientists around the world, are not yet fully understood. A stress factor may activate mechanisms which will result in adaptation to the existing conditions and maintaining the normal functioning of the cells and organisms. Such adaptive mechanisms can be passed on to daughter organisms and lead to the development of genotypes resistant to a given stress factor. The research presented in this habilitation thesis is characterized by a multi-level, comprehensive approach, used to define the molecular basis of plant responses to environmental stresses. Inspired by the conclusions drawn from the analysis of the literature data described in the **P1** and **P2** publications, I decided to devote my research to the analysis of molecular responses to the conditions of abiotic stress of agricultural crops. Therefore, the aim of the research was to analyze the responses of native cultivars to environmental stress in a temperate climate. I chose two industrially important crops for my research: maize (*Zea mays*) and soybean (*Glycine max* L.). Maize was subjected to herbicidal stress and soybean to cold stress.

Detailed objectives:

Maize:

Herbicidal stress has not been studied extensively to date, despite the fact that maize has seen a varied phenotypic response to herbicides. The aim of my research was to understand the molecular mechanisms of increased resistance to herbicidal stress induced by RoundUp® herbicide, which have been observed in natural maize populations. In order to answer the above-mentioned scientific questions, bioinformatic methods were

used, thanks to which the details of the structure of the genomes of two maize lines differing in their sensitivity to herbicides, as well as molecular methods, thanks to which changes in the levels of gene expression, miRNA, and DNA methylation were analyzed. As a result of the conducted analyzes, differences and similarities in the genomes of maize, as well as changes in the level of gene expression between two maize lines more or less tolerant to herbicide, were identified. The results of the research (**publications P3**, **P4 and P5**) are a source of new data that will help answer the questions about the universality of plant responses to herbicidal stress.

Soybean:

Soy is a short-day plant with high thermal requirements, and none of the varieties introduced so far is completely resistant to low temperatures and frosts. In a temperate climate, cold is one of the most important factors that negatively affects the growth and yield of soybeans. Soybeans require relatively high temperatures, ranging from 15–22°C when emerging, 20–25°C during flowering and 15–22°C when ripe. Under moderate climatic conditions, soybeans may be exposed to cold stress mainly in two periods. The first period is the emergence and early vegetative stages of plant development (V1-V3), i.e. from the last ten days of April to mid-May. The flowering phase is the second most sensitive to cold weather, as the formation of pods is critical to the productivity of legumes.

The aim of my research was to learn and understand the differences resulting from the effects of cold stress in the pool of small non-coding RNA (sRNA) molecules and the degradome of three soybean varieties (Fiskeby V, Augusta and Toyomusume) and one soybeans wild ancestor - *Glycine soja* (PI 538411A). In addition to assessing changes in the small RNA pool and the degradome, changes in the phenotype of selected cultivars and changes in the expression of known genes involved in the plant response to cold stress were also examined. By comparing phenotypic symptoms of stress and molecular responses to stress, genes and short regulatory RNAs responsible for adaptation to stress conditions were selected in different varieties. Such a comprehensive approach allowed to recognize the mechanisms of the response of an economically important crop to low-temperature stress conditions (**publications P7 and P8**). Several varieties of soybean have been registered in Poland, which grow relatively well in our climate zone, but understanding the mechanisms involved in the response of soybean to cold stress will improve the cultivation of this plant in the temperate climate zone.

4.C. Plant material used in the research

Maize:

Two inbred maize lines selected for detailed molecular analysis showed the highest (line S79757) and the lowest (line S245) susceptibility to spraying with RoundUp® herbicide under field conditions (unpublished data, Prof. Kazimierz Adamczewski). These lines were obtained as a result of the crossing program under the research grant no. PBZ-MNiSW-2/3/2006. The two maize lines selected for the study were derived from a herbicide tolerance screening test performed on 25 inbred lines selected on the basis of genetic background, type of endosperm, and importance in breeding. At the highest concentration of the herbicide (RoundUp® 360 SL) (300 g, 1.0 l / ha), the damage level of the tolerant maize variety was 40%, while in the more sensitive line it was 85% (K. Adamczewski, unpublished data).

The research material was provided by prof. K. Adamczewski from the Institute of Plant Protection, and the seeds were obtained from the maize producer and breeder, prof. J. Adamczyk from Hodowli Roślin Smolice Sp. z o. o. IHAR Group. The seedlings were grown in a greenhouse under controlled temperature (22 ° C), humidity and lighting (16 h/8 h - light/dark). Equal seedlings were selected and divided into two groups: one was sprayed with Roundup® herbicide (1.0 l / ha, 300 g glyphosate) adjuvanted with AS 500 SL (4.0 l / ha) 2 weeks after emergence of the plants (stage 4- 5 leaves) and a second (control) group was grown without herbicide treatment. To understand both the early and late response to stress conditions, for each test variety, leaves were harvested from 6 plants at predetermined time points (6 hours and 7 days after Roundup®

application); the leaves were collected at the same time for both tested groups of plants. After harvesting the plant material, the samples were immediately frozen in liquid nitrogen and stored at -80 ° C.

Soybean:

In collaboration with prof. Zbigniew Broda and dr hab. Jerzy Nawracała from the Department of Genetics and Plant Breeding at the University of Life Sciences in Poznań, we selected 3 soybean varieties for our research: Augusta, Fiskeby V, Toyomusume and the wild species *G. soja*. One of them, the Fiskeby V variety, was grown in Sweden, is not sensitive to photoperiods or low temperatures. The Fiskeby V variety was bred by Dr. Sven A. Holmberg in Sweden, near the city of Norrkoping (58 ° 30′N). The cold tolerance of the Swedish variety Fiskeby V is believed to come from the Sakhalin Namikawa breed. The Augusta variety was bred in the Department of Genetics and Plant Breeding of the University of Life Sciences in Poznań and registered in 2002 in Poland. It was obtained from two crosses: in the first step, a cross was made between Fiskeby V and the PI 194643 line and the line 104 was obtained; in the second step, line 104 was crossed with line 11 (wild *G. soja*). Line 11 *G. soja* grows in the natural environment of the Far East of Russia at latitudes close to Poland and has a long-day tolerance genotype. Therefore, Augusta has two sources of photosensitivity, and its cold tolerance comes from Fiskeby V. The seeds of the Augusta and Fiskeby V soybean varieties were provided by Prof. J. Nawracała from the University of Life Sciences in Poznań. Another object of research was *Glycine soja*, an annual species of wild soybeans native to China, partly Korea, Japan and Russia. It is the wild ancestor of the *Glycine max* varieties grown currently. This genotype belongs to the "000" group of earliness, that is, it is insensitive to cold. *Glycine soja* (accession number PI 538411A) was harvested near the Amur River (Russian Far East) at latitude: 52 ° 58′39 ″ N and longitude: 127 ° 21′44 ″ E. For control purposes, we also decided to analyze a soybean variety belonging to I group of earlyness, sensitive to low temperatures. It is the Toyomusume variety that is grown on the Japanese island of Hokkaido, mainly for the production of tofu and is not adapted to the climatic conditions of Poland.

Prior to sowing, soybeans were inoculated with *Bradyrhizobium japonicum* (HiStick® Soy, BASF) to induce nodule formation. The Augusta, Fiskeby V, Toyomusume and *G. soja* varieties were planted in pots filled with a 3:1 mixture of universal potting soil and sand. Plants were grown under controlled environmental conditions in a phytotron at 20°C, 60% relative humidity and a 16:8 hour photoperiod (light:dark) before the experiments. Plants were divided into three groups, with each group subjected to cold stress at a different stage of development (Table 1). The first batch of plants was stressed in VE stage (seedlings) by keeping them at 4°C for 48 hours in Percival chambers. The next batch of plants was exposed to 8°C for 120 h (5 days) in the V1 growth phase (first trifoliate). The last group of plants was stressed at 14°C during the day and 7°C at night for 168 hours (7 days) during the R1 growth phase (beginning of flowering). In the control and treated groups, 20 to 30 soybean plants were grown.

Table 1. Scheme of the chilling stress treatment.

Stage of soybean growth	**Optimal growth temperature**	**Stress temperature (day/night)**	**Duration of stress conditions**
VE – seedlings	20°C	4°C	48 h
V1 – vegetative	20°C	8°C	120 h (5 days)
R1 – reproductive	20°C	14°C /7°C	168 h (7 days)

VE – seedling emergence stage, V1 – first trifoliate stage , R1 – the beginning of flowering

4.D. Research results

In sections 4.D.1.-4.D.2. detailed goals and results for particular research issues / objects are presented.

4.D.1. Analysis of the effect of herbicidal stress on native maize varieties differing in sensitivity to Roundup®.

Maize:

Currently, maize ranks third in the global sowing structure (world production is estimated at 1100-1200 million tons, [3]), and its acreage is constantly growing, both in Poland and in the world. Maize is used in the food industry for the production of groats, flour, oils, but also for direct consumption or as semi-finished products. There is an increasing interest in maize as a typical industrial plant, which is used in the alcoholic fermentation process to obtain ethanol (both food and fuel), high-quality starch, it is used for energy purposes by burning (straw, cob cores) or biogas production (biomass). Maize straw is also a raw material for the production of paper and insulation materials. The dominant cultivation target, however, remains the fodder industry, where maize is used as a material for whole plant silage or grain and cob forage. Due to its importance and numerous applications, it is also used as a model organism to learn about many cellular processes, e.g. meiosis, mitosis, transposon function, recombination and genetic imprinting.

Modern agriculture, as a consequence of routine agrotechnical procedures, such as fertilization or spraying with insecticides / herbicides, introduces many exogenous substances into the cultivation of plants, which can reach high local concentrations and which are abiotic stressors for growing plants. Half of the pesticides used today are herbicides. The best known active ingredient of many herbicides is glyphosate - a selective, systemic compound, which inhibits 5-enolpyruvate shikim-3-phosphate synthase (EPSPS) by interfering with the synthesis of aromatic amino acids and their derivatives [4]. Glyphosate was first used to prevent sprouting of weed seeds. However, to obtain better crop protection, it was necessary to use it as a post-emergence herbicide. Maize (*Zea mays*) as a plant grown in a wide row spacing is particularly vulnerable to weed infestation, therefore herbicide spraying is often used in the early stages of seedling growth to reduce competition for light, water and minerals. Herbicidal stress has not been studied extensively so far, despite the fact that maize has shown a varied phenotypic response to herbicide use.

Genome (P3 publication)

The genome of maize, which has been duplicated several times, is made up of 10 chromosomes of 2.3 * 10^9 nucleotides. About 40,000 genes that produce protein products are coded in the maize genome, but the vast majority of it, as much as 85%, are sequences of various types of transposable elements [4, 5]. Comparative studies have confirmed the enormous genetic variability of even closely related maize lines, which far exceeds that found in other eukaryotic organisms. It has been shown that between any two selected maize lines, single nucleotide polymorphisms (SNPs) occur on average every 100 nt. The maize genome is characterized not only by SNPs or deletion/insertion (indel) polymorphisms, but also by differences in the structure of chromosomes, or altered location of genes and repeated elements [7, 8].

The aim of my research was to investigate the genetic differences between the tolerant and sensitive maize lines. To overcome the complexity of the maize genome, two sequencing technologies were used: Illumina (400bp and 500bp libraries, producing very accurate sequencing readings) and PacBio SMRT (two 8kb and 11kb insertion "mate-pair" libraries, generating less precise readings). Long PacBio readings, which are able to cover significant chromosomal regions and enable the identification of large deletions, insertions, duplications or inversions [9], allowed the detection of structural differences between the genomes of selected inbred maize lines. A total of 11,172 structural variants representing 6,062 insertions and 5,110 deletions were identified (only variants with 5 or more readings were examined). Most of the variants were in non-coding regions, such as upstream and downstream regions, and intron and intergenic regions.

Ilumina readings were used in the tolerant and sensitive maize genomes to identify minor structural changes such as SNPs and indels. After matching each dataset read with the reference genome of the B73 maize line,

changes in SNPs and indels that are only present in the tolerance line were noted. The result was an extensive list of genetic changes only seen in the RoundUp® tolerant lineage. This approach resulted in the identification of 4,068,829 SNPs and 729,866 indels, of which 113,775 SNPs and 15,277 indels were found in the protein coding regions.

The vast majority of variants were present in the intergenic regions (25.13%), the regions upstream and downstream (24.9% and 25.18%, respectively) and intron regions (17.56%). Sequencing data was deposited in the ENA (European Nucleotide Archive) database under the number PRJEB31400 (https://www.ebi.ac.uk/ena/data/view/PRJEB31400).

Gene ontology (GO) analysis showed that among the genes with deletion, the most abundant GO terms in the 'molecular function' category were iron ion binding, methyltransferase activity, transferase activity, and RNA binding, while in the 'biological processes' category the most abundant the terms were glycogen biosynthesis, K48 protein-coupled deubiquitination, carbohydrate metabolism, and metal ion transport. It is noteworthy that some of these genes have also been identified as part of the heat, biotic stress (nematodes), and oxidative stress [10-12] responses. On the other hand, among the genes affected by insertions, the most common GO terms in the category of "molecular function" were RNA binding, SUMO transferase activity, and hydrolase activity. In the 'biological processes' category, the most commonly used GO terms were protein sumoylation, cell growth, ATP hydrolysis, and cellular response to an extracellular stimulus. In the last category, two MYB transcription factors were identified.

To understand changes in the genome that may be responsible for the herbicidal stress tolerance phenotype, genes and pathways involved in glyphosate metabolism, such as the shikimic acid pathway, were investigated. We first analyzed the gene variants of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) as the best known example of an enzyme inhibited by glyphosate [13-14]. To date, several ways to overcome glyphosate susceptibility have been described; for example, amino acid substitution at the enzyme active site, gene copy number variation and gene overexpression. For the tolerant maize line tested, no large structural variants were identified, only a few SNPs and indels within or near the EPSPS gene were identified (Fig. 1). Importantly, all identified changes had a moderate effect on gene expression according to VEP (variant effect predictor) and none of them were located in the coding region.

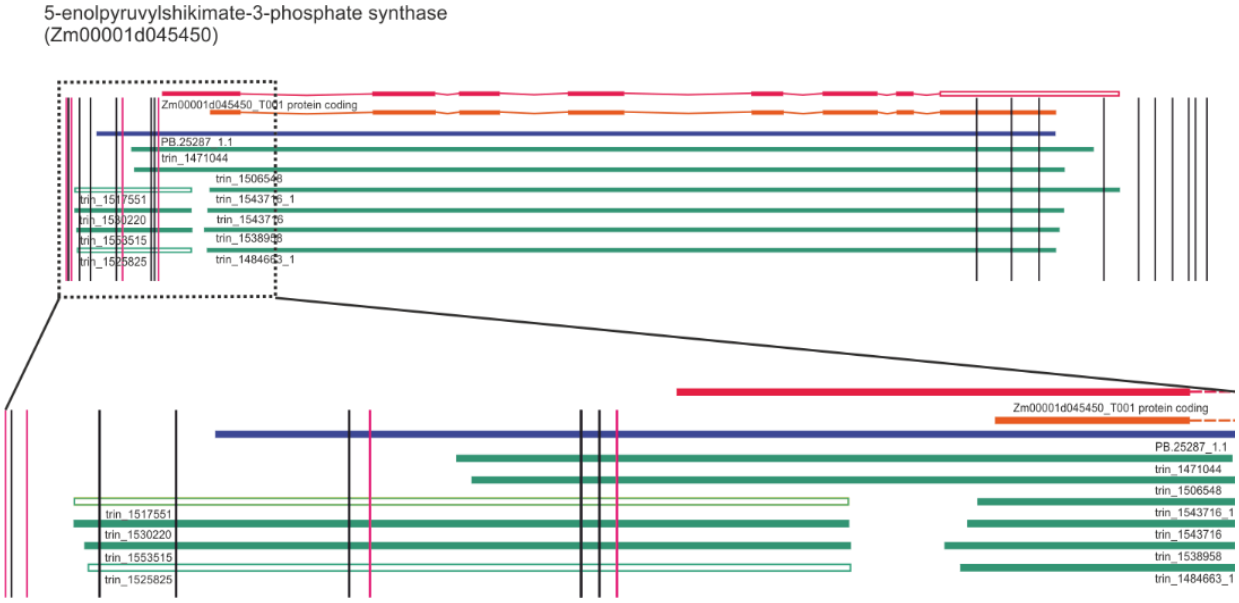


Figure 1. Location of identified SNPs (black) and indels (pink) on the *EPSPS* gene.
In red, the *EPSPS* gene structure according to the version 4 annotation of the maize genome, and in orange, the version 3 annotation have been given. Blue bars represent the publicly available full-length EPSPS transcript isoform identified with the iso-seq approach; green bars represent publicly available Trinity-assembled transcripts from RNA-seq data.

In the case of chorismate synthase chloroplastic and bifunctional 3-dehydroquinate dehydratase/shikimate dehydrogenase chloroplastic, the effect on gene expression was high, changes affected splice sites and caused frame shifts. Other identified structural changes that may result in increased tolerance to glyphosate were located in the genes encoding the phosphate transporters. Previously, it was suggested that phosphate transporters 1 and 2 may be involved in the active transport of glyphosate into plant cells [15-18]. Other identified transporters, with SNP and indel type changes, are involved in the distribution of inorganic phosphate, 3-phosphoglycerate, triose phosphates and, to a lesser extent, phosphoenolpyruvate (PEP), which is a substrate for the shikimate pathway. Another group of genes, where structural variants with potentially large expression effects were identified that caught our attention in the context of the herbicide stress response, were genes encoding proteins involved in the extrusion of multidrug and toxic compound intrusion (MATE) compounds. This family of proteins is one of the most conserved and largest families of transporters in plants and acts as membrane carriers for drugs and synthetic compounds, as well as organic acids, plant hormones and secondary metabolites [19].

The presented results of the analysis from the sequencing of two inbred maize lines give us insight into the molecular pathways that could potentially be involved in the glyphosate tolerance trait. The variants and changes identified in the coding sequences are a good starting point for further biochemical and genetic analyzes, especially those related to genes encoding enzymes of the shikimate pathway (bifunctional 3-dehydroquinate dehydratase/shikimate dehydrogenase and chorismate synthase). The described changes identified in the coding sequences may lead to an increase in the efficiency of the shikimate pathway and thus compensate for the decreased EPSPS activity induced by glyphosate. In addition, variants with a high impact on gene expression have been identified in the genes encoding the phosphoenolpyruvate carboxylase and its regulator, phosphoenolpyruvate carboxylase kinase. The potential decrease in phosphoenolpyruvate carboxylase activity may lead to increased PEP availability, providing another level of compensation for EPSPS inhibition. Other tolerance mechanisms may be related to altered glyphosate transport by phosphate transporters or multidrug and toxic extrusion proteins. All the above-mentioned mechanisms can lead to changes in the effective intracellular concentration of glyphosate and allow plants to adapt to the conditions of herbicidal stress.

Methylome (P4 publication)

Cytosine methylation at the C5 position in DNA plays a key role in the regulation of gene expression, the activity of transposable elements, defense against foreign DNA, and the inheritance of specific gene expression patterns. The relationship between exposure to stress, biotic and abiotic, and sequence-specific changes in DNA methylation was hypothetical until it was shown that stress can induce changes in gene pattern and expression levels [20-21]. Transcriptional gene silencing is associated with hypermethylation of gene promoter sequences, while post-transcriptional silencing of genes is associated with hypermethylation of transcribed or coding sequences [22]. In plant genomes, methylated cytosine (m5C) residues are found in three sequence contexts: symmetric CG and CNG, and asymmetric CNN (where N is A, T, or C).

The aim of the study was to identify changes in the levels and patterns of DNA methylation that may underlie *Z. mays* resistance to herbicidal stress conditions (SONATA grant, National Science Center, UMO-2011/01/D/NZ9/03631, PI - Dr. Agata Tyczewska). The MSAP (Methylation-Sensitive Amplification Polymorphism) technique and 24 pairs of primers were used to study changes in the level and pattern of DNA methylation in maize genomes. DNA amplification products, for each pair of primers separately, were separated for 18 h on 6% polyacrylamide gels with dimensions of 50x33 cm and visualized with Sybr Gold (Thermo Fisher Scientific).

In total, 888 and 826 distinct bands were obtained for the TL (tolerant line, S245) and SL (sensitive line, S79757) variety, respectively. Based on the obtained electrophoretic images, it was possible to analyze changes in the total level of DNA methylation in maize under the influence of the herbicide used (Fig. 2). Under control

conditions, the total methylation level of the 5′-CCGG-3′ sequence was 63.11% for TL and 59.38% for SL. The total level of DNA methylation ranged from 62.38% (6 hours after spraying) to 63.40% (7 days after spraying) for TL and from 57.87% (6 hours after spraying) to 60.90% (7 days after spraying) for SL. Herbicidal stress increased the number of fully methylated sequences in both tested lines, 6h after spraying. The change was very insignificant for TL (1.13%) compared to SL (18.64%) (Fig. 2). Seven days after spraying with the herbicide, a decrease in methylation was observed for the TL line (3.94%) and almost no difference was noted for the SL line (0.12%). Importantly, there were always more completely methylated loci than hemi-methylated loci.

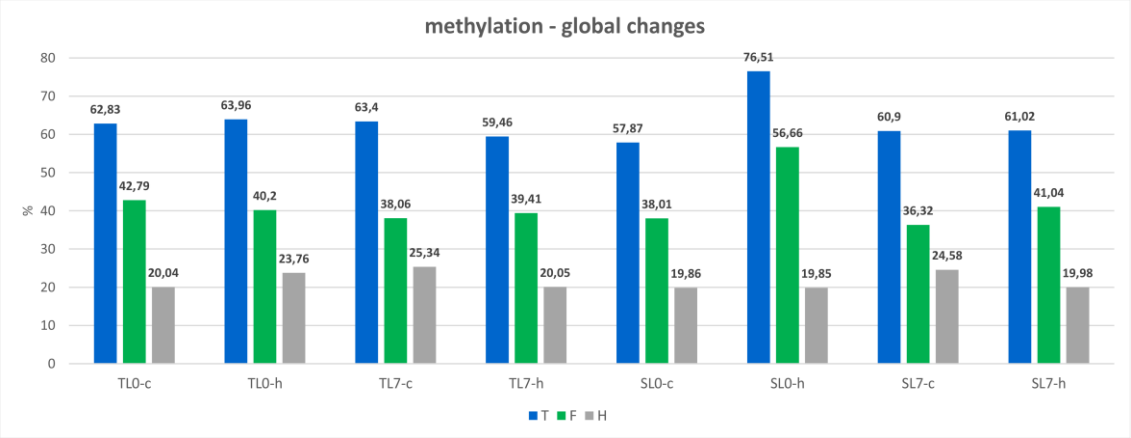


Figure 2. Global DNA methylation changes in TL and SL *Z. mays* lines resulting from herbicidal stress conditions.
T – total methylation level, F- fully methylated sites, H – hemimethylated sites. TL6h-C – TL line 6h after spraying, control; TL6h-H - TL line 6h after spraying, herbicide; TL7d-C- TL line 7 days after spraying, control; TL7d-H - TL line 7 days after spraying, herbicide; SL6h-C - SL line 6h after spraying, control; SL6h-H - SL line 6h after spraying, herbicide; SL7d-C - SL line 7 days after spraying, control; SL7d-H - TL line 7 days after spraying, herbicide. The biggest changes in DNA methylation in two tested *Z. mays* lines under herbicide stress conditions were observed in SL line 6h after herbicide application. These changes are attributed to a large increase (by 18,65%) in the number of fully methylated sites.

To analyze changes in cytosine methylation patterns under herbicidal stress in TL and SL lines, all possible band patterns (no change, demethylation and methylation, Table 2) were analyzed and counted.

Table 2. Analysis of DNA methylation changes in tested maize cultivars under the influence of Roundup® herbicide. The number of bands and the percentages of occurrence are given for the *Z. mays* lines tested and for all tested conditions.

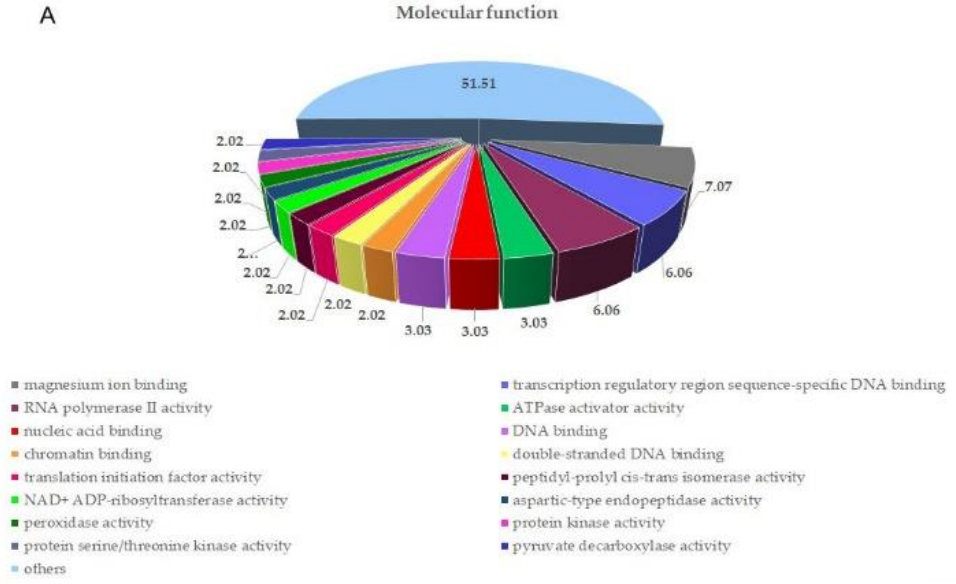
	Z. mays			
Class	***TL6h-C/TL6h-H***	***TL7d-C/TL7d-H***	***SL6h-C/SL6h-H***	***SL7d-C/SL7d-H***
	Wartość procentowa (liczba prążków)			
No change	*72,63% (645)*	*64,41% (572)*	*46,00% (380)*	*72,76% (601)*
Demethylation	*13,40% (119)*	*19,25% (171)*	*17,32% (143)*	*10,52% (87)*
Methylation	*13,96% (124)*	*16,32% (145)*	*36,68% (303)*	*16,70% (138)*

TL6h-C – TL line 6h after spraying, control; TL6h-H - TL line 6h after spraying, herbicide TL7d-C- TL line 7 days after spraying, control; TL7d-H - TL line 7 days after spraying, herbicide; SL6h-C - SL line 6h after spraying, control; SL6h-H - SL line 6h after spraying, herbicide; SL7d-C - SL line 7 days after spraying, control; SL7d-H - TL line 7 days after spraying, herbicide.

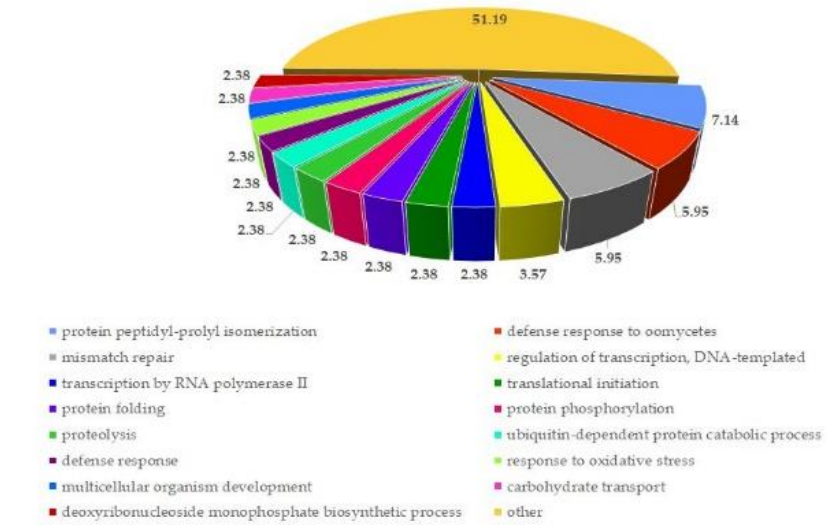
In the case of TL, the methylation of 72.63% and 64.41% of the 5'-CCGG-3 'sites did not change under the influence of the herbicide spraying, 6 h and 7 h days after spraying, respectively. There was a difference in demethylation events at two different time points in TL - 13.40% versus 19.25% - and differences in methylation events of -13.96% and 16.32% at 6 hours and 7 days after surgery, respectively. For SL with 826 bands, 46.00% and 72.76% of 5'-CCGG-3 'remained unchanged under stress, 6 h and 7 h days after spraying. There was a decrease in demethylation events after 7 days as compared to 6 hours after spraying (17.32% to 10.52%). The largest difference was observed for methylation events (19.98%), a decrease from 36.68% to 16.70% one week after spraying.

Selected DNA fragments were isolated from polyacrylamide gels and sequenced (Sanger's method) by an external company (Genomed, Poland). In total, the sequences of 197 DNA fragments were obtained, which were subjected to bioinformatic analysis using the NCBI and MaizeGDB databases. Out of 197 fragments, as many as 151 fragments were identified (with an accuracy greater than 90%) in at least one of the searched databases. Among the identified sequences were genes encoding transferases, transporter proteins, methyltransferases, hydrolases, transposons, ribosomal proteins, cytochromes and proteins involved in transcription and stress response (Figure 3).

A


Molecular function
51.51
7.07
6.06
6.06
3.03
3.03
3.03
2.02
2.02
2.02
2.02
2...
2.02
2.02
2.02
2.02
2.02
magnesium ion binding
RNA polymerase II activity
nucleic acid binding
chromatin binding
translation initiation factor activity
NAD+ ADP-ribosyltransferase activity
peroxidase activity
protein serine/threonine kinase activity
others
transcription regulatory region sequence-specific DNA binding
ATPase activator activity
DNA binding
double-stranded DNA binding
peptidyl-prolyl cis-trans isomerase activity
aspartic-type endopeptidase activity
protein kinase activity
pyruvate decarboxylase activity

B


Biological process
51.19
7.14
5.95
5.95
3.57
2.38
2.38
2.38
2.38
2.38
2.38
2.38
2.38
2.38
2.38
protein peptidyl-prolyl isomerization
mismatch repair
transcription by RNA polymerase II
protein folding
proteolysis
defense response
multicellular organism development
deoxyribonucleoside monophosphate biosynthetic process
defense response to oomycetes
regulation of transcription, DNA-templated
translational initiation
protein phosphorylation
ubiquitin-dependent protein catabolic process
response to oxidative stress
carbohydrate transport
other

C

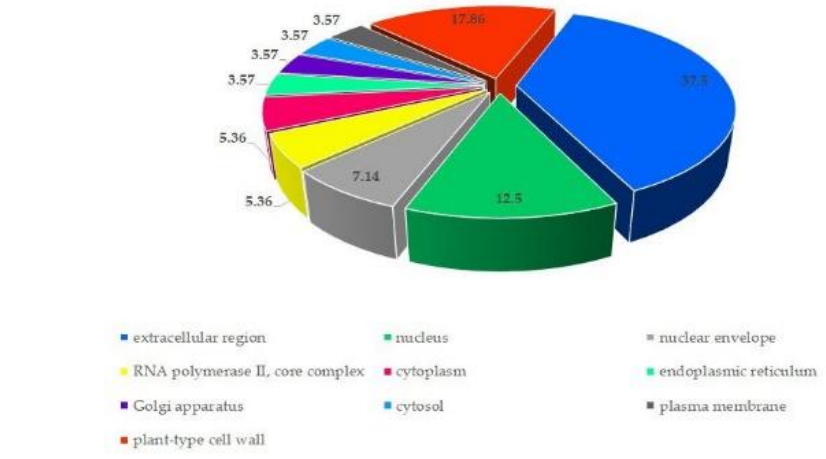
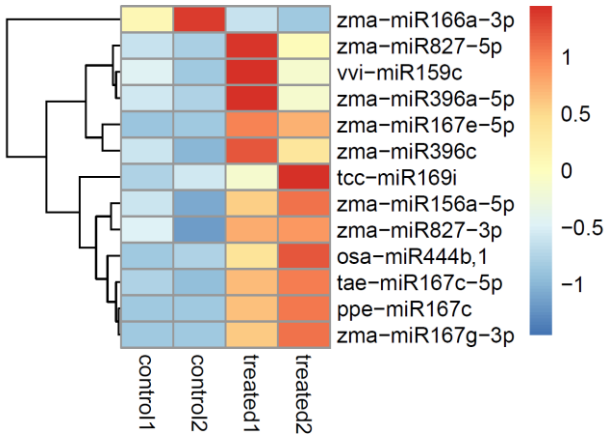

Cellular component
17.86
33.9
12.5
7.14
5.36
5.36
3.57
3.57
3.57
3.57
3.57
extracellular region
RNA polymerase II, core complex
Golgi apparatus
plant-type cell wall
nucleus
cytoplasm
cytosol
nuclear envelope
endoplasmic reticulum
plasma membrane

Figure 4. A graph showing the percentage of GO enrichment analysis in: (A) 'molecular function' category, (B) 'biological process' category, (C) 'cellular component' category.

The selective pressure exerted by the constant use of herbicides may force adaptive responses not only in weeds (a phenomenon that has been observed for many years), but also in crops. This analysis showed that herbicidal stress, depending on the natural susceptibility of *Z. mays* cultivars to herbicide, induced different changes in the levels and patterns of DNA methylation in the two tested maize cultivars. Such changes may, in turn, be reflected in the patterns and levels of expression of individual genome fragments, which may result in the differentiated response of organisms to stress conditions. The enormous changes in the level and pattern of DNA methylation observed for the SL 6h line after herbicide spraying (18.64%) may lead to deregulation of gene expression, which may eventually lead to plant death. On the other hand, the change in the level of DNA methylation in the TL line following the application of herbicides was very small (1.13%). It seems that the natural resistance of crops to herbicides is much more complicated than changing a single trait and is based on many mechanisms and different levels of regulation of the expression of genetic information (epigenetics, small non-coding RNA, changes in expression and composition of gene products) which together constitute the increase in the suitability of individual varieties to stress conditions.

miRNA (P5 publication)

Little is known to date about the involvement of miRNAs in plant response to herbicide spraying. The publication **P5** describes the role of miRNAs in the late response of maize to one of the most commonly used herbicides in the world - RoundUp®. Profiling of miRNA expression was performed in maize leaves 7 days after spraying. The aim of the study was to identify differences in the profile and expression levels of known as well as new miRNAs in the S79757 (SL) maize line under control conditions and after herbicide spraying. The cDNA libraries were prepared for sequencing with the TruSeq small RNA Preparation Kit (Illumina). The Genome Analyzer IIx (Illumina) was used to sequence 72 nt single reads. Short sequencing reads were matched with the full set of known miRNAs from Magnoliophyta (6547 precursors and 7956 mature miRNA sequences) (miRBase 21st edition). Sequence readings that revealed no similarity to known maize miRNAs, but were instead mapped to precursor regions coinciding with positions of known miRNAs from other organisms, were considered new conserved maize miRNAs. In this way, 13 miRNAs, present in other plants but not assigned to maize, were identified. The number of readings for the sequences identifying new miRNAs varied from 12 for stu-miR479 to 129 for ptc-miR6478; however, in all cases, mature miRNAs were observed in at least three of the four sequenced cDNA libraries (two control and two test replicas), confirming the reliability of the observation.



Expression of 51 representative miRNAs was observed, in this group we identified 13 miRNAs that showed statistically significant changes in the expression level (p value <0.05, FDR value <0.01) in the plants sprayed with the herbicide (Figure 4). It was shown that the expression levels of 12 miRNAs belonging to the miR167, miR396, miR159, miR156, miR169, miR444, and miR827 families were significantly increased (from 1.6 to 3.6), and one, miR166, decreased after herbicide spraying in RoundUp® sensitive line.

Fig 4. Expression of miRNAs with significant differential expression upon glyphosate treatment.
The clustered heatmap shows miRNA expression profiles. Individual rows representing particular miRNAs have been scaled to represent the expression level changes for different samples from the mean expression levels of given miRNA.

The colour scale represents the distance (positive value and red colour stand for expression level higher than mean, negative value and blue colour represent expression level lower than mean, 0 and yellow colour is equal to mean).

All miRNAs identified in maize SL line (S79757) with altered expression levels under the influence of herbicidal stress are described in the literature and their target mRNAs are well documented. However, to test whether new miRNAs differentially expressed after herbicide spraying differ in functional potential from known maize miRNAs, we predicted their target molecules. For all the differentially expressed miRNAs, we identified 264 transcripts corresponding to 122 genes, 48 of which encode uncharacterized proteins. Gene ontology enrichment (GO) analysis showed that most proteins with known function were localized in the nucleus and were involved in the regulation of the transcription process. The only enriched GO category not related to the regulation of gene expression was the regulation of or participation in the activity of the hydrolase, with 17 representatives. This function has mainly been attributed to ATP-binding proteins. Importantly, all predicted mRNA targets for miRNAs, including those identified for new members of the known maize miRNA families, were consistent with previous literature reports [23-25]. In the P6 publication the role of miR827-3p in maintaining Pi (inorganic phosphorus) homeostasis and response to stress induced by RoundUp® herbicide has been proposed (Fig. 5). Under conditions of optimal Pi level, the NLA (Nitrogen Limitation Adaptation) gene is usually expressed. The NLA protein is responsible for mediating ubiquitination and degradation of membrane phosphate transporters. The herbicide-dependent elevated level of miRNA-827 expression (related to the maintenance of phosphate homeostasis) most likely causes an increase in the accumulation of phosphate transporters in cell membranes, which in turn leads to increased glyphosate uptake instead of increased Pi uptake, and consequently disrupts Pi homeostasis in the body.

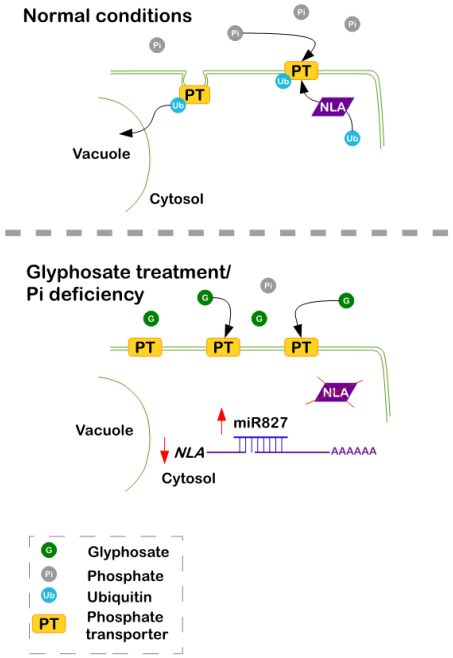


Fig5. miR827 role in Pi homeostasis and potentially in response to glyphosate.

In Pi (inorganic phosphorus) sufficiency conditions, NLA gene is normally expressed. NLA protein is responsible for mediating ubiquitination and degradation of membrane phosphate transporters. In Pi deficiency conditions as well as after glyphosate treatment, miR827, which targets NLA transcripts, is transcriptionally upregulated and as a consequence leads to the downregulation of NLA expression levels. As a result more phosphate transporters occur in cell membranes leading to increased Pi or glyphosate (depending on conditions) uptake.

4.D.2 soybean

Soybean belongs to the legume family of plants, comes from Southeast Asia, and was domesticated by Chinese farmers about 4500 years ago. The growth of the soybean acreage in the world is very dynamic. In the past 30 years, the world production of soybeans has tripled and in the 2021 season it exceeded 350 million tons. The main cultivation areas are: North America, Asia and South America. Soybeans are a very important raw material for the production of fodder and are also a valuable food for humans. Based on soybeans, products such as soybean oil, soybean meal, soybean groats, soy milk, tofu, tempeh and lecithin are produced. Soybean

preparations are also added to various preserves, including canned meats and sausages, to increase their nutritional value. Food products made from soybeans are particularly readily included in the diet by vegetarians as an alternative to meat. Soybeans contain a lot, as much as 30-45% of protein, which has an optimal amino acid composition for humans, especially compared to other plant proteins. In addition, soybeans are high in fat (~ 20%) and B vitamins. Most of the soybeans and soybean products used in Europe are imported. Among European countries, Poland is a relatively large outlet for soybeans and soybean products, but the vast majority of the domestic market is limited only to the consumption of imported goods. Imports of soybean meal are currently estimated at around 2.2 million tons per year, with very limited soybean cultivation. As a result, Poland is approximately 80% dependent on the import of high-protein feed [26-28].

At the core of all plant mechanisms of adaptation to stress is genetic information and the various mechanisms encoded in it regulating its expression, both at the transcriptional and post-transcriptional levels. The review publication **P6** describes the results of a literature analysis of the influence of biotic stresses (*F. oxysporum*, cust nematode, soybean mosaic virus) and abiotic stresses (drought, water stress, cold stress, salinity, phosphorus deficiency) on the growth and yielding of various soybean varieties and other economically important ones organisms. This article focuses on large-scale, high-throughput analyzes of transcriptomes (RNAs encoding proteins as well as RNAs that do not encode proteins - especially miRNAs) and proteomes under stress. The analyzes started with short non-coding RNAs - miRNAs and siRNAs. The roles of these molecules in adaptation to the stress conditions of drought and salinity as well as cold, maintaining homeostasis in the body, sulfur and copper metabolism and in response to elevated concentrations of heavy metals have been described. There have also been few reports on the role of siRNA in plant responses to stressful conditions. The functions of non-canonical non-coding RNAs are also presented. The first section is devoted to regulatory molecules derived from tRNA. They were discovered relatively recently, it was noticed that their amounts in organisms subjected to stress differ both in comparison to the unstressed control and in those stressed by different stresses. Short regulatory RNAs can also be derived from snoRNA or mRNA, and these are the subject of the next two sections. The last chapter of the **P6** publication is devoted to long non-coding RNA.

The scientific world is still looking for new varieties of crops characterized by greater tolerance to adverse environmental conditions, both biotic and abiotic, as well as having better quality and higher production efficiency. In the face of global climate change and the ever-increasing human population, the pressure to find new, better-adapted varieties is even greater than before, so we continually influence plant genomes by conventional breeding techniques or by methods discovered with the advancement of molecular biology. The plasticity of the soybean genome allowed for the expansion of its cultivation far beyond its place of origin, and thanks to its unique properties, it became the basis for today's agriculture. Advances in biotechnology and molecular biology in recent decades have radically changed our understanding of the regulation of gene expression and the response of plants to biotic and abiotic stresses. The examples of experiments and research described below show how complicated the relationship between plants and the environment is.

Phenotypic analyzes of two soybean cultivars resistant to cold stress (publication P7)

Two varieties less sensitive to cold (Fiskeby V and Augusta) were selected for phenotypic analyzes of soybean responses to cold stress. The impact of cold stress on the soybean phenotype was assessed by measuring several important parameters. Vegetative growth was determined by measuring plant height at stages V1 and R1 and calculating the number of days between growth stages. The growth rate was then calculated as the height difference between R1 and V1 plants divided by the number of days the plants grew from step V1 to step R1. Several parameters were measured during flowering, including the number of flowers on each plant, the number of pods on each plant, and the number of seeds in each pod. Finally, after harvesting, the seeds from the stress and control groups were weighed and counted. From these data, flowering efficiency was calculated by comparing the number of pods per plant with the number of flowers on each plant. The mean

number of seeds in a pod was quantified by dividing the number of seeds per plant by the number of pods per plant. Finally, the cold tolerance index was calculated as the average seed weight of the stressed plants divided by the average seed weight of the control plants.

A strong influence of cold on vegetative development (growth rate) was observed for the stressed Augusta cultivar in the V1 phase (growth slower by 45% compared to the control). Moreover, the analysis of variance (ANOVA) confirmed a statistically significant interaction between the variety and stress. A decrease in the flowering efficiency of stressed plants in the R1 phase was observed for both cultivars (the efficiency of forming pods decreased by 34% and 17%, respectively, in August and Fiskeby V). Additionally, the cold stress applied at the seedling stage reduced the flowering efficiency by 31% in the Augusta variety. Only the Augusta cultivar (31% reduction) when the plants were stressed in the R1 phase had a negative effect on the number of pods. Interestingly, further observations showed that the number of seeds per plant of both varieties changed after stress was applied in the R1 phase and showed a decrease of 20% and 29% for the Augusta and Fiskeby V varieties, respectively. In the case of Fiskeby V, the lower average number of seeds per pod was from reducing the number of seeds per plant while increasing the number of pods per plant. Conversely, in the Augusta variety, the number of seeds in the pod did not change significantly as a result of the stress, as the number of pods and seeds per plant decreased simultaneously. The last parameter used to determine the overall yield potential of the studied plants was the cold tolerance index. Remarkably, Augusta and Fiskeby V plants stressed at the stage of sowing and vegetative growth showed an increase of the indexes by ~ 10% compared to the control plants. A reduced seed yield was observed only in the Fiskeby V variety stressed in the R1 phase (reduction by 7.4%) and in Augusta plants stresses in the VE phase (reduction by 7.13%).

In order to compare the production potential of two soybean varieties (Augusta and Fiskeby V), in addition to the observation of phenotype changes under controlled conditions, at the Agricultural Research Station of the University of Life Sciences in Poznań (51 ° 41′37 ″ N, 17 ° 04′06 ″ E), a field experiment was carried out in the growing seasons of 2016 and 2017. At the physiological maturity stage, 10 plants of cultivar Augusta and Fiskeby V were manually harvested from each plot. Precipitation and air temperature were measured with a Vantage VUE 6357EU (Davis Instruments) ~ 400 m from the experimental field.

Conducting experiments in two consecutive growing seasons made it possible to compare the reaction of soybeans to natural moderate environmental conditions in the region of interest (Poland). Plants of both cultivars grown in the 2017 season showed reduced vegetative growth compared to the 2016 crops, as indicated by plant height measurements. In addition, reproductive growth parameters such as seed weight per plant and 1,000 seeds weight indicate poorer plant growth performance for both cultivars in 2017. As shown by the results of the analysis of variance (ANOVA), the number of seeds in a pod was influenced by the interaction between the cultivar and seasonal changes. The Polish variety showed much worse yields in the 2017 season, when the production of seeds per plant decreased to 13 g from 16.2 g in 2016, and the weight of 1000 seeds decreased from 18.2 g to 13.2 g. For plants of the Fiskeby V variety there was a decrease in seed weight per plant, from 13 g in the 2016 season to 9.5 g in 2017, and the weight of 1000 seeds decreased from 26.4 to 23.7 g. Unfortunately, some differences in the growth of soybeans in 2016 and 2017 resulted from meteorological anomalies such as hail and floods that occurred in 2017.

In the plants of the studied soybean cultivars (Augusta and Fiskeby V) subjected to cold stress, the expression profiles of five miRNAs, selected for research on the basis of the results of the literature analysis (publication P6), were investigated using the ddPCR method. In the unstressed (control) plants, the expression of the four miRNAs (miR169, miR319, miR397 and miR398) was confirmed in the trifoliate, unifoliates, cotyledons and roots of both cultivars grown under control conditions, while miR159 was not detected. Both miR397 and miR398 in variant Fiskeby V (at stages V1 and R1) showed similar trends as they were ~ 154- and 129-fold more abundant in leaves (single- and trifoliate). In Augusta, expression levels were ~ 94-fold and 57-fold higher for miR397 and miR398, respectively. Likewise, the expression level of miR169 was 5.5-fold higher in all terrestrial Fiskeby V tissues compared to that in roots and was 3.4-fold higher in the Augusta

variety. Increased amounts of miR319 (13.4 times higher in Augusta; 4.9 times higher in Fiskeby V) were detected in seedlings compared to those in single and trifoliate stages V1 and R1. The relative expression levels of the analyzed miRNAs in the stressed plants differed between the tested cultivars. In plants stressed during flowering (R1), cultivars Augusta and Fiskeby V showed contrasting expression patterns of miR169, miR319, miR397, and miR398, with the greatest difference for miR397 which showed an 80% decrease in Augusta expression level and a 250% increase in Fiskeby V. Similar trends were observed in plants stressed during vegetative growth (V1), with the exception of miR169 whose relative expression level in seedlings was similar in both cultivars. Importantly, the cold stress caused significant changes in the expression levels of miR169, miR319, miR397 and miR398 in the roots, compared to the above-ground parts of plants where miRNA synthesis was stable.

To understand the role of selected miRNAs in the response of selected soybean varieties to cold stress, the expression profiles of their target genes were examined, which were selected based on GO analysis. The four miRNAs and their 85 target genes were assigned to 48 molecular functions, 89 biological processes and 21st cellular elements. The most represented categories of cellular components were the nucleus and the transcription factor complex. Among the biological processes, DNA binding and transcription regulation were most often represented. DNA binding and protein binding were the most abundant molecular functions represented by all predicted target genes. Expression of all target genes was observed in cv. Fiskeby V, while expression of the miR397 target gene was undetectable in cv. Augusta. Interestingly, a negative correlation between the level of miRNA expression and their predicted targets was found mainly in the roots of seedlings. First of all, the overexpression of miR169 (0.46-fold and 0.88-fold) corresponded to a reduction of -2.64-fold and -1.11-fold of the expression of its target gene (nuclear factor Y subunit, NF-YA), respectively in Augusta and Fiskeby V. In the case of miR319 (Fiskeby V) there was a 0.7-fold increase in the amount of miR319 and a -1-fold decrease in the amount of its target gene encoding the GAMYB transcription factor. The expression of the laccase gene (Fiskeby V) was significantly increased (~ 2.65-fold) in trifoliates and unifoliates at stages V1 and R1; no negative correlation was found between miR397 expression and its laccase target gene. Another relationship was found between miR398 and its target gene (copper chaperone for superoxide dismutase (CCS) in the roots of Augusta cultivar seedlings, where miRNA expression decreased -1.73 times and the gene increased 0.33 times.

miRNA and degradom (P8 publication)

The publication **P8** describes the responses of four soybean cultivars (Augusta, Fiskeby V, Toyomusume and *G. soja*) to cold stress at the molecular level (miRNA and degradom). Observations on three plant tissues (seedling roots, cotyledons and trifoliates) allowed the study of the stress response in the early stages of soybean growth. To identify the miRNAs involved in the cold stress response, 72 sRNA libraries (for stressed samples and corresponding controls) were constructed and sequenced using Illumina technology. In addition, sequencing of the degradome (24 libraries) allowed the identification of potential miRNA target genes with different expression levels.

Readings from 72 sRNA libraries were compared to the soybean mature (and precursor) miRNA collection from miRBase (Release 22.1). In total, 321 known families of soybean miRNAs in all four variants were identified. In the soybean cotyledons at the VE stage, miR159 was the most abundant miRNA family, with the exception of Fiskeby V in which miR165 was the one with the highest expression level. In the roots of all plants harvested at the seedling stage (VE), the miR319 family was the most highly expressed, while in the soybean trifoliates harvested at the V1 stage, the miR398 family was the most abundant. In addition, among the highly expressed miRNA families, conserved miRNAs, miR159, miR165, miR166, miR167, miR319, miR396, miR398, miR408 and miR482, as well as legume specific miRNAs such as miR4414, miR1510, and miR35, dominated in all samples tested. On the other hand, among the miRNA families with the lowest expression levels, the ratio of conserved miRNAs to legume-specific miRNAs was significantly lower than in

the high expression fraction. Most of the conserved miRNAs, among the least abundant miRNA families, were transcriptional variants of their highly expressed counterparts. In addition, 348 new miRNAs were found in the four tested variants (213 miRNAs in Augusta, 220 in Fiskeby V, 224 in Toyomusume and 218 in G. soya), of which 129 miRNAs were common to all four variants.

In total, 162 differentially expressed miRNAs belonging to 137 families were identified in the stressed and control samples of four soybean cultivars. Among miRNAs with altered expression levels in all tested cultivars, 93 were downregulated in at least one variety, 28 of which were at least 5-fold. In contrast, 137 miRNAs were upregulated in samples of stressed plants in at least one cultivar, 41 of which were at least 5-fold (Fig. 6).

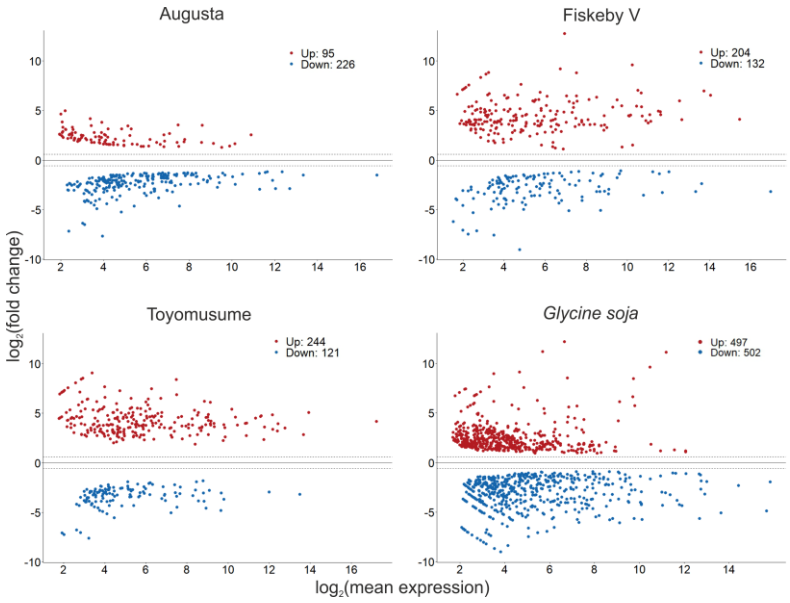
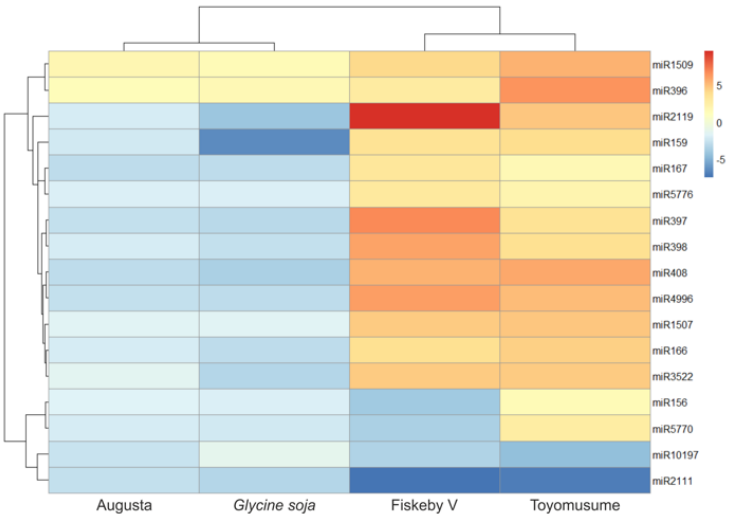
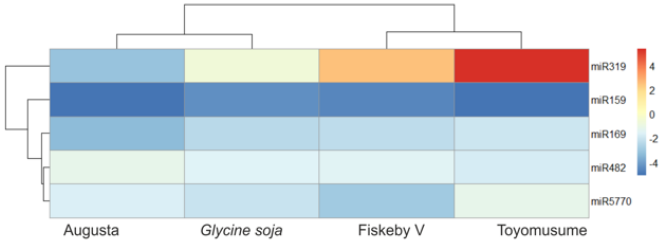


Figure 3. MA plots of differentially expressed miRNAs in four studied cultivars; red dots – upregulated, blue dots – downregulated.

The most pronounced reduction in expression levels during cold stress was found for miR169, miR166, miR159 and miR5037 in cotyledons (VE stage); for miR159, miR160 and miR171 in seedling roots (VE stage); and for miR167, miR2111, miR5371, miR5037, miR398, miR4416 and miR160 in trifoliates (stage V1). The most significant increase in expression levels was observed for miR6300, miR5368, miR6173 and miR1509 in cotyledons (VE stage); for miR319, miR9750, miR408 and miR2109 in seedling roots (VE stage); and for miR166, miR398, miR2119, miR399, miR4996, miR171, miR6300, miR5368 and miR169 in trifoliates (V1 stage), in all four varieties. Several miRNA families, including miR159, miR319, and miR482, showed differential universal expression in seedling roots, with miR159 and miR482 expression levels being decreased in all four varieties, while miR319 decreased in Augusta and *G. soja* and increased in Fiskeby V and Toyomusume. Similarly, trifoliates stressed at stage V1 had miRNAs with different expression levels in all soybean cultivars tested, including miR10197, miR1507, miR1509, miR159, miR166, miR2111, miR3522, miR396, miR398, miR408, and miR2111 with lowered and miR1509 and miR396 with increased expression levels. miR1507, miR159, miR166, miR3522, miR398, miR408 and miR4996 had reduced expression levels in Augusta and *G. soja*, and increased in Fiskeby V and Toyomusume (Fig. 7).

Importantly, several miRNAs showed distinct expression patterns in the cold sensitive variant Toyomusume and the cold tolerant strains of Augusta, Fiskeby V, and *G. soja*. It was found that in the roots of Augusta, Fiskeby V and *G. soja* seedlings, the expression levels of miR169 and miR5770 were decreased by 2.3-, 3.3- and 2.4-fold, and 2.9-, 1.7- and 2,1-fold respectively, while their expression did not change significantly in Toyomusume, suggesting their participation in the response to cold stress (Figure 9). Similar expression patterns were found in trifoliate (stage V1) for miR156 and miR5770, where expression levels were reduced by 1.7-, 3.9- and 1.9-fold, and by 2.1-, 3.6- and 2.3 -fold in the cultivars Augusta, Fiskeby V and *G. soja*, while their expression levels did not change significantly in the cultivar Toyomusume. Interestingly, 7 miRNAs (miR1507, miR159, miR166, miR3522, miR398, miR408, and miR4996) were upregulated in Toyomusume and Fiskeby V variants, while in Augusta and *G. soja* was reduced.





In addition, 18 new miRNAs were differentially expressed in the stressed samples. Among them, 4 miRNAs were found in more than one variety, two of which were common to the cotyledons and trifoliates of *G. soja*, one shared by the trifoliates of G. soja and Augusta, and one shared by the trifoliates of *G. soja* and Fiskeby V.

To verify the plausibility of the sequencing results, the expression levels of the four miRNAs (miR169, miR408, miR2109 and miR5770) were analyzed by ddPCR (Figure 10). The results of the analyzes showed that the expression patterns of the 4 selected miRNAs were mostly consistent with the sequencing results (except miR169 in Augusta and Fiskeby V and miR408 in Toyomusume). The fold-change discrepancy between individual samples can be attributed to differences in the sensitivity and specificity of the two techniques. Moreover, in ddPCR it is difficult to distinguish between individual miRNAs belonging to one miRNA family, which may further explain some of the differences observed between sequencing and ddPCR results.

From the results of degradome sequencing and subsequent analysis by CleaveLand software, we identified potential soybean miRNA target sequences that are involved in the cold stress response. A total of 2005 mRNA targets were identified in all 24 degradome libraries, of which 1183 belonged to category 0, 222 to category 1, 293 to category 2, 164 to category 3 and 143 to category 4, where category 0 represented the best match between miRNA and genes target. Further studies revealed potential miRNA targets with different expression, such as the GAMYB binding protein Glycine max (LOC732608), the transcription factor NAC Glycine max (LOC100814504) and superoxide dismutase [Cu-Zn] Glycine max (SOD1). The term GO has been assigned to 378 target genes controlled by 16 differently expressed miRNAs. Target genes have been described by 53 terms under the biological processes category, 31 terms under molecular functions, and 25 terms under the cellular compartment category. Terms broadly represented were "biosynthesis process", "cellular nitrogen compound metabolic process" and "cell protein modification process" in the category of biological processes; "ion binding", "DNA binding" and "oxidoreductase activity" in the category of molecular functions; and "cell nucleus", "protein-containing complex" and "membrane" in the category cell compartment. Furthermore, the results of the KEGG analysis indicated that 378 target genes were significantly enriched in 67 pathways, including purine and tyrosine metabolism, isoquinoline alkaloids biosynthesis, glycolysis and gluconeogenesis, and pyruvate and thiamine metabolism.

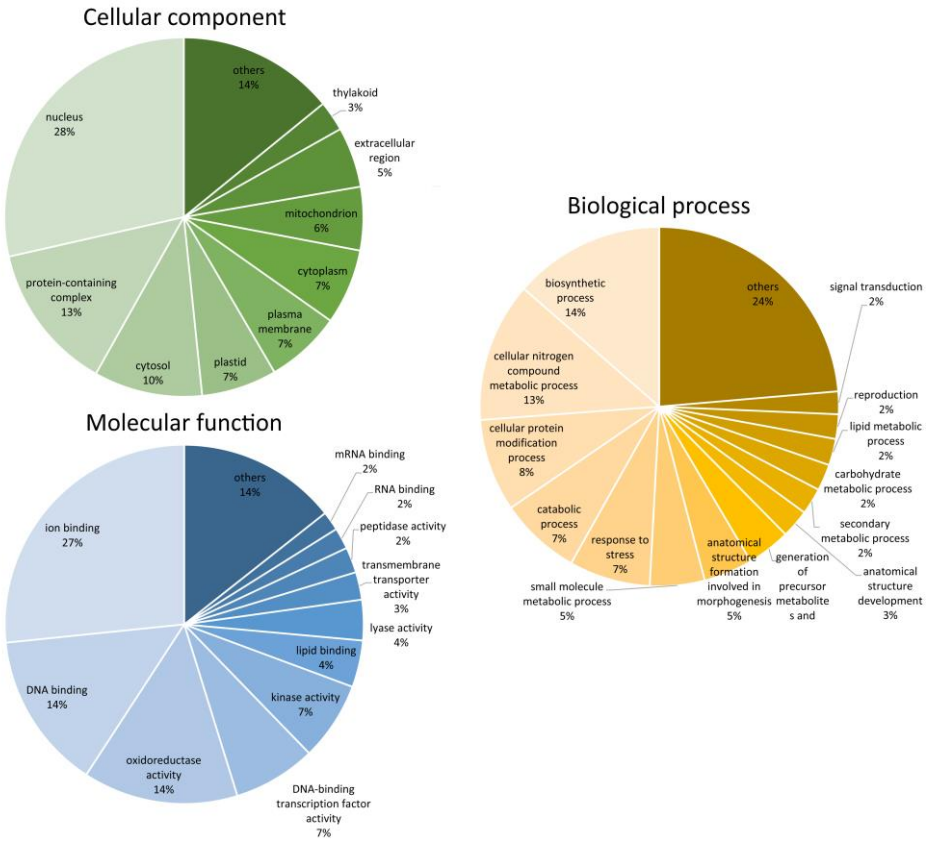


Figure 7. Gene Ontology (GO) analysis of target genes of differentially expressed miRNAs.

Three genes were selected for the analysis of changes in the level of gene expression in the roots and cotyledons of the four studied soybean cultivars: Glycine max putative phytocyanin (*Phyt*, NM_001251440.2), *Glycine max* transcription factor (NAC-19, NM_001255827.1) and *Glycine max* malate dehydrogenase [NADP] (GmMDH, NM_001369219.1). In the roots, after stress exposure, the expression of the *Phyt* gene was increased in the cultivars Augusta and Fiskeby V, in contrast to Toyomusume and *G. soja*, where a significant reduction in the expression level of this gene was observed. The expression level of the gene encoding transcription factor NAC-19 was significantly increased in the roots in all tested cultivars, and of the gene encoding GmMDH was increased in the roots in the cultivars Augusta, Fiskeby V and Toyomusume, a decrease in expression level by 50% was noted for *G. soja*. In cotyledons, the expression of a gene encoding phytocyanin was decreased (by at least 50%) in all cultivars except *G. soja*, where it was increased by 40%.

The expression level of the gene encoding transcription factor NAC-19 was increased in the cotyledons of all tested cultivars, and that of a gene encoding GmMDH was increased in Augusta and Toyomusume and decreased in Fiskeby V and *G. soja*.

Importantly, the expression level of the *nac-19* gene was significantly increased in the roots and cotyledons of all tested cultivars. This result correlated well with the observed reduction in the expression level of miR408 in the cotyledons. In the roots of the cultivars Augusta and Toyomusume, the levels of GmMDH were elevated or kept at the same level (in Fiskeby V). Only one cultivar (*G. soja*) showed a decrease in the level of GmMDH. Only in cotyledons of Augusta did it maintain an increased level of GmMDH expression. An increase was also seen in *G. soja*. In this study, an increase in the expression of the *Phyt* genes was observed in the roots of Augusta and Fiskeby V - two cold-resistant soybean varieties. On the contrary, in the cotyledons the expression level was decreased in the cultivars Augusta, Fiskeby V and Toyomusume. The different expression profiles included proteins involved in redox homeostasis and electron transports, which may be responsible to some extent for increased/decreased susceptibility to abiotic stresses.

Previous studies have shown that reprogramming the central metabolism of carbohydrates plays a key role in the acclimatization of plants to the cold [29-31]. These findings were confirmed in the above study, which showed that sugar metabolism was one of the pathways influenced by 16 miRNAs with varying expression levels identified during the analysis. In addition, sugars stabilize biological membranes, liposomes, act as osmoprotectants, and even stabilize the photosynthesis process during stress, as it has been shown that reduced photosynthetic capacity in plants is often accompanied by increased sugar accumulation [29, 32]. Cold stress also negatively affected photosynthesis and CO2 binding [32-34], which was further confirmed in the above studies, as enzymes involved in carbon binding, photosynthesis and nicotinate-nicotinamide metabolism were among the targets of the miRNA with altered expression levels under stress conditions cold. Glutathione, the organic sulfur storage, in reduced form (GSH) is an essential metabolite in various biosynthetic pathways such as detoxification and redox homeostasis [35]. To date, several reports indicate the involvement of glutathione in response to abiotic stresses [36-39]. At low temperatures, a high content of GSH and glutathione reductase activity have been detected in several plant species, indicating their potential contribution to cold tolerance and cold acclimation [40]. Glutathione metabolism was one of the pathways identified in the KEGG analysis in which three enzymes reductase, 5-oxoprolinase (ATP hydrolysis) and dehydrogenase (NADP +) were predicted as miRNA targets with altered expression levels during cold stress.

4E. The most important cognitive achievements of the presented research

1. Sequencing of two common maize genomes, varieties differing in resistance to herbicidal stress.
2. Identification of SNP changes and insertion/deletion polymorphisms in genes involved in the transformation of phosphate in maize TL line.
3. Identification of changes in DNA methylation patterns of two maize cultivars under the influence of herbicidal stress.
4. Identification of DNA sequences differently methylated under the influence of herbicidal stress of two studied maize cultivars.
5. Identification of miRNAs differentially expressed under the influence of herbicidal stress of two maize cultivars under the influence of herbicidal stress.
6. Determination of the role of miR827 in Pi homeostasis and in plant response to herbicidal stress.
7. Phenotypic analysis and identification of the adaptive abilities of two soybean cultivars (Augusta and Fiskeby V) subjected to cold stress.
8. Determination of changes in miRNA expression levels under the influence of cold stress in 4 soybean cultivars with different susceptibility and response to the above-mentioned stress.
9. Identification of 348 new miRNAs in soybeans.

10. Depiction of the molecular pathways involved in the soybean response to cold stress conditions (carbohydrate metabolism, photosynthesis and CO_2 binding, glutathione and glutathione metabolism).

4F. Summary

Maize and soybeans are crops derived from a warm climate. Maize has been cultivated in a temperate climate, including Poland, for many years. Moreover, it was shown that different native maize cultivars are characterized by different resistance to the applied herbicides. So I decided to carry out a molecular analysis of the basis of this diversity. On the basis of the obtained results, it was shown that the changes occurring in the studied varieties of maize are multilevel and multifactorial. Changes at each stage of the regulation of genetic expression have been identified, i.e. in genomes, transcriptomes, miRNA pools, and the degradome. Such broad changes suggest that in natural populations there are multiple mechanisms that collectively increase stress resistance, in contrast to genetically modified plants, where single changes are made at the genome level. As a result of the analyzes, thousands of structural variants were identified, also in protein-coding regions, in two variants that differ in susceptibility to RoundUp®. The genes and pathways involved in glyphosate metabolism were investigated and a number of changes that could affect gene expression levels was identified. It was also shown that herbicidal stress caused an increase in the number of fully methylated sequences in the sensitive line, 6h after spraying, by 18.64%. Among the identified sequences differently methylated under the influence of the herbicide were genes encoding transferases, transporter proteins, methyltransferases, hydrolases, transposons, ribosomal proteins, cytochromes and proteins involved in transcription and stress response. In the course of the research, miRNAs undergoing altered expression under the influence of herbicidal stress in the tolerant line were also identified and a response mechanism based on miR827-3p and phosphate transporters in cell membranes was proposed. It has therefore been shown that the natural resistance of maize to herbicides is much more complicated than just a single trait change (seen in genetically modified crops) and is based on multiple mechanisms and levels of regulation of the expression of genetic information (epigenetics, small non-coding RNA, changes in expression and composition of gene products), which together contribute to an increase in the resistance of individual plant varieties to stress conditions.

Soybeans are most exposed to cold stress in temperate climate conditions. For the analysis of soybean responses to cold stress conditions, 3 soybean cultivars were selected (two resistant - Augusta and Fiskeby V; one sensitive - Toyumusume) and *G. soja*. Changes in the expression levels of the short regulatory RNA pools and the degradome were studied in tissues of stressed plants at different stages of growth. A total of 321 known miRNAs were identified, and 348 novel miRNAs were predicted, of which 162 miRNAs, including well-conserved, legume- and soybean-specific miRNAs, and 18 novel miRNAs, respectively, had changed expression profiles. Interestingly, several miRNAs such as miR156, miR169 and miR5770 had similar expression patterns in Augusta, Fiskeby V and *G. soja*, which clearly contrasted from that in cold-sensitive Toyomusume variety. Altogether, the results suggest that these miRNAs may play a role in the chilling responses of soybean. Degradome analysis as well as GO and KEGG annotations allowed us to assign potential target genes to the differentially expressed miRNAs. Many of these genes were found to be related to plant abiotic stress response mechanisms such as ROS scavenging, flavonoid biosynthesis and regulation of osmotic potential. Phenotypic analyzes of two ‘resistant’ to cold soybean cultivars under controlled stress (phytotron) and in the field were also performed. Remarkably, Augusta and Fiskeby V plants stressed at the stage of sowing and vegetative growth showed an increase of the indexes by ~ 10% compared to the control plants. A reduced seed yield was observed only in the Fiskeby V variety stressed in the R1 phase (reduction by 7.4%) and in Augusta plants stressed in the VE phase. Under field conditions, it was shown that the Augusta variety is characterized by a higher yield (g of seeds) regardless of the applied stress, this variety turned out to be better adapted to Polish cultivation conditions, as shown by the results of the field experiment.

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5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

A. List of publications not included in the scientific achievement

Scientific publications in journals in the database (Journal Citation Reports (JCR):

1. Tomkowiak A, Bobrowska R, Kwiatek M, Spychala J, Kuczynski J, Tyczewska A, Kowalczewski PL, Weigt D, Kosiada T (2023) Analysis of miRNA expression associated with gene Lr34 responsible for resistance mechanisms to wheat leaf rust. Pak. J. Bot., 55(1): doi: http://dx.doi.org/10.30848/PJB2023-1(6)
   MNiSW (2019): **40** IF (2020): **1.101** IF (5-year): **1.066**

2. Woźniak E, **Tyczewska A**, Twardowski T (2021) A shift towards biotechnology: Social opinion in the EU. Trends in Biotechnology, 39(3):214-218, doi: 10.1016/j.tibtech.2020.08.001
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#article originally published in Oligonucleotides (ISSN 1545-4526), 01 Oct 2011, journal later changed its name to Nucleic Acids Therapeutics (https://www.liebertpub.com/action/doSearch?ContribAuthorRaw=Tyczewska%2C+Agata)

Total IF of publications not included in the scientific achievement: **48.132** (5-year IF: **45.134**) *
* Total Impact Factor (IF) was calculated on the basis of data from Web of Science (WoS), in accordance with the year of publication of a given item
Number of MNiSW points: **685** *
* The number of MNiSW points was calculated on the basis of the MNiSW score, in accordance with the year of publication of a given item

<u>Publications in journals other than those in the JCR database:</u>

1. Woźniak E, **Tyczewska A**, Twardowski T (2022) Public opinion on biotechnology and genetic engineering in the European Union: Polish consumer study. BioTechnologia. Journal of Biotechnology, Computational Biology and Bionanotechnology 103(2),
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6. **Tyczewska A** (2014) Human ribonuclease Dicer inhibition using RNA aptamers. BioTechnologia. Journal of Biotechnology, Computational Biology and Bionanotechnology 95(4), 259-263
MNiSW: **7**

7. Kurzyńska-Kokorniak A, Koralewska N, **Tyczewska A**, Twardowski T, Figlerowicz M (2014) RNA oligomers as microRNA production process regulators. BioTechnologia. Journal of Biotechnology, Computational Biology and Bionanotechnology 95(4), 272-275
MNiSW: **7**

8. **Tyczewska A**, Bąkowska-Żywicka K (2010) miR-122 silencing - a novel therapy for HCV infections? (in Polish). Biotechnologia, 3(90), 29-35
MNiSW: **6**

9. **Tyczewska A**, Figlerowicz M (2009) New face of the "RNA world" (in Polish). Nauka 2, 93-111
MNiSW: **9**

10. Bąkowska-Żywicka K, **Tyczewska A** (2009) The structure of the ribosome – short history. Biotechnologia 1(84), 14-24
MNiSW: **6**

11. **Tyczewska A**, Bąkowska-Żywicka K (2009) Recycling of eucaryotic ribosomes. Biotechnologia 1(84), 81-86
MNiSW: **6**

12. Bąkowska-Żywicka K, **Tyczewska A** (2009) Ribophagy – the novel degradation system of the ribosome. Biotechnologia, 1(84), 99-103
MNiSW: **6**

13. Bąkowska-Żywicka K, **Tyczewska A**, Twardowski T (2008) Genetically modified organisms – economy and safety (in Polish). Nauka 3, 59-65
MNiSW: **9**

14. **Tyczewska A**, Bąkowska-Żywicka K (2008) Animals as bioreactors – the future of pharmaceutical industry? (in Polish). Biotechnologia 3(82), 64-71
MNiSW: **6**

15. **Tyczewska A**, Twardowski T (2008) Genetically modified organisms, pro and con (in Polish). Bromatologia i chemia toksykologiczna 3, 197-201
MNiSW: **6**

16. Bąkowska-Żywicka K, **Tyczewska A**, Twardowski T (2008) Civilization diseases – therapeutic application of antisense strategy (in Polish). Nauka 1, 45-62
MNiSW: **9**

13. **Tyczewska A**, Bąkowska-Żywicka K, Twardowski T (2006) Therapeutic applications of aptamers, in: Na pograniczu chemii i biologii, vol. XIV, 175-203.

14. Figlerowicz M, **Tyczewska A**, Figlerowicz M (2006) The influence of small regulatory RNAs on the viral infection - new strategies in HCV therapy (in Polish). Przegląd Epidemiologiczny, 60 (4), 693-700
MNiSW: **4**

15. Bąkowska-Żywicka K, **Tyczewska A**, Twardowski T (2006) Mechanism of the peptide bond synthesis – controversions (in Polish). Postępy Biochemii, 52(2), 166-172
MNiSW: **5**

16. **Tyczewska A**, Sikora M (2005) Philadelphian list (in Polish). Biotechnologia, 4(71), 232-235
MNiSW: **6**

17. **Tyczewska A**, Figlerowicz M, Twardowski T (2003) RNA aptamers as potential antiviral drugs (in Polish). Biotechnologia, 2(61), 15-164.

Number of MNiSW points *: **184**
* The number of MNiSW points was calculated on the basis of the MNiSW score, in accordance with the year of publication of a given item

<u>Conference abstracts in magazines from the WoS list:</u>

1. Kuczynski J, **Tyczewska A**, Gracz J, Twardowski T (2019) Small RNA-Seq highlights importance of microRNAs in soybean chilling stress, FEBS Open Bio, 9(1), 311-312
MNiSW (2019): **70** IF (2019): **2,231** IF (5-letni): **2,231**

2. **Tyczewska A**, Mahmoud M, Zywicki M, Karlowski W, Gracz-Bernaciak J, Twardowski T (2019) (Roundup (R) resistance trait traced in genomes of maize inbred lines, FEBS Open Bio, 9(1), 311-311
MNiSW (2019): **70** IF (2019): **2,231** IF (5-letni): **2,231**

3. Gracz J, **Tyczewska A** (2016) Changes in DNA methylation in maize under herbicide stress conditions, New Biotechnology 33(S), S167-S167, Meeting Abstract: P19-4
MNiSW (2016): **30** IF (2016): **3,813** IF (5-letni): **3,755**

4. Kuczynski J, Gracz J, **Tyczewska A** (2016) Analysis of cold-regulated microRNAs in soybean, New Biotechnology 33(S), S163-S163, Meeting Abstract: P18-32
MNiSW (2016): **30** IF (2016): **3,813** IF (5-letni): **3,755**

5. **Tyczewska A**, Zywicki M, Gracz J, Karlowski W (2016) Changes in expression of miRNAs involved in stress responses and senescence in glyphosate-treated maize, New Biotechnology 33(S), S160-S161, Meeting Abstract: P18-24
MNiSW (2016): **30** IF (2016): **3,813** IF (5-letni): **3,755**

Total IF of conference publications: **15,901** *
* Total Impact Factor (IF) was calculated on the basis of data from Web of Science (WoS), in accordance with the year of publication of a given item
Number of MNiSW points: **230** *
* The number of MNiSW points was calculated on the basis of the MNiSW score, in accordance with the year of publication of a given item.

Patents:

1. European patent under the PCT procedure no. 2 255 002 "Method to inhibit ribonuclease Dicer, ribonuclease Dicer inhibitor, and use of RNA aptamers as ribonculease Dicer inhibitors**", 02.04.2014,** Figlerowicz M, **Tyczewska A**, Twardowski T, Szopa A, Kietrys AM

2. Patent no. 212696 „Inhibitor rybonukleazy Dicer (Ribonuclease Dicer inhibitor)", Patent Office of the Republic of Poland, **27.11.2012**, Figlerowicz M, **Tyczewska A**, Twardowski T, Szopa A, Kietrys AM

Scientific publications in the review:

1. Public perception of plant gene technologies worldwide (2022) Woźniak E, Tyczewska A, Perisic M, Beniermann Anna, Eriksson D, Vangheluwe N, Gheysen G, Cetiner S, Abiri N and Twardowski T – in: GM Crops and Food (Biotechnology in Agriculture and the Food Chain)

Other scientific projects:

1. **Bioeconomy and sustainable development. Social perception of biotechnology, GMO and bioeconomy** - cooperation with Dr. Ewa Woźniak-Gientka and prof. Dr. hab. Tomasz Twardowski (Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań)
2. **Analysis of wheat response to abiotic stress** - cooperation with Dr. hab. Agnieszka Tomkowiak (Poznań University of Life Sciences)

Additional activity:

2022 co-editor of the Special Issue of the EFB Biotechnology Journal entitled: "Circular and sustainable bioeconomy: legal and social aspects", https://www.journals.elsevier.com/efb-bioeconomy-journal/call-for- papers / circular-and-sustainable-bioeconomy-legal-and-social-aspects

2018 until now - Editor of the journal "Postępy biochemii", https://postepybiochemii.ptbioch.edu.pl/index.php/PB/about/editorialTeam

2016 until now - Deputy Editor-in-Chief of the quarterly "BioTechnologia. Journal of Biotechnology, Computational Biology and Bionanotechnology ", http://www.biotechnologia-journal.org/Journal/BioTechnologia-85

2015 until now - Inspector for GMM / GMO supervising genetic engineering plants and work with genetically modified organisms and microorganisms at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań

2010 co-editor of the 3/2010 issue of the quarterly 'Biotechnologia' on non-coding RNAs

2009 co-editor of the 1/2009 issue of the quarterly 'Biotechnologia' on protein biosynthesis

6. Presentation of teaching and organizational achievements as well as achievements in popularization of science or art

Scientific supervision of PhD students as an auxiliary supervisor:

1. **Auxiliary supervisor from June 2012 to April 2015**, substantive supervision from January 2011. Supervision of the doctoral thesis by Joanna Gracz- Bernaciak. Thesis topic: "Alternative splicing as a response mechanism to herbicidal stress in common maize". Defense 2015.
2. **Auxiliary supervisor from 2018,** substantive supervision from October 2015 to the present. Supervision of the doctoral dissertation of Mr. Jakub Kuczyński. The subject of work: Changes in miRNA expression and degradome under the influence of cold stress in 4 soybean cultivars characterized by different sensitivity to cold. Planned Defense 2022.

Scientific supervision of students:

Master's thesis supervisor:

1. *Aleksandra Klimek,* 2019, UAM: "Differential analysis of regulatory short RNAs in selected common corn lines under herbicidal stress"

Scientific supervisor of graduate students:

1. *Anna M. Kietrys* (2006-2008), Poznań University of Life Sciences
2. *Aleksandra Szopa* (2006-2008), Lodz University of Technology

Engineering thesis supervisor:

1. *Jakub Szymkowiak,* 2014, AMU "Analysis of changes in the level of DNA methylation of two selected *Zea mays* lines under the influence of herbicidal stress"
2. *Maciej Pszczoła,* 2016, AMU "Profiling the expression of selected miRNAs in soybean cold stress"

Scientific supervision over interns:

1. *Dobrosława Michalska,* 2014, UP, six-month internship, topics: analysis of the effect of herbicidal stress on two inbred maize lines differing in their sensitivity to the RoundUp herbicide
2. *Aleksandra Mazur,* 2014, UP, six-month internship, topics: analysis of the impact of herbicidal stress on two inbred maize lines differing in sensitivity to the RoundUp herbicide

3. *Monika Gazecka*, 2018, AMU, topics of practice: Analysis of changes in RNA levels in crops under the influence of abiotic stresses
4. *Martyna Dolata*, 2021, TEB Technical School Education, monthly internships, topics: organization of work in the laboratory, learning and application of health and safety, environmental and sanitary-epidemiological regulations, working with biological material in sterile conditions
5. *Wiktoria Zielnik*, 2021, TEB Technical School Education, monthly internships, topics: organization of work in the laboratory, learning and application of health and safety, environmental and sanitary-epidemiological regulations, working with biological material in sterile conditions
6. *Wiktoria Springer*, 2021, TEB Technical School Education, monthly internships, topics: organization of work in the laboratory, learning and application of health and safety, environmental and sanitary-epidemiological regulations, working with biological material in sterile conditions

Reviewing theses:

1. *Natalia Maria Dąbrowska*, 2015, Poznań University of Life Sciences. RNA-dependent transcriptional modulation of gene expression in *Bacillus subtilis* as exemplified by the lysine ribs switch

Internships in foreign research centers:

1. Austria, Vienna, Gregor Mendel Institute of Plant Molecular Biology, Austrian Academy of Sciences, Matzke team, postdoctoral fellowship (02.2009-10.2010)

2. Switzerland, Basel, Friedrich Miescher Institute for Biomedical Research, Ciosk team (May 15, 2017 - August 15, 2017)

Implemented grants / research projects:

1. COST Action CA18111 - Genome editing in plants - a technology with transformative potential. The implementing entity is the Swedish University of Agricultural Sciences (head: Dr. D. Eriksson), **role - researcher in the WG4 group**, implementation time: April 2019 - April 2023

2. Ministry of Science and Higher Education, activities promoting science, type of application - publishing activities, for 2017, applicant: Institute of Bioorganic Chemistry of the Polish Academy of Sciences. The grant was related to the co-financing of the quarterly BioTechnologia. Journal of Biotechnology, Computational Biology and Bionanotechnology ", implementation time: January 2017 - December 2018

3. National Science Center, competition 15, subtype OPUS, December 2014. Identification of molecular mechanisms involved in soybean's cold stress response in temperate climate conditions, project manager: prof. dr hab. Tomasz Twardowski, **role - main researcher**, implementation time: July 2015 - January 2020

4. National Science Center, competition 6, subtype MAESTRO, September 2012. Multilevel approach to reveal elucive molecular basis of herbicide stress response in maize, project manager: prof. dr hab. Tomasz Twardowski, **role - main researcher**, implementation time: May 2013 - November 2019

5. National Science Center, competition 1, subtype SONATA, June 2011. Analysis of the epigenome and proteome in *Zea mays* lines resistant and sensitive to a herbicide**, head and main investigator - Dr. Agata Tyczewska**, implementation time: December 2011 - December 2015

6. National Science Center project, subtype OPUS, March 2011. Small non-coding RNAs and changes in plant degradation as a response mechanism to herbicidal stress conditions, principal investigator: prof. dr hab. T. Twardowski, **role - researcher**, implementation time: March 2011-March 2014

7. National Science Center, subtype OPUS, October 2010. Alternative splicing in common maize induced under conditions of herbicidal stress, principal investigator: prof. dr hab. T. Twardowski, **role - researcher**, implementation time: October 2010-October 2013

8. MNiI project, June 2009. Short RNA molecules as regulators of the enzymatic activity of the human Dicer ribonuclease, principal investigator: Dr. A. Kurzyńska-Kokorniak, **role - researcher**, duration: June 2009 - June 2012

9. RNA-dependent DNA methylation (postdoctoral fellowship, Austria), implementation period: February 2009 - October 2010

10. Searching for RNA inhibitors of Dicer and HIV-1 RT ribonuclease (PhD thesis), implementation period: September 2002 - June 2008.

Popularization of science:

2014 Seminar of the Scientific Society of Biotechnology Students "OPERON", University of Life Sciences in Poznań, April 24, 2014, Poznań, lecture "How do plants cope with stress? on the example of maize and herbicidal stress "

2017 Eurobiotech, 6th Central European Congress of Life Sciences, 11-14 September 2017, Kraków, Poland - workshop “To publish or not to publish?”, Lecturer, lecture topic: “How to increase the chance that your paper will be published? "

2019 Eurobiotech, 7th Central European Congress of Life Sciences, 23-25 September 2019, Kraków, Poland - workshop “To publish or not to publish?”, Workshop organizer and lecturer, lecture topic: “How to increase the chance that your paper will be published? "

2019 Participation in the Biologists' Night event, 11/01/19

2019 Participation in the Scientists' Night event 27/09/19

2020 Participation in the Scientists' Night event, 27/11/20

2021 Partner of the social campaign "We love R&D", the idea of which focuses on solving real problems of Polish science and R&D activities related to building a positive and interesting image of a scientist and innovator, commercialization of developed solutions through communication and popularization of science in society.

Activity in the institute's bodies and its committees:

Member of the Team for the Purchase of Strategic Equipment at ICHB PAN

Member of the Bioethics and GDPR Team in bioethical research at ICHB PAN

Activities in non-institute bodies:
Member of the Biotechnology Committee at the Poznań Branch of the Polish Academy of Sciences, term 2019-2023

7. **Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.**

Received awards and distinctions:

2011 Distinction of the publication "*Selection of RNA oligonucleotides that can modulate human Dicer activity in vitro (2011), Nucleic Acid Ther. 21 (5), 333-46 (Tyczewska A, Kurzynska-Kokorniak A, et al.)*, by F. Steele, editor of Nucleic Acid Therapeutics - one of the most interesting experimental works published in Nucleic Acid Therapeutics in 2011

2019 Scientific Award of the Institute of Bioorganic Chemistry of the Polish Academy of Sciences for the best review publication created at the Institute in 2018 for the work:
*Tyczewska A, Woźniak E, Gracz J, Kuczyński J, Twardowski T * (2018) Towards Food Security: Current State and Future Prospects of Agrobiotechnology. Trends in Biotechnology, 36 (12): 1219-1229, DOI https://doi.org/10.1016/j.tibtech.2018.07.008*

2020 Polish Intelligent Development Award 2020 in the category "Scientist of the future" for the implementation of the *NCN project "Analysis of the epigenome and proteome of common maize (Zea mays), a herbicide resistant and sensitive line"*

My scientific achievements to date include **37** original creative works, **2** patents (European and Polish), **28** conference announcements, **10** conference papers, 2 chapters in monographs. My published scientific achievements consist of **35** co-authored works and **2** author's works, of which I am the lead author in **16** publications, and I am a correspondent author in **14**. Of the **37** original creative works, **27** were published in English, including **15** in the journals of the Master Journal List.
The total amount of points obtained by me in accordance with the list of journals of the Ministry of Science and Higher Education, taking into account the scientific achievement, is **1,444**.
In the period before obtaining the doctoral degree, my scientific achievements consisted of 6 original creative works. A significant increase in the scientific and research achievements took place after obtaining the doctoral degree.
The total IF of my publications according to the Journal Citation Reports (JCR) list is **78.833** (5-year IF: **79.504**), the total IF of conference publications: **15.901**, h-index: **7**, number of citations: **143**, without self-citations: **130**.