PhD. Eng. Agnieszka Niedziela

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Attachment 5

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

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A. PERSONAL DATA

Name / names and surname: Agnieszka Katarzyna Niedziela
Previous surname: Fiuk (from 2001 to 2010)
Date and place of birth: 24th September 1975, Iłża, Poland
Academic degree: doctor (PhD)
Place of employment: Plant Breeding and Acclimatization Institute - National Research Institute, Department of Plant Physiology and Biochemistry, Laboratory of Molecular Markers, Radzików, 05-870 Błonie, Poland

B. SCIENTIFIC CAREER

Higher education:

1995-2000 Warsaw University of Life Sciences – SGGW, Faculty of Horticulture and Landscape Architecture, Department of Plant Genetics, Breeding and Biotechnology, Nowoursynowska 166 str., Warsaw, Poland

MSc Horticulture (plant genetics)
I prepared my master's thesis entitled „Characteristic of T2 tomato generation after transformation with npt II and thaumatin II genes" at the Department of Plant Genetics, Breeding and Biotechnology under the supervision of doctor Grzegorz Bartoszewski

Doctorate:

2001-2005 Polish Academy of Sciences Botanical Garden - Center for Biological Diversity Conservation in Powsin, 02-973 Warsaw, Prawdziwka 2 str., Poland

I prepared my doctoral thesis entitled „Morphogenic capacities of multicellular and single cell explants of gentians" at the Laboratory of Plant Biotechnology under the supervision of Professor Jan J. Rybczyński (Reviewers: Professor Ewa Kępczyńska, Professor Katarzyna Niemirowicz-Szczytt). Thesis defended with distinction on 21th December 2005 in Warsaw University of Life Sciences - SGGW. Date of receiving the doctor degree: 11th January 2006

Other forms of education:

Participation in trainings and scientific workshops:

1. „Application of Molecular Markers in Studies on Plants" 25-29 September 2002, Warsaw, Poland
2. „Application of novel cytogenetic and molecular techniques in genetics and breeding of the grasses." Centre of Excellence in Plant Agrobiology and Molecular Genetics PAGEN. 31 March - 2 April 2003, Poznań, Poland

3. „Molecular and cytogenetic diagnostics in plant breeding.” Centre of Excellence in Plant Agrobiology and Molecular Genetics PAGEN. 27-30 April 2004, Poznań, Poland
4. „Plant genome mapping and search for markers linked to agricultural traits.” Centre of Excellence in Plant Agrobiology and Molecular Genetics PAGEN. 18 October 2004, Poznań, Poland
5. „Alternative plants for balanced agriculture.” Centre of Excellence in Plant Agrobiology and Molecular Genetics PAGEN. 7-8 September 2005, Poznań, Poland
6. „MSB” Service for Molecular Biology „Course for molecular phylogenetics.” 22-24 June 2006, Warsaw, Poland
7. „Planning and inference of statistical analysis in agricultural research.” IHAR-PIB. Radzików, 20-22 November 2007, Warsaw, Poland.
8. „Statistical modeling of bioinformatics data.” Wrocław University of Environmental and Life Sciences & European Social Fund. 25-29 June 2012, Wrocław, Poland.
9. „The R system in population genetics.” QuantUp. 30-31 August 2012, Radzików, Poland

C. PROFESSIONAL EXPERIENCE

2001- 2007
Polish Academy of Sciences Botanical Garden - Center for Biological Diversity Conservation in Powsin, Laboratory of Plant Biotechnology; positions: intern engineer (2001); biologist (2001); research assistant (2001-2006); adjunct (2006-2007).

2007 - until now
Plant Breeding and Acclimatization Institute - National Research Institute in Radzików, Department of Plant Physiology and Biochemistry, Laboratory of Molecular Markers; position: adjunct (2007- until now)

(2011 - maternity leave - 6 months)
(2016/2017 - maternity and parental leave - 9 months)

D. INDICATION AND DESCRIPTION OF THE SCIENTIFIC ACHIEVEMENT SPECIFIED IN ARTICLE 16, SECTION 2 OF THE ACT OF 14 MARCH 2003 ON ACADEMIC DEGREES AND ACADEMIC TITLE AND DEGREES AND TITLE IN THE ARTS (DZ. U. [JOURNAL OF LAWS] NO. 65, ITEM 595 AS AMENDED BY SUBSEQUENT LEGISLATION)

1. TITLE OF THE SCIENTIFIC ACHIEVEMENT

A series of five monothematic scientific publications under a common title:

Identification of molecular markers for the evaluation of the aluminum tolerance of triticale (x *Triticosecale* Wittmack) breeding materials.

2. PUBLICATION CONSTITUING THE SCIENTIFIC ACHIEVEMENT

P1). Niedziela A., Orłowska R., Machczyńska J., Bednarek P.T. (2016) The genetic diversity of triticale genotypes involved in Polish breeding programs. SpringerPlus, 5:355.

[$IF_{5\text{-years}}$ = 1.195; IF_{2016} = **0.982**; $MNiSW_{2016}$ = **25**]

My contribution to this work consisted in developing a research concept and planning experiments. I was responsible for the isolation of genomic DNA from leaves as well as preparation of the samples for DArT analysis. On the base of DArT results, I assessed genetic similarity of plant material and population structure. I participated in the analysis of genetic diversity of the tested lines. I was responsible for preparing tables and figures. In the case of the given manuscript, most of the data collection, writing the manuscript and replying the reviewers' comments were on my side. I estimate my percentage share at 70%.

P2). Niedziela A., Bednarek P.T., Cichy H., Budzianowski G., Kilian A., Anioł A. (2012) Aluminum tolerance association mapping in triticale. BMC Genomics, 13:67.

[$IF_{5\text{-years}}$ = 3.938; IF_{2012} = **4.397**; $MNiSW_{2015}$ = **35**]

My contribution to this work consisted in planning experiments and carrying out most of the molecular analyzes (except of DArT). I was responsible for phenotyping the plant materials using physiological test dedicated towards Al-tolerance. I was also responsible for PCR analysis using SSR and AFLP techniques, and preparation of the DNA samples for DArT analysis. I participated in association mapping and the identification of markers associated with Al tolerance. I was responsible for preparing tables and figures incorporated in the ms and participated in writing the ms. I estimate my percentage share at 55%.

P3). Niedziela A., Bednarek P.T., Labudda M., Mańkowski D.R., Anioł A. (2014/ Online 13 listopada 2013) Genetic mapping of a 7R Al tolerance QTL in triticale (x *Triticosecale* Wittmack). Journal of Applied Genetics, 55/1: 1-14.

[$IF_{5\text{-years}}$ = 1.743; IF_{2013} ($_{\text{First Online: 13 Nov 2013}}$) = **1.902**; $MNiSW_{2013}$ = **20**]

My contribution to the creation of this work consisted in planning experiments and the phenotyping plant materials, following genotyping two F2 mapping populations. I was responsible for the isolation of genomic DNA from leaves, PCR analysis using SSR, AFLP, and ALMT gene-specific molecular markers (along with selection PCR conditions), and preparation of the DNA samples for DArT technique. I prepared samples for biochemical analysis (detection of malic and citric acid). I have a significant participation in the construction of genetic maps and QTL identification. After preparing all the data in the form of tables and figures, I participated in writing the manuscript. I estimate my contribution to 50%.

P4). Niedziela A., Mańkowski D., Bednarek P.T. (2015) Diversity Arrays Technology-based PCR markers for marker assisted selection of aluminum tolerance in triticale (x *Triticosecale* Wittmack). Molecular Breeding, 35:209.

[$IF_{5\text{-years}}$ = 2.235; IF_{2015} = **2.108**; $MNiSW_{2015}$ = **35**]

My contribution to this work consisted in developing a research concept, planning experiments and carrying out all the molecular analyzes. On the base of statistical analysis I proposed markers for the selection of triticale lines towards to aluminum tolerance and determined the chromosomal localization of these markers on the genetic maps based on F2 mapping populations. I prepared all tables and figures. I have a significant contribution to the manuscript in the field of the text and replying to the reviewers' comments. I estimate my contribution to 50%.

P5). Niedziela A. (2018) The influence of Al^{3+} on DNA methylation and sequence changes in the triticale (× *Triticosecale* Wittmack) genome. Journal of Applied Genetics. https://doi.org/10.1007/s13353-018-0459-0.

[$IF_{5\text{-years}}$ =1.743; IF_{2017}=**1.756**; $MNiSW_{2017}$ =**20**]

This study was designed and performed by myself and included research concept, planning experiments, carrying out analyzes, interpretation of the results, writing the manuscript and replying the reviewers' comments. My contribution is 100%.

Total 5-years IF for the presented achievement: **10.854**
Total IF according to the year of publication: **11.145**
The total number of MNiSW points: **135**
Statements of co-authors on their own contribution in the preparation of the works constituting the above-mentioned scientific achievement are included in the **Annex 8**.

3. SYNTHETIC DISCUSSION OF THE SCIENTIFIC ACHIEVEMENT

Introduction

Triticale (x *Triticosecale* Wittmack) is a synthetic cereal crop originated from a cross between wheat (*Triticum durum*, 2n=28=AABB or *Triticum aestivum* L., 2n=42=AABBDD) as a seed parent and rye (*Secale cereale* L., 2n=14=RR) as the pollen parent [1]. The first obtained and tested triticale forms were octoploids (2n = 56 = AABBDDRR), while currently the majority of cultivars grown in the world and all Polish varieties are hexaploids (2n=42=AABBRR).
Triticale breeding in Poland was initiated in the 1960s by Professor Tadeusz Wolski, who selected the first winter cultivar Lasko released in 1982. Currently (August 2018), 62 triticale varieties (13 spring and 49 winters) are registered in the Polish National List [2]. Among them, 54 come from two leading Polish companies: Plant Breeding Strzelce Ltd., Co. IHAR-PIB Group (25 varieties) and DANKO Plant Breeding Ltd. (29 varieties).
For many years Poland had been the largest triticale producer in the world [3] with the present area of cultivation 1.3 million ha, which covers 17,1% of the available farming space. Triticale grain is mainly used for feeding and human consumption, but some varieties are suitable for baking purposes [4]. Moreover, triticale is a potential energy crop and putative source of biomass for bioethanol production [5]. The species is characterized by high yielding potential, good health and favorable nutritional value of grain [4]. One of the severe factors limiting of its cultivation may be soil acidification [6]. Acidic soils (pH>5) constitute at least 50% of Polish arable land [7]. The most important causes of soil acidification on agricultural land are natural soil, and climatic conditions leading to calcium and magnesium leaching from the soil, industrial and transport pollution, as well as increased mineral fertilization, observed in recent years [7]. Decreasing the pH amounts to increasing the aluminum concentration in the soil solution. When the pH is less than 5, the aluminum is present as soluble $Al(OH)^{2+}$, $Al(OH)_2^{+}$ and $[Al(H_2O)_6]^{3+}$ ions, which can be uptaken by the roots [8]. The most significant symptom of aluminum toxicity in plants is inhibition of root growth, as well as reduction of lateral roots and root hairs [9], which results in a yield declining [6].

Al-tolerance in plants is based on two mechanisms [9, 10]. The first one relies on the inhibition of the Al uptake by roots (external tolerance), whereas the second involves the immobilization and detoxification of Al ions inside the cells (internal tolerance) [10]. Most of the major crop plants are typically Al excluders. Only a few cultivated species such as tea, hydrangea or buckwheat can accumulate Al^{3+} ions in vacuoles of leaves and shoots cells [9, 10]. Plant response to aluminum stress depends on the activation of many genes, the expression of which is also induced under numerous abiotic [11] and biotic [12] stresses. The most frequently genes involved in Al tolerance are cysteine synthase, S-adenosylmethionine synthase, O-methyltransferase, oxalate oxidase, malate dehydrogenase, pyruvate dehydrogenase, superoxide dismutase, glutathione s-transferase and ascorbate peroxidase [13-16]. Although the trait is multigenetic [17, 18], organic acids such as malate, citrate, and oxalate are the essential compounds chelating Al^{3+} ions and thus performing detoxification [9]. Two gene families responsible for organic acid exudation in cereals has been described by now. The ALMT (aluminum-activated malate transporter) is responsible for malate whereas MATE (multidrug and toxin efflux) for citrate exudation. In rye genes belong to both families were located on the

7R chromosome at a distance of about 25cM [19, 20]. Al-induced genes code for malate and citrate in wheat are located on the 4DL [21] and 4BL [22] chromosomes, respectively. It was also proved that putative STOP1 (sensitive to proton rhizotoxicity 1) transcriptional factor on the chromosome 3R in rye [20] and 3BL in wheat [23] might have regulatory activity affecting the expression of the locus codes both organic acids. The location of ALMT genes in rye and wheat results from the synteny of chromosomes 7R and 4D and these genes are orthologs [24, 25]. It is also possible, that location of the other genes involved in Al-tolerance can results from synteny of 7B and 4B, as well as 3R and 3B chromosomes [20, 26]. Recognized Quantitative Trait Loci (QTL) of Al tolerance in wheat and rye cover a significant part of the phenotypic variance of the trait. The ScALMT and ScMATE rye loci located on the 7RS chromosome of three independent F2 populations accounted for 60% and 40% of the variation [20], respectively. In contrast, in wheat, the level of the explained variance of the trait depends on the mapping population studied and the method of measurement and was up to 56%, 51% and 49% for loci located on 4DL, 4BL and 3BL chromosomes [22, 27, 28], respectively.

Tolerance mechanisms for Al stress in triticale have not been systematically investigated so far. It is presumed, that in the case of this species they may be a function of the interaction of wheat and rye genes [29]. Studies with additive and substitution lines suggest that the rye genome may inherit a high degree of aluminum tolerance in hexaploid triticale, and the genes of the trait are located on chromosomes 3R, 4R and 6R [29, 30, 31]. However, the weaker response of triticale to Al stress, than rye suggests a suppressive effect of the wheat genome [31]. It was also demonstrated in substitution lines, that chromosomes D from the wheat genome might have a beneficial impact on Al tolerance in triticale [30]. Increased expression of genes located on genome D in response to Al stress has also been documented in octoploid triticale [32].

Despite genetic regulation, it is also not excluded, that epigenetic factors can be involved in Al tolerance [33, 34]. Such a notion is supported by studies suggesting, that the regulation of gene expression during adaptation to adverse environmental conditions may be dependent on epigenetic mechanisms such as DNA methylation [33]. Epialleles appearing in the process may arise as a change in methylation patterns induced by Al stress. The induced changes may be inherited in subsequent generative cycles, increasing the adaptive abilities of a given genotype [33]. By now, there were no systematic studies devoted to epigenetic aspects of Al tolerance in cereals, including triticale. Such studies may be fundamental for the understanding of genome functioning and explanation, why some traits, despite many years of selection programs, do not give fully phenotypically uniform lines.

Both genetic and epigenetic studies can be the source of markers for Marker Assisted Selection (MAS). The MAS technique allows fast and reliable assessment of a large number of individuals for the presence or absence of a trait and distinguishes homozygotes from heterozygotes at a very early stage of development. It also allows the breeders to become independent from the influences of many environmental factors, that may interfere with the expected response of plants in field conditions, and to identify individuals with the desired genes even if the trait is multigenic [35]. Application of MAS reduces field works and expenditures on the selection process, which should lead to a shortening of the breeding cycle, the length of which, according to breeders, is the primary limiting factor in breeding progress [36]. Unfortunately, in Poland marker techniques are not widely used by breeders, due to the lack of the necessary base and markers useful in the selection of materials [36]. The failure to implement

molecular selection may significantly diminish the competitiveness of Polish breeding on the domestic and foreign markets in the coming years. Based on studies conducted on rye and wheat, the selection of cereals towards increased tolerance to Al with the use of molecular markers is a challenging but feasible task. Nevertheless, the B1 and B4 markers were selected in rye at the distance of 0.4cM from the ScALMT gene locus and allowed for the differentiation of tolerant and sensitive plants in the tested F2 population [24]. Recently, the markers RZ891, B6, B11 and B26 closely linked to Al tolerance gene in rye were assessed [25, 37]; however their usefulness in the selection of tolerant plants was not tested on diverse plant material. In wheat, two allelic forms of the TaALMT1 gene were found: ALMT1-1 present in the tolerant line ET8 and ALMT1-2 in the sensitive line ES8 [21, 37]. The two forms of the gene originated from the point mutations within the sequence of the fourth exon of TaALMT gene. Moreover, the primers for markers identified in the area of exon 4 [21], intron 3 [38] and promoter region [39] were designed and used to assess the genetic relationships within the TaALMT1 gene in 457 wheat forms [40]. The obtained allele combinations divided the studied plant material into 22 haplotypes [40]. None of the markers, however, were strictly correlated with Al-tolerance.

Until now, the study identifying markers related to Al-tolerance in triticale and potentially useful for MAS have not been carried out.

Research hypothesis

Triticale is a poorly investigated species, whose economic importance in the world and Poland is still growing. Its breeding can be limited by the lack of the forms sufficiently tolerant to Al^{3+} ions. This limitation is even more significant with the increase of soil acidification, as the result of leaching of alkaline components from soil, and human activity (inefficient use of nitrogen fertilizers, acid rain). In triticale, the trait may result from the interaction of rye and wheat genomes, where several relatively strong QTLs were determined. The QTLs (and relevant genes) are widespread in Polish materials, which makes it possible to identify markers useful for MAS. Nevertheless, the Al-tolerant QTLs do not explain the total phenotypic variance of the trait. Therefore, researches verifying the hypothesis that the trait may also have an epigenetic background exhibited at the methylomics level are needed.

The aim of the research

The main aim of the study was to extend the knowledge of Al-tolerance genetic and epigenetic background at the molecular level in triticale using modern marker techniques and statistical approaches including genetic and association mapping for the assessment of the DNA markers for MAS purposes.

The following researches were undertaken to achieve the goal:

1. evaluation of genetic variation and analysis of putative population structure of the available genetic pool of polish breeding triticale lines and forms (P1);
2. identification of the Al-tolerant QTLs and the determination of their number by distinguishing their chromosomal location in triticale genome (P2, P3);

3. search for DNA markers associated/linked with Al-tolerance QTLs within triticale lines and varieties used in Polish breeding programs as well as F2 mapping populations (P2, P3);
4. selection of markers useful for MAS within the available gene pool (P4) and
5. verifying whether Al-tolerance in triticale involves changes in DNA methylation pattern (P5).

Discussion of the scientific achievement

P1). Niedziela A., Orłowska R., Machczyńska J., Bednarek Piotr T. (2016) The genetic diversity of triticale genotypes involved in Polish breeding programs. SpringerPlus 5:355.

Triticale is a very young artificial hybrid, that not undergone long-term natural evolution, like other cereal species [1]. For that reason, its genetic variation is limited, and available accessions in the world differ relatively slightly [41, 42]. Most probably, the similar situation takes place in the case of triticale forms available in Poland. Nevertheless, due to the different directions of breeding programs as well as diverse climatic and environmental conditions, several gene pools could have been selected.

The objective of the study was to estimate genetic diversity and population structure of winter and spring triticale genotypes incorporated into Polish breeding programs and delivered by Plant Breeding Strzelce Ltd., Co. - IHAR-PIB Group.

Plant material for the analysis consisted of 232 triticale lines and registered varieties. The tested plant materials were genotyped using DArT markers (Diversity Arrays Technology). In total 3117 DNA markers were obtained. Identical triticale forms, as well as redundant, monomorphic and markers with many missings, were removed from the analysis. Computations were performed in PAST Software [43]. For the identification of similar or identical triticale forms, genetic distances among them were calculated according to Jaccard's [44] and clustered using the unweighted paired group method with an arithmetic mean (UPGMA) method. The forms were assumed to be identical if the differences between them did not exceed 5% and if their molecular profiles, except but missing markers were identical. Only one representative of the given redundant group was retained, and information about removed forms was saved for further purposes. A similar analysis was performed for the identification of redundant markers. Among 232 genotypes, there were 22 spring and 49 winter forms having nearly identical counterparts (similarity >95%) that were substituted by their representatives. The redundancy of DArT markers resulting from the presence of the additional copies of some DNA sequences equaled about 42%. Finally, the study was conducted on a set of 161 diverse triticale forms (17 spring and 144 winters) and 1829 DArT markers.

The population structure was evaluated using Principal Coordinate Analysis (PCoA) (PAST software) and the Bayesian approach using STRUCTURE 2.2.3 [45] and STRUCTURE HARVESTER [46] software. The averaged genetic structure was estimated in CLUMPP [47]. Indices of genetic diversity, including the percentages of polymorphic markers (P%), Shannon's diversity index (I) and Analysis of Molecular Variance - AMOVA were calculated in GenAlEx 5.3 EXCEL add-in [48]. The following formula estimated polymorphic information content (PIC) of dominant bi-allelic data: $PIC = 1- (p^2 + q^2)$, where "p" is the frequency of present alleles and "q" is the frequency of null alleles [49].

Based on Principal Coordinate Analysis (PCoA) the first two principal coordinates explained the only small fraction of genetic variance (8.7% and 6.5%, respectively). Nevertheless, three groups of data represented by most spring together with some winter forms plus two putative clusters of winter forms could be recognized.

Population structure followed by Bayesian analysis recorded weak stratification (delta K= 58.4) of plant materials into three groups (Pops). In the first group (Pop1) all 17 spring together with 17 winter forms were clustered. The second (Pop2) and the third (Pop3) groups were represented by 101 and 26 winter forms, respectively. The number of polymorphic markers shared by representatives of each group corresponded to 43.7%, 40.7% and 30.7% for Pop1, Pop2, and Pop3 respectively. PIC and I values showed similar trend and were the highest in Pop1 (PIC = 0.319, I = 0.478) and Pop2 (PIC = 0.309, I = 0.466), and the lowest in Pop3 (PIC = 0.234, I = 0.356). AMOVA analysis of the model-based populations Pop1, Pop2, and Pop3 showed that 14% of the variance was due to among-population differences.

Summing up, the triticale genotypes exploited in Polish breeding programs in Plant Breeding Strzelce Ltd., Co. - IHAR-PIB Group exhibit weak populations differences among the groups; however, a relatively high variation within each of the groups was detected what may simplify the identification of Al-tolerant markers identification.

P2). Niedziela A., Bednarek P.T., Cichy H., Budzianowski G., Kilian A., Anioł A. (2012) Aluminum tolerance association mapping in triticale. BMC Genomics, 13:67.

variation in the gene pool of triticale cultivated in Poland creates the possibility to find molecular markers associated with Al-tolerance. Association mapping is a method of choice allowing the identification of a broad spectrum of markers associated with the trait within available plant materials. The method can be used both for the identification of markers with strong and weak associations. The effectiveness of the analysis depends, among others, on the size of the mapping population, the reliability of phenotyping and DNA markers used for genotyping. Moreover, it is known, that breeding crops may be under selective/adaptive pressure. It cannot be excluded that genome areas under such pressure will be responsible for the expression of tolerance to Al in triticale. Additionally, they can be a source of molecular markers useful in future analysis.

This study aimed at identifying markers associated with Al-tolerance present within triticale polish breeding pool. Moreover, markers under positive and balanced selection pressure were assessed as they may also be linked with the trait due to the breeding process.

Advanced 232 triticale breeding forms described in P1 were used in the experiment. The physiological test of Al-tolerance described by Anioł [50] was performed. Triticale seeds were sterilized, germinated and transferred for polyethylene net floated in a tray. The tray was filled with medium containing essential macronutrients. After three days the plants were transferred to the same medium supplemented with $AlCl_3$ at a concentration of 16ppm. After 24h roots were washed with water, and seedlings were placed again in the medium without $AlCl_3$ for 48h. The experiment was performed under controlled-environment growth cabinet (POL-EKO-APARATURA, ST500 B40 FOT10) conditions at 25°C, photoperiod 12/12h day/night, light intensity 40W m-2 and aeration. In order to assess the level of plant tolerance to Al, roots were

stained with Eriochrome Cyanine R resulting in dark purple color in damaged regions of roots. Al treatment leads to irreversible damage of apical meristem of not-tolerant seedlings. Tolerant and moderately tolerant seedlings preserve their ability to root grow after the Al^{3+} ions were removed from the solution. The newly growing root parts are not stained with Eriochrome Cyanine R. The length of the regrowth measured in millimeters reflects the level of plant tolerance to the presence of toxic Al^{3+} ions. Individuals were classified according to the length of regrowth as I) non-tolerant, 0.0-0.2 cm; II) moderately tolerant, 0.2-0.5 cm and III) tolerant, above 0.5 cm. Among the tested forms, 111 was in the group I, 70 in group II and 51 in group III.

Plant material (232 triticale forms) were genotyped using AFLP (Amplified Fragment Length Polymorphisms) and SSR (Simple Sequence Repeats) markers, except for DArT markers described in P1. In total 3289 DNA fragments for each individual representing given triticale form were obtained. Polymorphic markers were assigned to the chromosomes for future statistical analysis. Unassigned markers were group together. Each chromosomal marker set was checked for the presence of identical or similar plant forms as well as marker redundancy using agglomeration analysis (UPGMA) in PAST software [43]. Genetic structure was studied with STRUCTURE [45], STRUCTURE HARVESTER [46] and CLUMPP [47] software. For association study, the General Linear Model (GML) and Multiple Linear Model (MLM) [52] implemented in TASSEL software [51] were applied. For MLM analysis, kinship matrices adequate for dominant markers were evaluated using SPAGeDi software [53]. Besides, marker-trait associations were tested using SML technology [54] by DArT Pty Ltd. Markers reflecting genomic regions under putative positive and balancing selection were identified by Mcheza software [55]. Markers associated with Al-tolerance as well as under positive and balancing selection pressure were located on rye [56] and triticale [57] genetic maps saturated with DArTs. Such information proved to be valuable in estimating the putative location and the number of QTLs via comparative mapping approach. The presence of a QTL was assumed when associate markers grouped within the same region of the map. Moreover, markers present within the region of the QTL are potentially useful for future purposes.

Fifty-three markers associated with Al-tolerance and forty-nine markers under selection pressure were identified. The associated markers were assigned to the 3R, 4R, 6R and 7R chromosomes. Structure analysis revealed grouping of the individuals into two groups with robust data structuring in the case of the 3R ($\Delta K = 3100$), 4R ($\Delta K = 8300$) and 7R ($\Delta K = 15300$) chromosomes. The 6R set exhibited weak structuring ($\Delta K = 88$) with two putative groups. The first group consisted of moderately tolerant forms. The second one was composed of non-tolerant forms. Most of the associated markers were present on available maps of triticale and rye. Those markers were linked with 126 molecular markers on the maps. Indirect location of QTLs associated with aluminum tolerance suggests that there might be one, two, three and one QTL on 3R, 4R, 6R and 7R chromosomes, respectively.

The selected markers were considered as candidates for MAS if transformed to sequence-specific markers.

P3). Niedziela A., Bednarek P.T., Labudda M., Mańkowski D.R., Anioł A. (2014) Genetic mapping of a 7R Al tolerance QTL in triticale (x *Triticosecale* Wittmack). Journal of Applied Genetics 55/1: 1-14.

Associative mapping allows the identification of many QTLs in the studied plant material, but without information about the location of genetic markers on linkage maps, it cannot be clearly stated, how many QTLs are involved in the trait. For this purpose, model biparental populations are usually created and used for QTL detection.

The study aimed to locate QTLs and identify molecular markers linked to Al-tolerance in triticale using specially designed biparental mapping populations. Moreover, the purpose of this study was to verify, which gene is responsible for the expression of the trait in the given case.

Two biparental mapping populations, namely MP1 and MP15 were derived via crossing individual double haploid plants cv. Bogo differed in Al-tolerance. Each of the two populations comprised 94 individuals and was phenotyping using standard Al-tolerance physiological test according to the procedure described in publication P2. Amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), diversity arrays technology (DArT), and specific markers were used to profile the F2 mapping populations. In total 1060 DNA fragments were obtained, but those with a profile showing similarity >95% were merged using agglomeration analysis (UPGMA) in PAST software [42]. Individuals and markers with missing data below 70% as well as markers, that show significant distortion at the 5% level were omitted from further analysis using R software with installed qtl package [58]. Finally, for construction of the linkage groups, 96 and 99 markers for MP1 and MP15 were used, respectively. The analysis was performed in R/qtl package [58] using functions est.rf (), pull.rf (), formLinkageGroups (), orderMarkers (), calc.errorlod () and pull.map (). Mapping of quantitative trait loci (QTLs) was performed using function cim() with Haley-Knott regression method, Kosambi map function, error probability equal 0.001. A 1000-permutations was used to evaluate empirical LOD thresholds for QTL significance determination at 0.05 alpha-value level. Explained phenotyping variance for a single QTL model was assessed using fitqtl() function. Phenotypic trait distributions in mapping populations were assessed for normality with a Shapiro-Wilk normality test using SAS/STAT 9.2 software [59]. Association between molecular markers and Al-tolerance was tested using the non-parametric Kruskal-Wallis test implemented in MapQTL 5.0 software [60].

Broad sense heritability coefficient (h2) for aluminum tolerance was estimated using Restricted Maximum Likelihood (REML). Malic and citric acids were detected using enzymatic methods [61] and a commercially available assay kit (K-CITR 07/11, Megazyme International Ireland Ltd.), respectively. Statistical analysis of heritability coefficient and biochemical data was performed using SAS/STAT 9.2 software [59].

Linkage analysis allowed identification of three groups assigned to 5R, 7R and 2B chromosomes for both mapping populations. The limited number of detected linkages and they poor saturation resulted from a homozygous genetic background of parental forms choose for crossing. Assignment of the linkage groups to the species chromosomes was possible due to the known chromosomal assignment of DArTs and SSRs. **Composite interval mapping (CIM) allowed identification of a single QTL, that mapped to the 7R chromosome in both mapping**

populations. The 7R QTL explained 25.3% (MP1) and 35.9% (MP15) of phenotypic variance. The B1, B26, and Xscm150 markers were 0.04cM and 0.02cM apart from the LOD function maximum in the MP1 and MP15 populations, respectively. The analysis using Kruskal-Wallis test revealed that B1, B26 and Xscm150 markers located on chromosome 7R were associated with Al-tolerance (p=0.0001; K = 22.23 for MP1 and K = 31.28 for MP15) in case of both mapping populations. Broad-sense heritability coefficient (h2) for Al-tolerance equaled to 0.996 and 0.994 in case of MP1 and MP15 populations, respectively.

The obtained results confirmed the conclusions of association analysis (P2) and other studies [29, 30] indicating the dominant role of the rye genome in Al tolerance in triticale. Biochemical analysis confirmed the presence of malic acid in a treatment solution containing Al^{3+} ions. Moreover, the B1 and B26 sequence-specific markers were previously linked with the A*lt4* locus at the distance of 0.4cM and 0.05cM, respectively [24, 37]. The locus encodes the ScALMT1 [19], which is known to be involved in aluminum tolerance in rye via malate efflux mechanisms. Thus, in rye and triticale, the same gene located on chromosome 7RS may control at least part of the aluminum tolerance response.

The presented analyses provide data concerning the location and function of the main gene related to aluminum tolerance in triticale. It has been proven that in investigated F2 populations the trait is determined mainly by the ALMT gene originating from the rye genome.

P4). Niedziela A., Mańkowski D., Bednarek P. T. (2015) Diversity Arrays Technology-based PCR markers for marker assisted selection of aluminum tolerance in triticale (x *Triticosecale* Wittmack). Molecular Breeding 35:209.

The identification of numerous DArT markers associated or linked to aluminum stress in triticale as well as markers under selection pressure gave the possibility to convert them to specific PCR conditions. Such markers can be used to identify tolerant plants, and in the future to select forms with the desired genotype.

This study aimed to use available information on DArT markers associated with loci on chromosomes 4R, 6R, and 7R to evaluate PCR-based molecular markers for Al-tolerance QTLs applicable to the differentiation of available gene pool and in the future also to the broad genetic pool of triticale. Besides, the chromosomal location of these markers using biparental mapping populations and verification of their usefulness for breeding purposes in triticale was accomplished.

Forty-nine DArT markers associated with Al tolerance (P2) were searched for their DNA sequences availability. Forty available DArT marker DNA sequences were analyzed in CLC Main Workbench software version 6.0 [62] to identify primer pairs for their amplification. The thermal profile for each primer pair was tested in gradient thermocycler (PTC-225 Peltier Thermal Cycler; MJ Research). PCR-based markers were tested for segregation in preselected 161 non-redundant accessions (38 tolerant, 26 medium tolerant and 97 intolerant to Al) described in publication P1. Sixteen primer pairs generated monomorphic banding patterns or a weak and/or unclear product. Three, eight, and thirteen markers from chromosomes 4R, 6R, and 7R, respectively, resulted in polymorphic PCR products of expected sizes and segregated in the same way as their corresponding DArTs. Some of those markers had their redundant counterparts. After selecting only one marker from the redundant group, three, six and four

original PCR markers assigned to the 4R, 6R and 7R chromosomes were obtained, respectively. All of the these thirteen markers were dominant. The DArT sequences for markers located on 3R chromosome were unavailable.

Spearman rank correlation coefficients between converted markers ('c' is added to the DArT marker name) and Al-tolerance of plant materials were calculated using the CORR procedure in SAS 9.3 software (SAS Institute, Cary, NC). The significance of the association between evaluated molecular profiles, including markers with the highest Spearman rank correlations with tolerant and non-tolerant accessions, was assessed via Pearson's Chi-Square test ($\chi 2$) [63]. The relative strength of an association between two variables was evaluated by calculating the Phi (Φ) factor [64].

Correlation coefficients between the average value of root regrowth and 13 original PCR-based markers (c) associated with Al tolerance were highly significant for 10 of them. **The highest correlation was observed for rPt-508078c, rPt-401828c, and rPt-505154c markers and therefore they were considered for selection purpose. All these markers and their redundant counterparts were assigned to the chromosome 7R.**

Marker rPt-401828c was identified almost exclusively among Al-tolerant accessions (33 out of 38 tolerant plants), with only 7 of 97 non-tolerant accessions carrying it. Hence rPt-401828c was highly correlated with Al tolerance ($r = 0.523$, $p > 0.0001$). Another marker, rPt-508078c, was not detected in any tolerant accessions but was present in 104 non-tolerant and moderately tolerant plants with a correlation to lack of Al tolerance of $r = - 0.628$ ($p > 0.0001$). Finally, the marker rPt-505154c was present in 25 tolerant and all non-tolerant and moderately tolerant accessions, equating to the lowest correlation with the trait among the three markers evaluated ($r = - 0.414$, $p > 0.0001$).

The chi-square test indicated a significant association ($\chi^2 = 84.539$, $p < 0.0000$) between individuals classified into three phenotypic groups (tolerant, moderately tolerant, and non-tolerant) distinguished according to Al response and the molecular profile of the rPt-401828c marker. If two markers were combined, assuming all four dominant profiles (I-0/0, II-0/1, III-1/0, and IV-1/1) generated by markers rPt-508078c and rPt-401828c, then the χ^2 was 106.715 ($p < 2.2e-16$). Finally, when the third marker, the rPt-505154c, was included in the analysis, the chi-square statistic increased slightly: $\chi^2 = 110.953$ ($p < 2.2e-16$). The strengths of the associations (Φ) between one, two, or three marker profiles, and the trait were 0.724, 0.814 and 0.830, respectively.

The converted specific PCR markers were tested on F2 biparental mapping populations, namely MP1 and MP15 described in the publication P3. After re-mapping, two markers: rPt-508078c and rPt-505154c were located in the 7R chromosome linkage groups. The rPt-508078c was located at the distances of 1.2cM (MP1) and 2.2cM (MP15) apart from the maximum value of the LOD function within the QTL region. The rPt-505154c mapped 21cM from the QTL maximum in the MP1 population.

To sum up, forty-nine DArT marker sequences were used to evaluate sequence-specific PCR based markers. Three of the converted markers differentiated the available gene pool regarding Al-tolerance. The marker showing the highest correlation with the trait were closely linked with QTL responsible for Al-tolerance in MP1 and MP15 mapping populations.

P5) Niedziela A. (2018) The influence of Al^{3+} on DNA methylation and sequence changes in the triticale (× *Triticosecale* Wittmack) genome. Journal of Applied Genetics. DOI: 10.1007/s13353-018-0459-0.

Plants growing under stressful conditions may be subjected to genetic and/or DNA methylation pattern changes. Aluminum (Al) is one of the most recognized factors inducing abiotic stresses in plants growing in soils under acidic conditions. However, it is not clear how broad is the level of changes evoked by the presence of aluminum in soil and whether the stress activates epigenetic changes exhibited at the DNA methylation level. By now, there were no systematic studies devoted to epigenetic aspects of Al tolerance in triticale.

This study aimed to analyze the influence of aluminum stress on methylation of genomic DNA in the leaves and roots of tolerant and not tolerant genotypes.

Five Al-tolerant (T) and five non-tolerant (NT) triticale inbred lines (S10) provided by the Plant Breeding Strzelce Ltd., Co. - IHAR-PIB Group were exposed to Al stress according to the physiological test described in P2. Total genomic DNA was isolated from pooled leaves and root tips of stressed in the presence of 15, 20 and 30 ppm of aluminum ($AlCl_3$), as-well-as control materials. RP-HPLC (Reversed Phase-High Performance Liquid Chromatography) quantification of overall DNA methylation was performed to calculate the percentage of deoxycytidine methylation concerning the total content of cytidine. The MSAP (Methylation Sensitive Amplification Polymorphism) [65] and metAFLP (methylation Amplified Fragment Length Polymorphisms) methods were used to assess changes in the methylation level of the 5'-CCGG-3' and 5'-GGTACC-3' sequences under the influence of aluminum stress, respectively. The differentially amplified MSAP fragments were recovered from the gel and sequenced. The sequence homology of MSAP fragments to the know genes was searched in BLASTN against the non-redundant database (NR) in NCBI.

Out of the three techniques used only RP-HPLC revealed differences between tolerant (T) and non-tolerant (NT) lines. The direction of the observed changes depends on phenotype and organ but not on the stress dose. **Al-treatment increases the level of global DNA methylation in roots of tolerant (T) lines (~0.65 %). However, the demethylation (~1.0 %) of DNA in non-tolerant (NT) lines was observed.**

MSAP method showed alternations in the level of methylation between control and Al-treated plants. Demethylation of the 5'-CCGG-3' sites reached approximately 3.97% and 3.75% for T and NT lines, respectively and prevailed over *de novo* methylation, which was observed only in two tolerant lines affected by Al stress and reached only 0.23%. No change in DNA methylation quantitative characteristics of leaves of NT and T lines due to Al stress was observed. Three of the MSAP fragments showed homology to known genes involved in abiotic stress: receptor-like protein kinase, histone-lysine N-methyltransferase, and peptide chain release factor subunit 1-2-like.

Genome methylation (GM) value reflecting methylation within sequences 5'-GGTACC-3' analyzed with metAFLP approach equaled to ~5.0 % regardless of the genotype (T, NT) and the presence or absence of Al^{3+} ions in the medium (control, stress). However, the metAFLP approach showed that Al stress elevated the level of sequence variation (SV) in roots and leaves. In roots, SV reached 0.97% and 0.87% for NT and T lines, respectively. In leaves, SV was lower in comparison to the roots and equaled to 0.04% and 0.14% for NT and T lines.

In this study, it has been demonstrated that **exposure triticale lines to Al^{3+} ions induced genome-wide changes in DNA methylation/demethylation in roots of T and NT plants, but not leaves. These changes are likely related to limited regions of the genome or are located outside the areas of the sequence recognized by endonucleases using in MSAP and metAFLP methods. Moreover, Al as a toxic and mutagenic metal could contribute to the sequence changes that prevailed in roots and to a smaller extent in leaves.** DNA methylation changes regarding Al stress in T and NT triticale lines assessed based on analysis of root DNAs show that epigenetic aspects of Al stress need further investigations.

Summary

The scientific achievement presented above relies on a monothematic publication cycle, the goal of which was to extend the knowledge of Al-tolerance genetic and epigenetic background in triticale and identified of the DNA markers for MAS purposes. The implementation of the set objectives required an assessment of the diversity of the triticale gene pool grown in Poland, identification of markers associated with Al-tolerance, verification whether identified markers locate within the Al-QTL and obtain specific markers useful for differentiating plant materials. The analysis of methylome for T and NT were also of interest. The hypothesis, that the trait may be conditioned by epigenetic factors was verified in this researches. Plant material consisted of 232 triticale forms tested for aluminum tolerance using physiological test with the medium supplemented with Al^{3+} ions. Depending on the analysis, different DNA markers were used for genotyping: DArT, SSR, AFLP, PCR-specific, metAFLP, and MSAP. Statistical data were developed using PAST, STRUCTURE 2.2.3, STRUCTURE HARVESTER, CLUMPP, SPAGeDi, TASSEL, Mcheza, R, MapQTL 5.0, GenAlEx 5.3, SAS/STAT 9.2, BLASTTN, CLC Main Workbanch softwares. Applied statistic methods allowed to obtain reliable results, which successfully published in journals from the IF list. It was revealed that the tested triticale pool shows a slight degree of structuring and a relatively high level of diversity within individual subpopulations. Association and linkage mapping clearly indicates, that Al-tolerance genes relocate on the 7R chromosome, but it is also possible, that some of the weaker QTLs occur on the 3R, 4R and 6R chromosomes. It was proved for the first time that the ALMT gene responsible for the expression of malic acid transporters is responsible for Al-tolerance in triticale. The obtained result suggests, that the tolerance is determined by the rye genome and can be modified by the wheat genome. It should be emphasized, that the identified markers associated with Al stress in triticale are suitable for effective differentiation of the T and NT forms among test forms, after their conversion to selective PCR conditions. The results of preliminary studies on methylomes of T and NT forms are also noteworthy. The lack of changes in methylation patterns within the DNA of leaves of T and NT forms and the occurrence of such changes within the root suggests that Al stress probably influences methylom, but its effect may be 'selective' and limited to narrow regions of the genome. The way of planned research and designed of research tasks lead to the development of simple methods for differentiating T and NT forms, and in the future also for their application in MAS. Obtained results constitute an important step in understanding the Al-tolerance in triticale and shows, that the background is not only genetic, but probably also epigenetic. On the base of the obtained information, I will continue research in the field of RNA and protein gene expression evaluation

in order to get more comprehensive information on genes related to Al-tolerance in triticale, as well as epigenetic mechanisms controlling their expression. Due to high costs of such analyzes, I will apply for funding them to NCN or MNiSW.

The most important practical achievements:

The presented works have both application and scientific value. The most important practical achievement of the postdoctoral thesis was the selection of molecular markers associated with a different response of triticale to the Al stress. These markers can be used in the future in MAS programs. As a result of the multiplication of the examined material for the analyzes and its constant selection on media with aluminum, genetically uniform and homozygous triticale (S10) lines with increased tolerance were obtained. These lines are available for Plant Breeding Strzelce Ltd., Co. - IHAR-PIB Group and can be successfully used in breeding programs of this species. Besides, the lines could be used as research material for further experiments planned dedicated to understanding better the genetic and epigenetic mechanisms associated with Al-tolerance in triticale including transcriptomics and metabolomics.

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E. AN OVERVIEW OF OTHER SCIENTIFIC-RESEARCH ACHIVEMENTS

My scientific activity started in 1998 as a student of Warsaw University of Life Sciences, Faculty of Horticulture and Landscape Architecture where Master's degree course in the Department of Plant Genetics, Breeding, and Biotechnology under the supervision of Professor Grzegorz Bartoszewski began. The thesis was entitled "Characterization of T2 transgenic tomato plants with introduced neomycin phosphotransferase and thaumatin II genes." and was devoted to the introduction of the thaumatin gene into tomato genotypes, analysis of the expression of the gene and the influence the expression of the gene had on plant morphology, fruit taste, and salinity resistance. The research was supported by grant entitled "Development of tomato transformation technology (*Lycopersicon esculentum* Mill.) for breeding purposes." (KBN No. 5P06A02511). I was responsible for the selection of homozygous transgenic tomato plants cv. Beta, *ls* mutant (lateral suppressor) and *nor* mutant (non-ripening) on the medium supplemented with neomycin, determination of the number of transgene copy and correctness of its integration, as well as transgene transcript level (mRNA) and the presence of thaumatin in the leaves and fruits. In this work, plants morphology, fruit production and maturation, and plant tolerance to salinity stress were assessed. During the research, the following blotting techniques were used: Southern blot, Northern blot and Western blot.

The study revealed that twelve of the fourteen tested transgenic tomato lines were characterized by the presence of one locus, while in two lines, the transgene was integrated into two and four transgene loci. All plants with single locus and a line with two integration sites revealed high protein expression at the level of mRNA in leaves. In the case of plants with four copies of the gene, the level of thaumatin II expression at the mRNA level was shallow. It was probably due to the interactions between homologous fragments of multiple copies of the transgene introduced into the plant. A similar analysis made for the fruit revealed the presence of mRNA in two of the three tested lines. Western blot analysis confirmed the presence of sweet protein in the leaves of ten transgenic tomato lines (out of fourteen tested) and in the fruits of two *nor* mutants (among seven lines tested). The experiment with transgenic plants growing on the medium supplemented with NaCl showed a beneficial effect of thaumatin on plant growth of *ls* lines. Similar properties have not been observed for *nor* mutant and cv. Beta transgenic lines. The results of the research were published in an article entitled „Modification of tomato taste in transgenic plants carrying a thaumatin gene from *Thaumatococcus daniellii* Benth." (**IIA1.2** in the list of publications) also, partly presented in a review article (**IID1.5** in the list of publications).

After graduation, I continued my scientific career in the Polish Academy of Sciences Botanical Garden - Center for Biological Diversity Conservation in Powsin. In January 2001, I embarked on a research study in the Department of Plant Biotechnology to gather material for the doctoral dissertation entitled „Morphogenic capacities of multicellular and single cell explants of gentians." Part of that research was granted by the project „Somatic hybridization of gentians using somatic embryogenesis and transformation." (MNiSW No. 3P04C03723)." The supervisor of the thesis was Professor Jan J. Rybczyński. The study aimed at the evaluation of the morphogenic potential of a single cell and multicellular explants of five selected Gentiana species (*G. kurroo*, *G. cruciata*, *G. tibetica*, *G. lutea* and *G. pannonica*) and the analysis of regenerants obtained via somatic embryogenesis. The explants originating from seedling (root, hypocotyls, and cotyledon), leaf and cell suspension were used. Protoplasts of green leaf mesophyll cells and cell suspensions constituted single cell explants. Somatic embryogenesis was induced on MS medium containing auxins: 2,4-D, NAA and dicamba and cytokinins: zeatin, TDZ, CPPU, BAP, and kinetin at various concentrations and combinations. The different efficiency of somatic embryo production and dependence on species, explant origin, the type of plant growth regulators as well as their concentration was revealed. Moreover, dicamba and NAA stimulated somatic embryogenesis on the leaf explants. The best results were obtained for *G. kurroo*, whereas left explants of *G. lutea* did not form somatic embryos. The seedling fragments had greater morphogenetic abilities and were characterized by more homogeneous response then leaves, that regenerated callus tissue, roots as well as somatic embryos. Among seedling fragments, the highest efficiency of somatic embryos was observed on cotyledons and was induced on MS medium supplemented with 0.5 mg/l 2,4-D and 1.0 mg/l kinetin. In the case of protoplasts, both the age and origin of the tissue had a significant impact on the development of culture. Cell divisions of the green protoplasts isolated from mesophyll tissue of the leaves appeared only when the youngest leaves of the seedlings were used as the tissue explants. Among 54 combinations tested in protoplast cultures isolated from cell suspensions differing in the type of culture, type of medium, fraction size and origin of the suspension, the best results were obtained for the cotyledon origin suspension in agarose droplets on MS medium (without NH_4NO_3) + 30 g/l glucose + 3.0 g/l glutamine + 2.0 mg/l BAP + 1.0 mg/l dicamba + 0.1 mg/l

NAA + 80.0 mg/l adenine sulfate + 9.0% mannitol. Interestingly, in protoplast cultures of young cell suspensions, it was possible to obtain direct somatic embryogenesis from a single protoplast. During the research, I established cooperation with PhD Henryk Bilski (The Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology in Warsaw), and with a team of Professor Mieczysław Kuraś (Institute of Botany, Faculty of Biology, University of Warsaw), what allowed me to accurately visualize the whole embryo development process using transmission and scanning electron microscopy. A more detailed analysis using two-dimensional electrophoresis (2-DE) for the different developmental stages of somatic embryos was performed under PhD Andrzej Kalinowski supervision in the Institute of Plant Genetics, Polish Academy of Sciences, Poznań. The obtained results indicated that the most significant changes in protein profiles appeared between globular and dicotyledonous embryos. Partly, the study focused on the analysis of regenerants derived via *in vitro* cultures. The establishment of cooperation with Professor Jolanta Małuszyńska (Department of Plant Anatomy and Cytology, University of Silesia in Katowice) and Professor Elwirą Śliwińska (Department of Plant Genetics, Physiology and Biotechnology, UTP University of Science and Technology in Bydgoszcz) allowed assessing the number of chromosomes and DNA content in plants that originated via somatic embryogenesis. Cytometric analysis revealed variability of the DNA level depending on the origin of the regenerant. The highest variability of DNA content was observed for plants obtained in protoplast cultures, what can be explained by the occurrence of numerous spontaneous fusions. Any changes found in the tested pool of regenerants originated from leaf explants and hypocotyl-derived cell suspensions. The DNA content was correlated with the number of chromosomes. Molecular analyses were conducted in the Department of Plant Biotechnology and Physiology, Plant Breeding and Acclimatization Institute in Radzików under the supervision of PhD Piotr Bednarek. The AFLP analysis showed the presence of variation in the case of *G. kurroo* regenerants. Its level depended on the explant type used for the regeneration. The highest variability (18.5%) was obtained for regenerates that originated from the cotyledon-derived cell suspension. Moreover, together with Professor Helena Gawrońska (Faculty of Horticulture, Biotechnology and Landscape Architecture, Warsaw University Of Life Sciences) and Professor Bożena Borkowska (Department of Plant Physiology and Biochemistry, Institute of Horticulture in Skierniewice) it was proved that 0.3% sucrose concentration in regeneration medium positively affects the photosynthetic abilities of *G. kurroo* regenerants.
Direct participation in most experiments and data analyzes allowed me to become familiar with various analytical techniques mentioned above. All obtained results were included in the dissertation and published after it defense (**IIA2.3-8, IID1.1-4**, and **IID2.4, 6, 7** in the list of publications). Some of them were presented at scientific conferences in the form of oral presentations (**IIK1.2-5** and **IIK2.4** in the list of scientific achievements) or posters (**IIIB1.1, 3, 4, 5, 7, 8** and **IIIB2.19, 23** in the list of scientific achievements).

Another research relevant to the project (MNiSW No. 3P04C03723) undertaken by me was the development of conditions for the fusion of gentian protoplasts. The optimal alternating current (AC) during fusion of protoplasts isolated from mesophyll tissue of *G. cruciata*, *G. pannonica*, and *G. tibetica* seedlings, as well as *G. kurroo* and *G. cruciata* cell suspensions (in various combinations), was determined and equaled 67 V/cm. Direct current (DC) depended on the species. The highest fusion efficiency (6.72%) for a combination of mesophyll protoplasts of *G. cruciata* with cell suspension-derived protoplasts of *G. kurroo* using DC equal to 1.33

kV/cm was achieved. Moreover, the highest number of heterokaryons (4.80%) for a combination of mesophyll protoplasts of *G. tibetica* and cell suspension-derived protoplasts of *G. cruciata* after the application of DC equal to 1.17 kV/cm was observed. The obtained results were presented in the form of publications (**IID2.6** on the scientific achievements list) and poster (**IIIB1.1, 2** and **IIIB2.20-22, 24** on the scientific achievements list).

In 2007, I began working at the Plant Breeding and Acclimatization Institute - National Research Institute, Department of Plant Physiology and Biochemistry under the supervisor of Professor Andrzej Anioł. In the years 2007-2010 I was the main contractor of the project No. PBZ/2/3/2006 funded by the Ministry of Science and Higher Education entitled: „Identification and mapping of molecular markers of tolerance to aluminum in cereals." During the project, the cooperation with PhD Grzegorz Budzianowski and late PhD Henryk Cichy from Plant Breeding Strzelce Ltd., Co. - IHAR-PIB Group. was established. In 2012-2017, I was also the head of the subject entitled: „Identification and mapping of molecular markers of aluminum tolerance in cereals." carried out as part of the statutory activity of IHAR-PIB. The results mentioned above formed the „scientific achievement" presented by me. During the research, I have become familiar with genotyping methods generating SSR, AFLP, metAFLP, MSAP and DArT markers. I have also used many statistical methods implemented in R-CRAN, JoinMap, MapQTL, WinQTLCart, MapChart software's and dedicated to the analysis of molecular data obtained for genetic mapping and QTL identification. In 2012, due to the invitation from Ph.D. Jordi Comadran to visit The James Hutton Institute (Scotland) my knowledge of R-CRAN and GenStat software was broaden.

My recent interests are related to the epigenetic aspects of aluminum tolerance in triticale. They prompted me to look for methods to analyze this phenomenon. One of such methods is MSAP analysis incorporated methylation-sensitive isoschizomers *Hpa*II and *Msp*I for DNA digestion. These endonucleases recognize the same 5'-CCGG-3' sequences, but display differential sensitivity to external and internal cytosine methylation. Unfortunately, there is no consistent way allowing interpretation of the MSAP outputs, and the results obtained by different researchers are difficult to compare. For this reason, we decided to investigate the interpretation approaches used up to now and analyze in detail the putative events affecting *Hpa*II and *Msp*I restriction site sequences of control and stressed materials depending on the location of the methyl group and bound to molecular marker profiles. We develop an innovative method (**IIA2.1** in the list of publications) allowing for the quantification of cytosine methylation status including *de novo* methylation and demethylation within CG and CHG sequences (where H = C, T or A) reflecting two symmetric methylation types.

Another mainstream of my research is the analysis of the cytoplasmic male sterility phenomenon in crops towards its use in heterosis breeding. I have participated in two projects under the supervision of Professor Piotr Bednarek. The grants were financed by the Polish Ministry of Agriculture and Rural Development and were entitled: „Identification of molecular markers associated with maintenance of pollen sterility genes in triticale with cms *Tt*." (No. HOR hn 801-12/14, No. MRiRW: 15) and „Identification of molecular markers associated with pollen fertility restoration in rye (*Secale cereale* L.) with CMS Pampa." (No. HOR hn 801-12/14, No. MRiRW: 21). The projects are dedicated to the identification of DNA molecular markers (DArT, DArTseq, GBS) linked to or associated with pollen sterility genes in triticale with cms *T. timopheevi* and with pollen fertility restoration genes in rye with CMS Pampa.

My contribution to the projects mentioned above includes deriving subsequent generations of the RIL lines in the field (mainly spikes isolation), crossing of the mother lines (cms *Tt*) with RILs (determination of the phenotype based on the number of kernels per spike), the collection of materials for DNA isolation, isolation and preparation of DNA for molecular analysis, preparing molecular data for further statistical analysis and performing association mapping. As part of the projects, I did a scientific visit in the United States Department of Agriculture - Agricultural Research Service (USDA-ARS), Kansas State University (USA) I had the opportunity to familiarize myself with the GBS procedure (genotyping-by-sequencing) combining the use of restriction enzymes for the controlled reduction of genome complexity with Next Generation Sequencing (NGS) technology. GBS markers, as well as DArTseq and silicoDArT were applying for development of the genetic maps for five triticale mapping populations. Similar analyzes was carried out for rye using DArTseq and silicoDArT markers. Genotypic and phenotypic data were used for composite interval mapping (CIM) and association mapping study for both crop species. The results showed, that a pollen sterility trait is expressed by numerous genes, explaining the limited variance of each. Current results were presented at both national and international conferences (**IIIB2.1, 3, 5- 8, 10** on the list of scientific achievements).

Perspectives for further research

The results of my research have inspired me to search for mechanisms controlling the response to aluminum stress in triticale, as well as related genes/proteins. As a part of the MRiRW/IHAR-PIB subject (DS 1-1-03-4-03), I performed two-dimensional gel electrophoresis (2-DE) for proteins isolated from root tips of T and NT triticale lines treated with aluminum stress and under control conditions. I have identified 25 proteins showing different expression under Al stress in NT line. Al stress did not cause permanent changes in the roots of T line. The results of these analyzes are to be presented in the form of publications.

I also cooperate with Dr. Jacek Żebrowski from the Department of Plant Physiology, University of Rzeszów. This cooperation aims to identify biochemical changes in the roots of the T and NT lines of triticale under the influence of Al stress using a Fourier spectrometer (FTIR - Fourier transform infrared spectroscopy). The results of the FTIR analysis are currently being developed.

The next task to be undertaken by me is the analysis of DNA methylation profiles in the triticale genome and analysis of the transcriptome of plants subjected to Al stress and under control conditions. This study will aim to determine the correlation between changes at the DNA methylation level caused by Al stress and its effects on observed changes in gene expression. The DNA sequences that differentiate the T and NT lines will be compared to the sequences in the databases for the identification of sequence homology to know genes and to define their relation to the tolerance genes. These studies, at the initial stage, will be conducted as part of the topic of MNiRW/IHAR-PIB subject (DS 1-1-03-4-03), and ultimately, a research project covering the presented subject is planned.

I am also a co-contractor of projects concern analysis of cytoplasmic male sterility in rye and triticale (HOR hn 801-PB-13/17). In the next two years, genotyping and phenotyping of RIL8 triticale populations will be carried out, as well as the identification of markers of the pollen fertility/sterility in rye and triticale, respectively. The final result will be the development

of a set of gene markers maintaining pollen sterility in triticale with cms *Tt*. For rye, selection of genotypes with desired combinations of pollen restorer genes is to be done using available plant materials based on identified molecular markers.

For many years I have also been cooperating with Professor Jan J. Rybczyński from the Botanical Garden - Center for the Preservation of Biological Diversity of the Polish Academy of Sciences in the field of somatic embryogenesis in gentians. The results of this collaboration are two publications (item **IIID2.4** in the scientific achievements list and the work entitled „Somatic embryogenesis of species in the family of *Gentianaceae* and its biotechnological application." being under revision in Frontiers in Plant Science).

F. LIST OF THE MOST IMPORTANT SCIENTIFIC ACHIEVEMENTS

1. LIST OF PUBLICATIONS ISSUED PRIOR AND AFTER RECEIVING DOCTOR DEGREE

Table 1. Arrangement of scientific works

Published in	Before receiving doctor degree	After receiving doctor degree	Sum
Journals with IF	2	14	16
Journals from B list	3	5	8
Conference materials	1	0	1
Monographs / chapter monographs in English	1	1	2
Monographs / chapter monographs in Polish	0	1	1
Sum	**7**	**21**	**28**
As the first author	1	15	17
Corresponding author	1	10	12
Publication type			
Original manuscripts	5	15	20
Review	2	6	8

Table 2. Number and presentation type

Presentation type	Before receiving doctor degree	After receiving doctor degree	Sum
International conferences	5	21	26
National conferences (posters/oral presentations)	10	8	18
Workshops	0	5	5
Sum	**15**	**34**	**49**
Oral presentations:			
International	0	0	0
National	3	2	5
Posters:			
International conferences	4	20	24
National conferences	5	5	10
Oral presentations in total	**3**	**2**	**5**
Poster presentations in total	**9**	**25**	**34**

Presentations in total	**12**	**27**	**39**

Table 3. Arrangement of reviews

Journal	Year	Number of reviews
After receiving doctor degree		
Acta Physiologiae Plantarum	2007	2
Acta Physiologiae Plantarum	2008	6
In Vitro Cellular and Developmental Biology – Plant	2008	6
Acta Physiologiae Plantarum	2009	6
In Vitro Cellular and Developmental Biology – Plant	2009	1
Acta Physiologiae Plantarum	2010	5
In Vitro Cellular and Developmental Biology – Plant	2010	2
Acta Physiologiae Plantarum	2011	2
In Vitro Cellular and Developmental Biology – Plant	2011	1
Acta Physiologiae Plantarum	2012	5
Plant Cell, Tissue and Organ Culture	2012	1
In Vitro Cellular and Developmental Biology – Plant	2012	2
Acta Physiologiae Plantarum	2013	2
In Vitro Cellular and Developmental Biology – Plant	2013	3
Acta Physiologiae Plantarum	2014	8
Plant Cell, Tissue and Organ Culture	2014	4
Acta Physiologiae Plantarum	2015	3
In Vitro Cellular and Developmental Biology – Plant	2015	1
Plant Cell, Tissue and Organ Culture	2015	3
Acta Physiologiae Plantarum	2016	2
In Vitro Cellular and Developmental Biology – Plant	2016	1
Plant Cell, Tissue and Organ Culture	2016	4
Plant Breeding	2016	1
Acta Physiologiae Plantarum	2017	3
Acta Physiologiae Plantarum	2018	2
Sum	**2007-2017**	**77**

2. BIBLIOMETRIC INDICES

Table 1. Arrangement of parametric assessment of scientific accomplishments#

Parameter	Before receiving doctor degree *	After receiving doctor degree **	Sum***
Impact factor (IF)	1.209	20.642^	21.851
Ministry of Science and Higher Education	35	347	382
Citations (WoS)	4	156	160
Index Hirscha (WoS)	1	7	8

Searched for (WoS): Niedziela A * or Fiuk A * or Niedziela Agnieszka * or Fiuk Agnieszka * taking into consideration the previous surname.

^ One manuscript was published in 2013 with DOI number. In case of this manuscript IF from 2013 was taken into account.

* Seven manuscripts published before 2006 were considered (including 2 manuscripts from WoS list before receiving doctor degree).

** Twenty one manuscripts published since 2006 until 2018 (28.08.2018) were considered (including 14 manuscripts from WoS list after receiving doctor degree).

*** All 28 manuscripts were considered until 28.08.2018.

3. SCIENTIFIC EXPERIENCE

I present the other scientific and didactic achievements in the „List of published scientific papers and information on didactic achievements, scientific cooperation, and popularization of science", constituting **Annex 6** of the documentation.

Signature