Dr Krystyna Rybka

Screening diagnostics of plant physiological condition based on normalized values and parameters of Chl *a* fluorescence

Self-Description of the achievements for scientific advancement

Plant Breeding and Acclimatization Institute- National Research Institute

Biochemistry and Plant Physiology Department

Radzików, January 18, 2019

Table of Contenets

1. Personal data

Name and Surname: **Krystyna Rybka**

Place of employment: Biochemistry and Plant Physiology Department
Plant Breeding and Acclimatization Institute-National Research Institute,
IHAR-PIB in Radzików
05-870 Błonie, Poland

2. Diplomas and academic degrees - including the name, place and year of obtaining them, and the title of the doctoral dissertation

1984 MsC in chemistry, specialization inorganic chemistry
Warsaw University, Chemistry Department, MsC dissertation title.:
„Elektroreduction of tris(acetylo-acetono-)cobalt III on a mercury electrode"
Supervisor: prof. dr hab. Marek Kalinowski

1993 PhD in agricultural sciences, agronomy-biochemistry
Plant Breeding and Acclimatization Institute-National Research Institute
Biochemistry and Plant Physiology Department, PhD Thesis title: :
„The relationship between the properties of non-starch polysaccharides and the digestibility of protein in the grain of rye inbred lines"
Supervisor: prof. dr hab. Konstancja Raczyńska-Bojanowska
Distinction by the IHAR-PIB Scientific Council

3. Information on previous employment in scientific units

2001-present	adiunkt, Biochemistry and Plant Physiology Department, IHAR-PIB
1998-2001	post-doctoral fellow at University California- Riverside, USA
1996-1998	adiunkt, Biochemistry and Plant Physiology Department, IHAR-PIB
1994-1996	post-doctoral fellow at National Institute of Agrobiological Resources-Tsukuba, Japan
1993-1994	adiunkt, Biochemistry and Plant Physiology Department, IHAR-PIB
1984-1993	asystent, Biochemistry Department, IHAR

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4. Indication of achievement for scientific advancement towards habilitation, in accordance with the legal provisions announced in: (Dz. U. nr 65, poz. 595 ze zm.)

a) Title of scientific achievement

Screening diagnostics of plant physiological condition based on normalized values and parameters of Chl *a* fluorescence

b) Publications included in the habilitation achievement, $\sum pkt_{MNiSzW} = 165$; $\sum IF = 11.969$

Review papers

H1 **Rybka K*** (2009) TILLING i FOX-hunting: nowe metody analizy funkcjonalnej genów. Postępy Biologii Komórki 36:539-554; Distinguished as the best article of the Journal in 2009. http://ptbk.mol.uj.edu.pl/download/nagrody/update2014/2009-artykul.pdf
Rybka K. (2011) Tilling and fox-hunting: new methods for functional analysis of genes. Advances in Cell Biology 3:1-16 https://doi.org/10.2478/v10052-011-0001-6
[pkt_{MNiSzW} = 15; IF = 0.1; my participation - 100%]

H2 **Rybka K***, Nita Z (2015) Physiological requirements for wheat ideotypes in response to drought threat. Acta Physiologiae Plantarum e37:1-13
http://link.springer.com/article/10.1007/s11738-015-1844-5
[$pkt_{MNiSzW2015}$ = 25; IF_{2015} = 1.86; my participation - 90%]

Experimental papers

H3 Żurek G, **Rybka K*(co-first author)**, Pogrzeba M, Krzyżak J, Prokopiuk K (2014) Chlorophyll *a* Fluorescence in Evaluation of the Effect of Heavy Metal Soil Contamination on Perennial Grasses. PLoS ONE 9: e91475
https://doi.org/10.1371/journal.pone.0091475
[$pkt_{MNiSzW2015}$ = 35; IF_{2014} = 3.324; my participation - 45%]

H4 Prokopiuk K, Żurek G, **Rybka K*** (2019) Turf Covering for Sport Season Elongation Cause No Stress for Grass Species as Detected by Chl *a* Fluorescence. Urban Forestry & Urban Greening, UFUG_2018_308_R2, waiting for a final approval
[$pkt_{MNiSzW2015}$ = 40; IF_{2014} = 3.521; my participation - 45%]

H5 Nykiel M, Lisik P, Dębski J, Florea BI, **Rybka K*** (2019) Chl *a* Fluorescence and Proteomics Reveal Protection of Photosynthetic Apparatus in Tolerant but not in Susceptible to Dehydration Wheat Cultivar. Biologia Plantarum 63: 287-297. https://bp.ueb.cas.cz/ corproof.php?tartkey=bpl-000000-6032 // on line first: 13.12.2018
[$pkt_{MNiSzW2015}$ = 25; IF_{2016} = 1.424; my participation - 45%]

H6 **Rybka K***, Janaszek-Mańkowska M, Siedlarz P, Mańkowski D (2019) Machine learning in determination of water saturation deficit in wheat leaves on basis of Chl *a* fluorescence parameters. Photosynthetica 57(1) https://ps.ueb.cas.cz/corproof.php?tartkey=phs-000000-2133) // on line first: 13.12.2018
[$pkt_{MNiSzW2015}$ = 25; $IF_{2017/2018}$ = 1.740; my participation - 45%]

* I am the author for correspondence in all and 1st author in 4 publications reported as the Achievement

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c) Synthetic summary of included publications

I. Introduction

The size of agricultural crop yield, as the final derivative of many parameters, among which resistance to environmental stress (biotic and abiotic) is an important feature, for years has been influence the crop market value. As a result of cooperation with the breeding company HR-Strzelce S.A. IHAR Group, I began to be interested in practical aspects of scientific research. So, I started working towards assessment of the physiological response of plants to abiotic stresses. I wrote several reviews and as a habilitation achievement I am quoting two (**H1** and **H2**). The first (**H1**, 2009) relates to mass and high-throughput methods of functional analysis of genes: TILING (*Targeting Induced Local Lesions IN Genomes*) and FOX-hunting (*Full-lghgh cDNA Over-eXpressing gene hunting system*). I discuss there the progress in applications of molecular biology techniques for plant genomes studies, according to approaches: "top-down/Forward" and "from mutation to gene" (Bottom-up/Reverse). In both approaches, the phenotype evaluation is crucial for the success of the conducted research (**H1**, Tab. 1) on a par with methods of data collection and processing. Writing this publication I have seen clearly that systems of massive phenotyping and big data elaboration adjusted for plant breeding purposes are, so-called, bottleneck of modern breeding (**H1** and (Rybka, 2017). For the reason, that **H1** article was awarded as the best publication in 2009 in the journal "Postępy Biologii Komórki" (on so-alled List A at that time, Editor in Chief: Prof. M. Olszewska from University of Lodz) I present this paper as an achievement.

ANALIZA FUNKCJONALNA	
Klasyczna (ang. *Top-down*)	Molekularna (ang. *Bottom-up*)
Białko Fenotyp	Białko fenotyp
⇓ Mapowanie QTL/asocjatywne	⇑ Wytwarzanie i analiza białek
Gen/Geny	Gen/Geny
⇓ Transformacja Wybranymi klonami DNA	⇑ Identyfikacja genów
Mutanty	Mutanty
⇓ Identyfikacja fenotypów	⇑ Identyfikacja fenotypów
Test komplementacji	Mutageneza

In the second review article (**H2**, 2015) I discuss the problem of drought in European temperate climate zone and phenotypic requirements that will have to meet well-yielding wheat genotypes under periodic water shortages, predicted by 2050. In **H2**, I focus on physiological, molecular and biochemical bases of drought tolerance and discuss experimental publications of three research teams: Vadez from ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, India), Blum from ARO (Agricultural Research Organization, Volcani Center, Israel) and Zagdańska- IHAR (Plant Breeding and Acclimatization Institute, Poland) and from 2001- SGGW (Warsaw University of Life Sciences, Poland) as well as on publications from Rothamtsted Research (UK) regarding statistical prediction of crop yield (Sirius model) in conjunction with the HadCM3climate model (Wolf et al. 1996; Jamieson et al. 1998; Brooks et al. 2001; Porter i Semenov 2005; Asseng et al. 2013; Semenov i Stratonovitch 2013; Semenov et al. 2014). From the Sirius model, it appears that genotypes with a well-developed root system will be able to yield better under predicted conditions of periodic water shortages in our country. The Vadez et al. publications are focused on the problems of efficiency of water uptake by roots (Vadez et al 2008, Bhatnagar-Mathur et al 2008, Kholova et al 2010a, Kholova et al. 2010b, Belko et al. 2013, Craufurd et al 2013, Vadez et al 2013a, Vadez et al 2013b, Kholova et al 2014, Vadez et al 2014). Blum's work are focused on the effectiveness of water use by plants (Blum 2009, Blum 2011). Zagdańska's publications concern biochemical mechanisms of drought tolerance to drought (Grudkowska and Zagdańska 2004, Grudkowska i Zagdanska 2010, Gietler et al 2017). The a biochemical approach to abiotic stresses tolerance is also known to me directly from joint work and Seminars in the Department of Biochemistry and Plant Physiology IHAR-PIB. Discussion in one review (**H2**) about theoretical and practical aspects of plant tolerance to water shortages in parallel with data modeling approach was an important step in my understanding of scientific tasks towards work for modern breeding.

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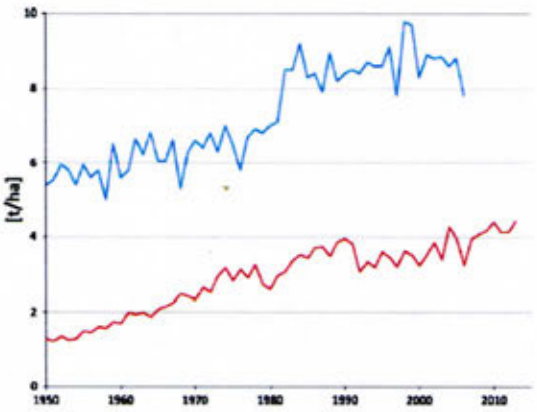
Water deficiencies in plant tissues are induced not only by soil drought but also by other abiotic stresses, such as: disturbance of ion balance in the soil (most often salinity or presence of heavy metal ions) and suboptimal temperatures (both too low and too high) (Kacperska 2004, Grudkowska i Zagdanska 2010, and Rucińska-Sobkowiak 2016). Literature data indicate that weak or gradually increasing stress influences the degree of hydration of cellular polymers, induces cell walls interaction with the cell membranes, causing the activation of LRK kinases (*Receptor-Like Kinase*), ion channels and changes in redox balance, which cause the subsequent activation of hormones (mainly ABA) and biochemical pathways. All these events in turn may lead to acclimation, i.e. adaptation of cellular metabolism and plant growth rate to suboptimal environmental conditions. Negative environmental stimuli, which appear rapidly and with high intensity, cause depolarization of cell membranes and induce phospholipid signaling. This can lead to increased production of reactive oxygen species (ROS), accumulation of H_2O_2, lipid peroxidation, protein modifications and increased synthesis of hormones such as jasmonic acid and ethylene. Violent and strong or weak and long-lasting negative environmental stimuli induce the same signaling pathways, leading to irreversible modifications and finally to cell death (Levitt 1985; Kacperska 2004; Dobra et al. 2010; Aroca et al. 2012; Gietler et al. 2016a; Gietler et al. 2016b; Bilska-Kos et al. 2016; Rucińska-Sobkowiak 2016; Sobkowiak et al. 2016; Gietler et al. 2017).

In case of extreme climate events, it is impossible to counteract the threat of a significant decline or even loss of crop yields based on plant breeding methods. However, because the way plants respond to environmental stimuli is genetically determined, selection of tolerant genotypes for abiotic stresses increases the ability for plant acclimation, which in consequence guarantee a relatively stable yield of new varieties, when unfavorable weather conditions happen. The trend of continuous yield growth resulting from this approach can be seen on the example of wheat yield in the UK and Poland during the last half-century (**H2**, Rybka and Oleksiak 2016) (see: graph, blue wheat yield line - GB, red - Poland).



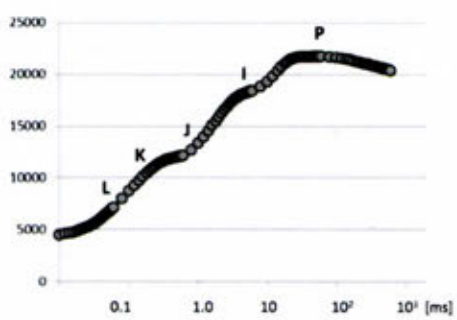
In breeding programs in Poland the selection towards improvement of tolerance to abiotic stresses is carried out indirectly, by assessing the stability of yields in regions and years. Clear breeding progress can be seen, e.g. in increasing drought resistance (**H2**, Rybka and Oleksiak 2016). In cereal breeding, phenotypic traits are taken into account, such as: later heading, as the plants have more time to produce more ear buds; fast grain filling, which is especially important during drought; fast movement of assimilates from leaves to grains; shorter and stiff stem (to increase the planting density on m^2 without fear of lodging); higher weight of 1000 grains as well as their high bulk weight; resistance to sprouting and tolerance to diseases. Therefore, the following morphological features are recorded in details: plant height, ear length, location and surface of flag leaves and phenological features: the date of beginning of vegetation after the winter resting period, the terms for heading and grain ripening (**H2**). There is no direct, common, screening method that could be used to assess the tolerance of plants to abiotic stresses. Therefore, as a habilitation achievement, I present the screening diagnostics of plant physiological condition based on normalized values and parameters of Chl *a* fluorescence in a way that was appreciated by Professor Strasser, H3 reviewer, one of the fathers of Chl *a* fluorescence studies (Tsimilli-Michael i Strasser 2013, retrospective publication). I quote the sentence from R2-stage of H2 review (the review is available from the Journal web-page: PONE-D-13-49265R1): „...Conceptual simplifications are often a big help to understand the main trends as long as more detailed descriptions are possible and remain on the track versus the trough...". In breeding programs, low-cost methods are required, technically simple

to perform and with high throughput. Measurement of Chl *a* fluorescence, when properly organized, meets these conditions (Rapacz et al. 2015; Sulkiewicz and Ciereszko, 2016).

The concept of interpretation of fluorescence data Chl *a* for the purpose of screening the physiological state of plants is based on 2 principles: 1 / tissue dehydration and rehydration dynamics are visible symptoms of reaction to many negative abiotic environmental stimuli (Kacperska 2004) causing changes in the conformation of cellular polymers; 2/ Chl *a* fluorescence reflects disorders in physiological and biochemical processes occurring in green tissues, with ascorbate and glutathione as a "redox hub" of cell metabolism (Foyer and Noctor 2011; Lázaro et al. 2013). The concept is presented on the following studies of : 1/ lawn grasses grown on soils contaminated with heavy metal ions (**H3**); 2/ grasses covered with agrotextile for the growing season prolongation (temperature stress) (**H4**); 3/ spring wheat seedlings subjected to dehydration (**H5**). In habilitation achievement works I use approach based on normalization of the raw data as well as the Chl *a* fluorescence parameters. I also present the possibility of using Chl *a* fluorescence parameters to model the Water Saturation Deficit (**H6**). The model was developed for wheat seedlings grown under controlled conditions. The numerous of scientific papers are describing the theoretical bases of Chl *a* fluorescence and photosynthesis and their disturbances under the influence of negative environmental stimuli (Garstka 2007, Janik et al, 2010, Stirbet and Govindjee 2012, Tóth et al. 2013, Tsimilli-Michael and Strasser 2013, Lázaro et al. 2013, Paunov et al 2018).

Chl *a* fluorescence is a natural phenomenon, characteristic for all photosynthetic organisms. It involves the reemission of excess photons that have neither entered the phase of light-dependent reactions nor their energy has been dispersed as an exothermic reaction of the xanthophyll cycle. Although the fluorescence phenomenon usually reemit no more than 1-8% of absorbed light, it can infered from it about the physiological state of the examined green tissue. The fact of reemission of light by Chl *a* was metioned for the first time in 1834. Era of modern research on CO_2 assimilation and energy transformations in cells, was begun by Wartburg and Kautsky, in '40 of the 20th century (Kalaji et al. 2012). However, until development of semiconductors and light emmiting diodesthe physiological studies on a massive scale were impossible (Tsimilli-Michael i Strasser 2013; Kalaji et al. 2012; Stirbet i Govindjee 2012; Stirbet et al. 2014).

Measurements of Chl *a* fluorescence, induced by a light of specific energy and wavelength can be performed both on previously darkened and on un darkened leaves. The data, recorded with decreasing sampling frequency and presented on the logarithmic time axis show the induction curve of fluorescence (Kautsky curve). The curve includes a rapid increase of fluorescence from the moment of induction of the phenomenon by a stream of light (O) to the maximum value (P) reached after 1 [s], followed by a decrease to the stationary level T. With measurements lasting 3 [s] only the initial fragment is visible. Easily visible inflection points on the curve, appeare in fixed time intervals and are marked with the letters J, I and P. These letters form the name of the JIP test (in other words: OJIP), the way of fast Chl *a* fluorescence analysis based on fluorescence parameters calculated on the basis of data raw with time-specific points (Tsimilli-Michael i Strasser 2013). They allow for a description of changes in structure and in functioning of the photosynthetic apparatus, mainly the photosystem II (PSII). Inflection points L and K are usually invisible on the raw data charts. The JIP test was developed based on the physical equations of energy flows used to describe the energy flow in thylakoidal membranes of PSII. Inflection points L and K are usually invisible on the raw data charts. The test was developed at the end of the '70s, but is in common use, in applied sciences, since the dissemination of devices based on semiconductor diodes and computers (Tsimilli-Michael i Strasser, 2013). A list of parameters and a description of their physical meaning is put in the Materials and Methods.



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The list includes only the parameters calculated by the software of PocketPeA fluorimeter that was used in presented research. In addition to comparison of particular parameters values, among which F_V/F_0 and F_V/F_M are widely known and used, I focus on the interpretation of curves plotted in the logarithmic time scale from double normalized fluorescence values (Oukarroum et al. 2009; Paunov et al. 2018) especially from the time range from 0.05 do 2 [ms], which is covering the time in which the first stage of stable stage of separation electrons in Electron Transfer Chain (ETC) takes place (Oukarroum et al. 2009).

In response to environmental stimuli, the shape of the fluorescence curve changes. Increased value at the starting point (F_O) indicates a decrease in the efficiency of PSII energy trapping, while the decrease in the maximum fluorescence value depends on complex factors: leaf structure determining the absorption of actinic light, re-absorption of already emitted fluorescence, Chl *a* content as well as from concentrations of particles quenching fluorescence. The minimum fluorescence value corresponds to the Q_A quinone oxidation state, while the maximum its reduction. This part of the curve illustrates the dynamics of light-induced electron flow through the Electron Transfer Chain (ETC) in chloroplasts: from phenotypin to plastochinone, cytochrome b6f, plastocyanin to PSI receptors. ETC damage and the reduction of electron capture capability by the PSI slowdown the flow of electrons. The time needed the maximal value to be reached is extended; colloquially, this can be described as a "flattening" of the recorded curve. Analysis and interpretation of changes in chlorophyll fluorescence can be the base for inference about the physiological state of plants.

II. Scientific goal

Presentation of the concept of universal screening system based on Chl *a* fluorescence, in order to identification of plants tolerant to environmental abiotic stimuli inducing tissue dehydration, i.e. WSD (*Water Saturation Deficit*) increase, as one of the first physiological reactions.

Abiotic stresses inducing tissues dehydration:

- unfavorable water balances (**H5**)
- unfavorable temperatures (**H4**)
- disturbance of ion balance in soil (**H3**)

As a habilitation achievement, the research on the influence of heavy metal ions (**H3**) and temperature (**H4**) on perennial grasses as well as studies on dehydrated wheat seedlings (**H5** and **H6**), are presented. The physiological status of plants was assessed by Chl *a* fluorescence detection.

III. Materials

The material for studies consisted a list of varieties from the *Poacae* family: perennial grasses and spring wheat. Perennial grasses were grown under field conditions (**H3**, **H4**). The **H3** experiment, on the usefulness of perennial grasses for phytoremediation, was carried out in Silesia, on a site near a closed mine of cadmium, lead and zinc ores (50.4°N/018.9°E) and on a referential site (25 km north) (50.6°N/018.88°E). The following cultivars and varieties were tested: Wiwena, tall oat grass (*Arrhentherum elatius* L.); Broma, rescuegrass (*Bromus carinathus* Hooker et Arn); Bamar, tall wheat grass (*Elytrigia elongata* Nevski); Brudzyńska, smooth brome grass (*Bromus inermis* Leyss) and Rahela, tall fescue (*Festuca arundinacea* Schreb). The **H4** experiment was conducted on lawn plots at the IHAR-PIB in Radzików (52.21°N/20.64°E). The studies were conducted to investigate whether turf covering can affect the length of the sport season usage of the grassy sports fields (Prokopiuk 2016, doctoral dissertation). Fluorescence measurements were additionally carried out in order to explore the concept of the universality of the physiological assessment of plants based on Chl fluorescence. In this experiment, the following species were tested: perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa*

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pratensis L.) and red fescue (*Festuca rubra* L.). Both experiments were carried out on adult plants, for 2 (**H3**) and 3 (**H4**) vegetation seasons. During the **H3** experiment, in contrast to the **H4** one, no fertilization or care treatments were carried out. The experiments **H5** and **H6** were conducted on spring wheat seedlings (*Triticum aestivum* L.), varieties Ethos and Zebra, as well as on advanced breeding lines from HR-Strzelce Sp. z o.o. The IHAR group. Seedlings were grown in rolls, in hydroponics on Hogland's medium enriched by Knopp solution in phytotron (**H5** and **H6**) or in microplates in a greenhouse (**H6**).

IV. Methods

The main research data were collected by measurements of Chl *a* fluorescence, performed on the adaxial side of leaves darkened for 30 min. before measurement, with the PocketPeA portable fluorimeter (Hansatech Instruments, King's Lynn, Norfolk, UK). Fluorescence was induced by saturated red actinic light with energy of 3500 [$\mu mol \cdot m^{-2} \cdot s^{-1}$] and then recorded in discontinuous mode for 3 [s], covering more than the increasing part of the fluorescence curve. Intervals of data recording increased from 10 [μs] for the first 300 [μs] duration of the measurement to 100 [ms] for times longer than 0.3 [s]. In total, in each measurement, the instrument recorded 124 values that were analyzed as: i) ΔW_{OJ} curves (**H3-H5**); ii) Chl *a* fluorescence parameters calculated by dedicated PocketPeA software (**H3-H6**); iii) in the graphic form of the so-called HeatMap, illustrating the grouping of normalized Chl *a* fluorescence parameters (**H4**, **H5**) as well as iv) WSD modeled by the supervised neural network on the basis of fluorescence parameters (**H6**).

Parameters specified and calculated by the PocketPeA software::

$F_O \approx F_{50}$ [ms] – minimal fluorescence value measured after 50 [μs];

F_1, F_2, F_3, F_4, F_5 – fluorescence values at time points: 0.05, 0.1, 0.3, 2.0 and 30 [ms] after the activation of actinic light, corresponding to fluorescence at the starting point, fluorescence value after 100 [μs] and fluorescence at inflection points K, J, I on the rising part of the OJIP curve

$F_M = F_P$ maximal fluorescence value

t_{FM} time [ms] to reach the maximal fluorescence

Area area between the increasing part of the OJIP fluorescence transient and the y-axis

F_V the value of maximal variable fluorescence calculated as $F_V = F_M - F_O$

F_V/F_M the value reflecting the force of the light reactions

RC_{ABS} the value reflecting the amount of active reaction centers per absorption]

$(1-V_J)/V_J$ measure of forward electron transport in ETC towards PSI

PI_{ABS} (*ang.: Performance Index*) the value interpreted as an index of processing efficiency of photons absorbed

Krzywe ΔW_{OJ}, double normalized curves of time-varying differences between the fluorescence values measured for plants grown under control conditions and under conditions a reaction to abiotic stress in leaves, calculated in Excel, according to given equation, drown using a macro written in VisualBasic;

$$\Delta W_{OJ} = \left[\left[\frac{F(t)-F(O)}{F(J)-F(O)}\right]\right] stress - \left[\left[\frac{F(t)-F(O)}{F(J)-F(O)}\right]\right] contr.$$

Complementary research methods are: qualitative visual assessment of perennial grasses (**H3**, **H4**); biometric evaluation of grasses (**H3**); weight analysis in assessing the yield of the green biomass (**H3**, **H4**) and leaf WSD in [%] (**H5**, **H6**); flame spectrophotometry in assessment of HM ions concentration in soil and leaves (**H3**), bi-directional electrophoresis of proteins and analysis of differentiating proteins after digestion with trypsin in the MS-MS system (**H5**).

Statistica software was used for ANOVA analysis of variance and determination of Duncan homogeneous groups and also for statistical modeling of data with the use of unsupervised and supervised neural networks. Unsupervised networks were used for cluster analysis with Manhattan (**H4**) and

Euclidean (**H5**) distances and for the principal components analysis (PCA) (**H3**, **H5**). Supervised networks were used in WSD modeling based on Chl *a* fluorescence parameters (**H6**).

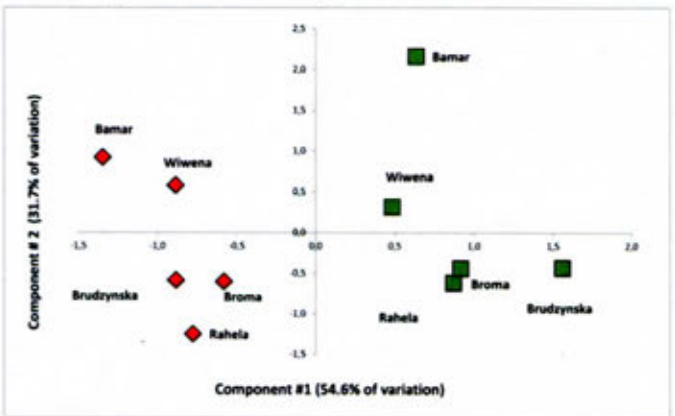
V. Presentation of papers declared as an achievement

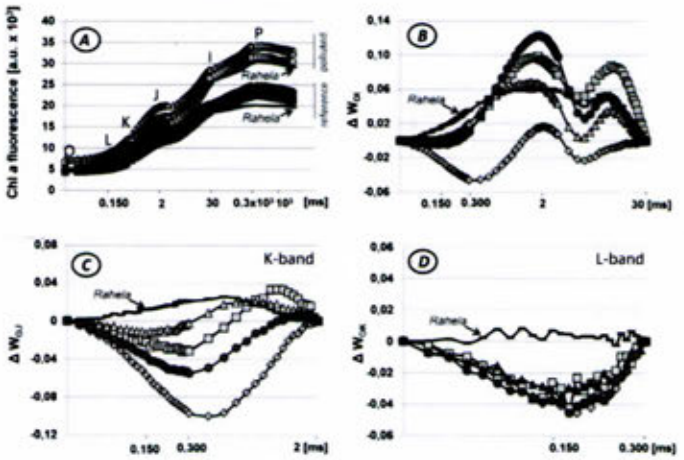
The work on screening of the physiological response of plants to abiotic stresses I began from studies on the usefulness of perennial grasses as phytoremediators using normalized Chl *a* fluorescence values and parameters (**H3**: *Chlorophyll a Fluorescence in Evaluation of the Effect of Heavy Metal Soil Contamination on Perennial Grasses*)

The experiment was carried out in frames of PW3-3-00-0-03 financial program in years 2008-2013. The usefulness of grasses for phytoremediation purposes was tested. Such species and cultivars were searched, which on one hand, would have the ability for bio-accumulation of heavy metal (HM) ions in terrestrial tissues and on the other hand, it would maintain a good physiological condition despite soil contaminations. Flame spectrophotometry and Chl *a* fluorescence data analysis allowed to create a system for screening of cultivars suitable for phytoremediation of contaminated environments.

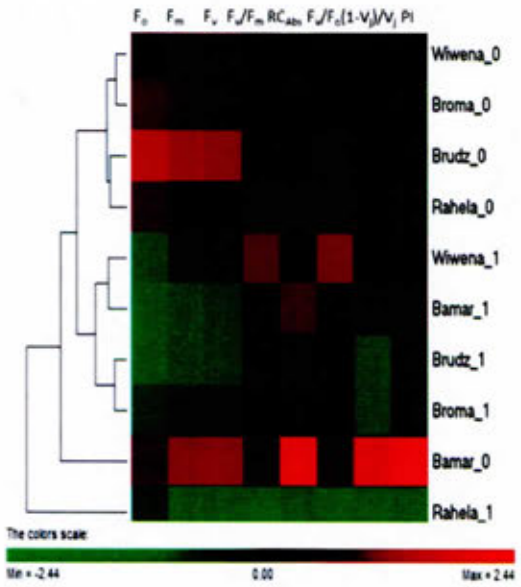
In the leaves of plants grown on soils contaminated with HM ions, disturbances of photosynthesis arise under the influence of single or accumulated phenomena: i) direct interaction of HM ions with thiol, histidyl and carboxyl groups of proteins; ii) accumulation of excess ROS that causes oxidative damage to cellular polymers; iii) substitution of cations in the centers of active proteins and chlorophyll. The ions of Hg, Cu, Cd, Ni or Zn can be the substituents of the central Mn^{2+} ion in the PsbO protein which stabilizing the PSII, forming chlorophyll-metal complexes, thereby reducing the PSII quantum yield (Paunov et al., 2018). In field studies, disturbances in Chl *a* fluorescence result from the synergistic interaction of HM ions and weather conditions. In selection of plants useful for phytoremediation those in a better physiological condition and at the same time with a higher concentration of HM ions in above-ground parts are chosen.

The PCA analysis of the Chl *a* fluorescence parameters showed grouping of the examined objects due to the exposure to HM ions in accordance to sign of the component #1; HM ions exposed plants were classified in IInd and IIIrd Quadrants of the Cartesian Plane (minus sign of component 1). Two components explained 86% of the variation; the first included values: F_M, F_V and F_1-F_5 and the second one: RC_{ABS}, $(1-V_J)/V_J$ and PI. However, based on the analysis of the profile of double standard differential curves (*vide*: chart on the next page), the Rahela variety was distinguished. That cultivar was characterized by the highest concentration of HM ions absorbed in the leaves. The inflection point K on the ΔW_{OJ} curve calculated for Rahela had a small positive value as opposed to the negative values on the curves calculated for the remaining varieties. The K point illustrates the rate of water photolysis and the number of electrons transported along the ETC; its positive value means a slowdown in electron flow from chlorophyll reaction centers (RC) in PSII, as a result of lower OEC activity in PSII (*Oxygen Evolving Complex*) and downturn of the process of water photolysis. Negative values of inflection points on the ΔW_{OJ} curves, reflecting slightly faster electron flow in the ETC of plants from the contaminated environment as compared to the reference ones may indicate the participation of ascorbate as an electron donor (Lázaro et al. 2013; Srivastava et al. 1997; Tóth et al. 2007). Normalization in points O and K (ΔW_{OK} curve) enables the visualization of the inflection point L, which negative value indicates a stronger grouping of antennas in PSII of plants from the contaminated area as compared to the



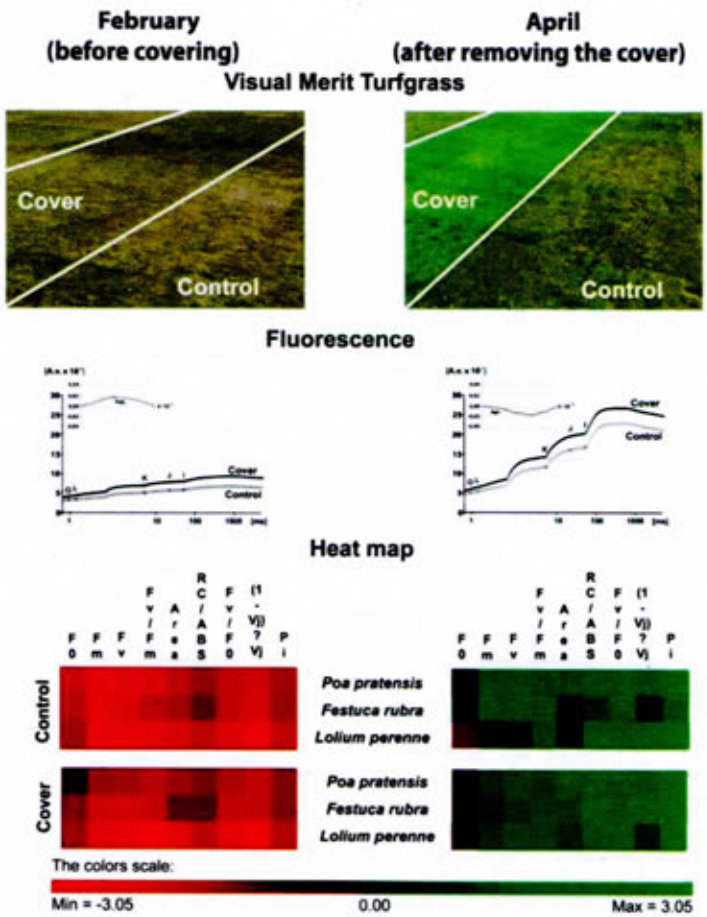


control (Gururani et al. 2012; Janik et al. 2010; Tóth et al. 2013), which is a fact for all varieties, except for Rahela. The similarities in the shape of calculated curves for all varieties except for Rahela may suggest a different physiological response to stress in this variety. Based on the results obtained, it was concluded that all tested varieties, with Rahela exception, took only such HM amount from the ground, which was not too toxic for them. In leaves of these varieties, acceleration of the photosynthesis, and thus of the entire metabolism, , was observed under the influence of HM ions, which is a typical physiological reaction of plants sensitive to stress. Tolerant plants usually trigger additional defense mechanisms and rebuild their metabolism for acclimatization to changed conditions (Foyer et al. 2012; Zagdańska 1995). Contrary to expectations, in leaves of Rahla there were no grouping of PSII antennas detected, despite the fact that it accumulated the largest amount of HM in biomass. Tall fescue, Rahela cultivar, in fact performed better under HM stress than the other varieties; its chlorophyll antennas did not aggregate. For the purposes of the presentation of habilitation achievement, I made a cluster analysis combined with the so-called heatmap, which also distinguished Rahela cultivar. The number "0" next to the name of the cultivar means the reference site of plants cultivation, while "1" the HM contaminated site. The color code in the case of the Rahela grown on HM contaminated soil indicates that the normalized values of parameters assume negative values, i.e. the size of each is lower than the average value. Lower values of these parameters are related to the weaker physiological condition of the plant. Fluorescence of leaves of Bamar cultivar from reference site is characterized by the highest relative values of parameters, which indicates its best, in the tested variety collection, condition. The **H3** publication is summarized by conclusions: 1/ Chl a fluorescence is a good tool in phytoremediation studies; 2/ The combined effects of elevated concentration of lead, cadmium and zinc in soil was manifested in alteration of some parameters of Chl *a* fluorescence as well as plant growth; 3/ Heavy metal soil contamination resulted in biomass yield reduction of cultivars of *Bromus inermis* 'Brudzynska' and *B. carinathus* 'Broma'; 4/ *Festuca arundinacea* 'Rahela', may absorb relatively high amount of HM ions from the soil, without significant reduction of biomass yield, what is expected in the case of bioremediation practices. Mentioned cultivar could be regarded as a good species for the phytoextraction of Cd-contaminated soil.



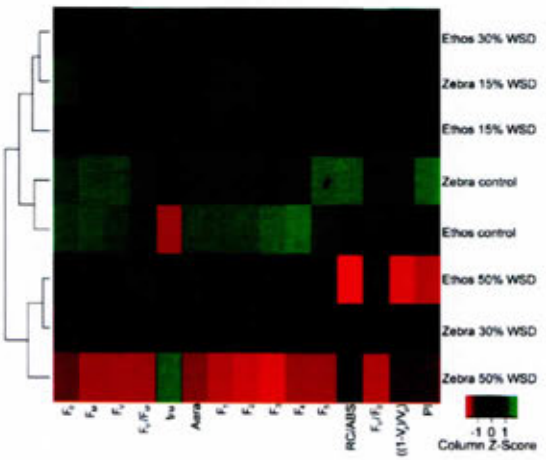
Then, I used the same methodological approach in studying the reaction of lawn grasses to turf covering (**H4**: *Turf Covering for Sport Season Elongation Cause No Stress for Grass Species as Detected by Chl a Fluorescence*).

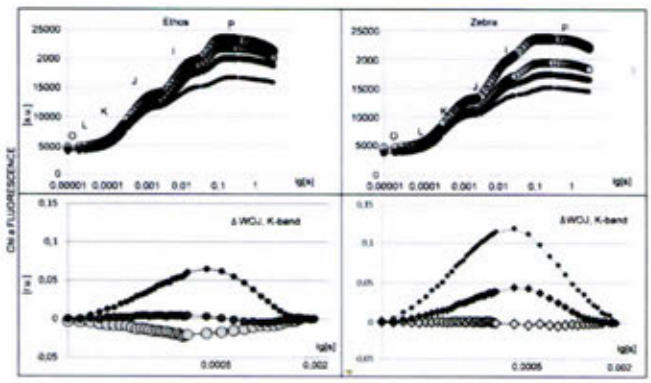
The experiment was carried out in parallel to the research conducted by Dr. Kamil Prokopiuk, IHAR-PIB, as part of his doctoral thesis entitled: "The influence of vegetation grass extension on the quality of football pitch grass" (Prokopiuk, 2016), supervisor dr hab. Grzegorz Żurek, prof. IHAR-PIB. The results of Chl *a* fluorescence measurements were not a part of the doctoral dissertation. The goal was to collect additional evidence that the pitches covering does not induce physiological stress in the leaves of studied grass species, in comparison with the non-covered turf. This argument is often raised by the opponents of this simple and cheap method of extending the season of grass pitches usage beyond the natural vegetation dates in countries with a cooler climate, according to UEFA competition dates, from mid-February to mid-December. The shape of fluorescence curves indicated *e.g.* by a very low value of the maximum fluorescence (F_P), that all tested species were in the winter dormancy state in mid-February, prior to covering. In late spring, which in the temperate climate is characterized by optimal growing conditions for grasses, *e.g.* the F_P values were high, which is characteristic for plants in good physiological condition. The shape of fluorescence curves in leaves of the control plants were characterized by similar shape of fluorescence curves, regardless of the species, while the fluorescence detected on leaves from the covered turf indicated physiological benefits resulting from the spring covering. The highest F_P value was recorded in *Lolium perenne* leaves, warm climate species, sensitive to low temperatures in winter and spring in temperate climate zone, while the lack of difference with control plants in shapes of fluorescence curves was recorded for *Festuca rubra*, a species tolerant to winter conditions. In June, the F_P value decreased in all species examined, which was associated with the phenomenon of typical at the beginning of the summer slowdown of growth and a weaker physiological state of grasses, assessed visually. Autumnal fluorescence measurements revealed the deteriorating condition of grasses, with the most visible effect of covering in case of *Lolium perenne*, and the smallest in case of *Festuca rubra*. The double normalized curves ΔW_{OJ} curves revealed no significant deviations from the zero value, suggesting no significant differences between the control and covered plants except for November, when for all tested species a positive impacts of covering were detected and in June with the exception of *Lolium perenne*. The detected impact of the covering on the fluorescence values recorded in months when the grasses were not covered is an interesting aspect of the issue, requiring further research. The publication includes a graphic abstract that can be found next to the text. It shows that in February species form both: control and covered plots were in winter dormancy state, whereas in April at the end of covering time were in better conditions.



While, to compare the response of wheat seedlings to dehydration of varieties differing in drought tolerance parallel to the physiological state detection using Chl a fluorescence detection I carried out proteomic analysis (**H5**: *Chl a Fluorescence and Proteomics Reveal Protection of Photosynthetic Apparatus in Tolerant but not in Susceptible to Dehydration Wheat Cultivar*)

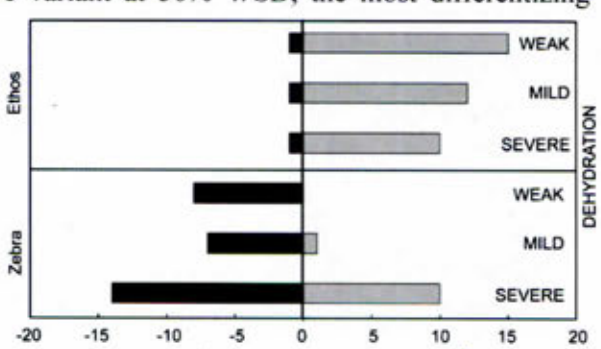
The experiments were carried out as part of NCN NN310079839 and NN304267540 grants on seedlings of 2 spring wheat varieties differing in dehydration tolerance, dehydrated, which induced 15, 30 and 50% of WSD in the leaves. As a result of the cluster analysis of fluorescence parameters, the tested materials were classified into two major clusters. Normalized parameters values lower than the average were calculated as a part of clustering analysis for seedlings of the sensitive variety (Zebra) dehydrated to 30 and 50% of WSD and seedlings of drought tolerant cultivar (Ethos) with a water deficit in leaves of 50% WSD. The second group, with normalized values of parameters higher than the average value, constituted the remaining materials with a subgroup of control plants of both varieties, with the highest





values of parameters, with the reversed value of t_{FM}. The classification of two samples dehydrated to 30% WSD into different clusters suggests that the PSII performance of a tolerant variety with such a dehydration was more similar to the control plants and that in leaves of the sensitive variety symptoms similar to that caused by 50% WSD could happened. Analysis of the shape of ΔW_{OJ} curves also showed a different response to dehydration in the leaves of the tested varieties. On the curves plotted for the tolerant variety, a small minimum appeared at 15% and a maximum at 50% WSD, with a value of about zero at medium dehydration. In contrast, in the sensitive variant, 30 and 50% WSD dehydration resulted in the appearance of positive inflection points. The analysis of chlorophyll fluorescence parameters showed that the majority of statistically significant differences arose under the influence of 15% WSD in the leaves of the tolerant cultivar, whereas in the sensitive variety the differences increased gradually.

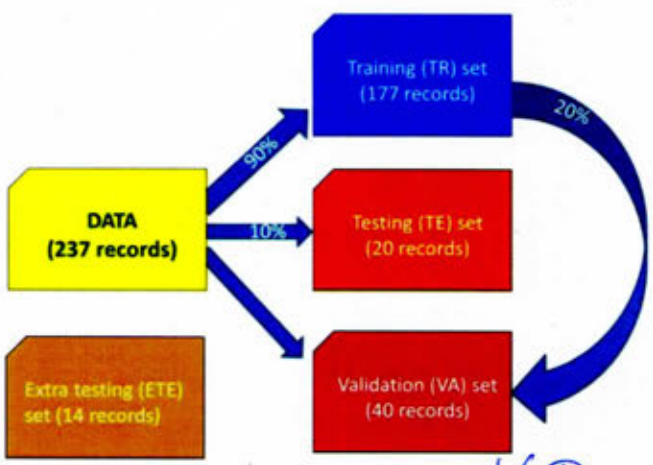
Modifications of the proteome had a slightly different pattern than changes in fluorescence parameters. Under the influence of weak dehydration in the leaves of the tolerant variety, the largest number of differentiating proteins appeared. There were over 90% of the proteins with increased expression (upregulated). In the leaves of the sensitive variant at 50% WSD, the most differentizing proteins appeared on the gels, both with increased and decreased (downregulated) expression. I will focus only on a fragment of the results, which I consider to be potentially important for the selection of new varieties with increased tolerance to drought. Only 3 proteins with reduced expression were found in the proteome of tolerant cultivar: chloroplast binding protein LHC a/b in PSII (15% WSD) and a



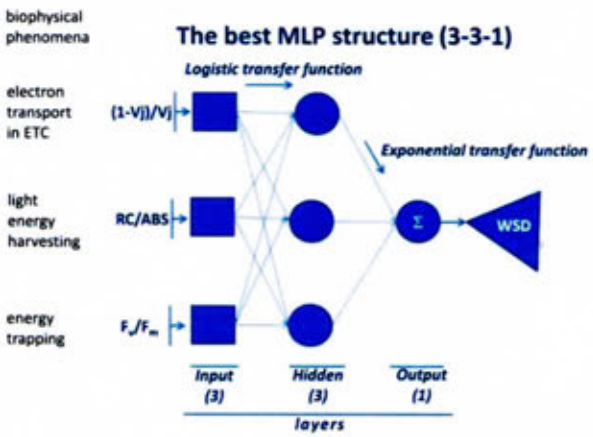
small subunit Rubisco only at moderate and severe tissue dehydrationrates (30 and 50% WSD). In leaves of the sensitive variety, Rubisco degradation has also been noted, both large and small subunits, at all stages of dehydration. This is a type of reaction to negative environmental stimuli characteristic for susceptible plants. That processes are accompanied by a decrease in photosynthetic efficiency, which can be inferred also from the shape of fluorescence curves as well as from the positive inflection points in ΔW_{OJ} diagrams (Pinheiro and Chaves 2011). In leaves of the sensitive variety, these processes are already visible in the initial phase of dehydration (Miazek et al. 2017). On the basis of the proteomic analysis, the increased expression of the Rubisco activase and carbonic anhydrase were detected at the initial stage of leaves dehydration of tolerant cultivar and the degradation of these enzymes in the leaves of the sensitive one. The increase of of Rubisco activase and carbonic anhydrase expression along with the degradation of LHC complexes in PSII in case of tolerant cultivar may indicate the protection of photosynthetic complexes from excess photons. In contrast, the increase in LHC expression in PSI may indicate increased cyclical phosphorylation and ATP production without NADPH production to stabilize the oxidative-reduction cell balance (Yadav et al., 2017). In leaves of the tolerant cultivar upon slight dehydration the processes indicating that the photosinthesis remains undisturbed are evidenced (Perdomo et al. 2017). Analysis of the entire data set led to the conclusion that the proteome modification and protection of the photosynthetic apparatus are reactions to the first stages of dehydration in leaves of tolerant cultivar. It was suggested that the selection of plants with stable and efficient photosynthesis under environmental stress is an approach towards obtaining new, well-yielding varieties.

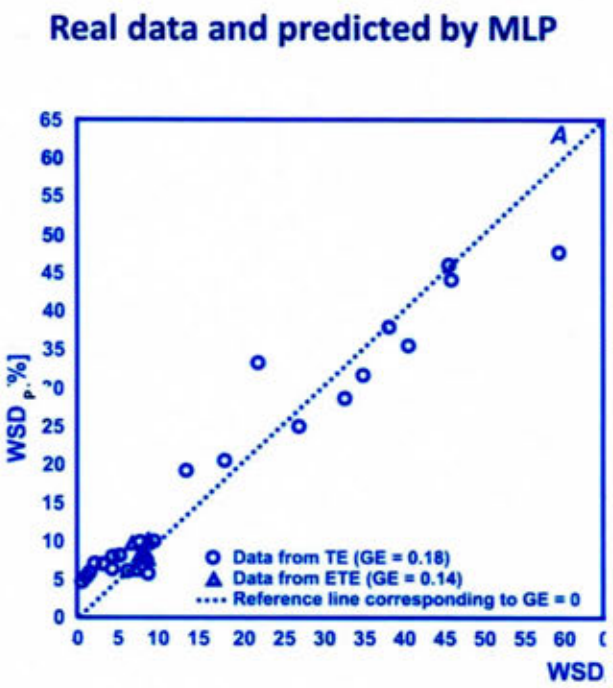
I also decided to check whether based on the parameters of Chl *a* fluorescence the model for prediction of tissue dehydration (WSD – *Water Saturation Deficit*) could be done (**H6**: *Machine learning in determination of water saturation deficit in wheat leaves on basis of Chl a fluorescence parameters*)

The experiments were carried out as part of grants NCN-NN304267540 and NCBiR-PBS3/B8/19/2015 using wheat seedlings and plants from ssd (*singe seed deccent*) breeding programs, (grown in controlled conditions) that differed in tolerance to dehydration. The aim of the experiment was to check whether an indirect WSD assessment could be done by modeling based on Chl *a* fluorescence parameters. Since WSD fluctuations are a simple phenotypic trait reflecting reaction to negative environmental stimuli (Kacperska 2004) I believe that inclusion of water saturation deficit (WSD), as a feature of a field screening, can be valuable for breeding progress. Unfortunately, mere WSD detection does not meet requirements of the screening test due to the low bandwidth. However, detection of Chl *a* fluorescence, in total measurement speed of one sample per 10-15 [s], meets those terms. The WSD prediction was realized by MLP (Multi-Layer Percepton) consisted of three layers. Single MLP input node corresponded to one input variable and the output node corresponded to WSD. Modeling was performed on 237 records of data including WSD determined by weight analysis and 13 fluorescence parameters calculated by the PocketPeA fluorimeter software: 90% of data entered the modeling as a "TR" training set (including 20% of the validation set (VA)) and 10% as a testing set (TE). A set of 14 data records were also introduced for modeling as an external testig set (ETE). The MLP task was to map input variables onto one output using weights adaptation and transfer functions (TF) to obtain high correlation coefficient (r) between experimental and predicted WSD values as well as the lowest global error (GE). Since neither the structure of the best MLP nor the best combination of TFs were known, MLPs with different number of



inputs as well as 4 different TFs (identity, logistic, exponential, and hyperbolic tangent) were tested to select model that met the requirements. During training inputs and outputs were normalised to obtain values in range 0–1. Each combination of input variables with a set of 4 test functions has been converted by 2000 artificial neural networks of the MLP type, giving a total of 62,000 models tested by statistical software. The MLP task was to map input variables onto one output using weights adaptation and TF to obtain high correlation coefficient (r) between experimental and predicted WSD values as well as the lowest global error (GE) and the lowest model complexity. The best model, predicting WSD with global error GE $\leq$ 0.18 and correlation coefficient r $\geq$ 0.98, was built based on 3 input variables, 3 hidden enurons and 12 weights. It is noteworthy that the best neural model was generated based on 3 Chl *a* fluorescence parameters describing functional activity at the three main stages of energy transformation in PSII: 1/ capture of the light photons (RC_{ABS} parameter reflects the relative number of PSII active centers absorbing light in a single event); 2/ energy trapping (F_V/F_M parameter refers to the maximum efficiency of the light reaction and energy trapping efficiency by PSII reaction centers, it is a value reflecting the power of light reactions); 3/ electron transfer in ETC (parameter ((1 - VJ) / VJ) reflects the efficiency of secondary electron transfer leading to stabilization of charges originally separated in the PSII reaction center) (Strasser et al. 1995; Strasser et al. 2004; Goltsev et al. 2016; Paunov et al. 2018; Stirbet et al. 2018). Essentially, dehydration of plant tissues leads to changes in hydration and polarization of cell macromolecules, which in turn affects conformational modifications of polymers, reducing their functionality and influencing on biochemical processes (Rumak et al. 2012; Malferrari et al. 2013; Ball 2017). Network sensitivity analysis provided information about the hierarchy of input data in the best model. For the correct WSD prediction of wheat seedlings grown in experimental conditions, the parameter ($(1-V_J)/V_J$) was carrying the most important information about WSD variability, about twice as important as RC_{ABS} and six times more important as F_V/F_M. The exclusion of the ($(1 - V_J)/V_J$) parameter from the model weakened WSD's prediction capability by more than eight times. Further studies are being carried out to collect data that takes into account the greatest variability of WSD in parallel with the Chl *a* fluorescence parameters to identify all factors that can modify fluorescence and consequently interfere with the correct WSD prediction. In 2018, we started WSD modeling based on field measurements. The model was generated by dr inż. M. Janaszek-Mańkowska (SGGW) using the Statistica software.





VI. Conclusions

Modern methods of functional analysis of genes and mapping based on molecular markers obtained by techniques generating large amounts of data require new high-throughput phenotyping methods of breeding materials and methods of processing 'big data' (**H1**)

Unsupervised machine learning (PCA analysis, cluster analysis) and supervised (neural networks) are approaches that allow processing of big data sets and can be used for plants physiological state description based on values and parameters of Chl *a* fluorescence (**H2-6**)

ΔW_{OJ} curves calculated and plotted on logarithmic time axis on the basis of fast Chl *a* fluorescent phase data allow for the differentiation of plant materials that are tolerant and sensitive to abiotic stresses (**H3-5**)

'Heat maps' based on hierarchical grouping of Chl *a* fluorescence parameters are tools for visual detection of differences in response to abiotic stress between tested plant materials (**H3-5**)

The MLP neural network enables WSD (*Water Saturation Deficit*) modeling based on Chl *a* fluorescence parameters by which allows estimation of the degree of leaf dehydration. The current model is functional for wheat seedlings grown under controlled conditions (**H6**)

Proteome modifications and protection of the photosynthetic apparatus are reactions to the first stages of dehydration of wheat seedlings of drought tolerant variety. The selection of plants with stable and efficient photosynthesis under environmental stress conditions is suggested as a selection approach (**H5**)

The proposed phenotyping methods can be used for the screening of breeding materials (a single fluorescence measurement lasts <10 sec).

VII. Bibliography

Aroca R, Porcel R, Ruiz-Lozano JM. 2012. Regulation of root water uptake under abiotic stress conditions. J Exp Bot 63:43-57.

Asseng S, Ewert F, Rosenzweig C, Jones JW, Hatfield JL et al. 2013. Uncertainty in simulating wheat yields under climate change. Nature Clim. Change 3:827-832.

Ball P. 2017. Water is an active matrix of life for cell and molecular biology. PNAS 114:13327-13335.

Belko N, Zaman-Allah M, Diop NN, Cisse N, Zombre G, Ehlers JD, Vadez V. 201. Restriction of transpiration rate under high vapour pressure deficit and non-limiting water conditions is important for terminal drought tolerance in cowpea. Plant Biol 15:304-316.

Bhatnagar-Mathur P, Vadez V, Sharma K. 2008. Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep 27:411 - 424.

Bilska-Kos A, Szczepanik J, Sowinski P. 2016. Cold induced changes in the water balance affect immunocytoloca-lization pattern of one of the aquaporins in the vascular system in the leaves of maize (*Zea mays* L.). J Plant Physiol 205:75-79.

Blum A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crop Res 112:119-123.

Blum A. 2011. Drought resistance – is it really a complex trait? Funct Plant Biol 38:753-757.

Brooks RJ, Semenov MA, Jamieson PD. 2001. Simplifying sirius: Sensitivity analysis and development of a meta-model for wheat yield prediction. Eur J Agr 14:43-60.

Craufurd PQ, Vadez V, Jagadish SVK, Prasad PVV, Zaman-Allah M. 2013. Crop science experiments designed to inform crop modeling. Agr Forest Meteorology 170:8-18.

Dobra J, Motyka V, Dobrev P, Malbeck J, Prasil IT, Haisel D, Gaudinova A, HavlovaM., Gubis J, Vankova R. 2010. Comparison of hormonal responses to heat, drought and combined stress in tobacco plants with elevated proline contents. J Plant Physiol 167:1360-1370.

Foyer CH, Noctor G. 2011. Ascorbate and Glutathione: The Heart of the Redox Hub. Plant Physiol 155:2-18.

Foyer CH, Neukermans J, Queval G, Noctor G, Harbinson J. 2012. Photosynthetic control of electron transport and the regulation of gene expression. J Exp Bot 63:1637-1661.

Garstka M. 2007. Strukturalne podstawy reakcji świetlnych fotosyntezy. Postępy Biologii Komórki 34:445-476.

Gietler M, Nykiel M, Zagdańska BM. 2016a. Changes in the reduction state of ascorbate and glutathione, protein oxidation and hydrolysis leading to the development of dehydration intolerance in *Triticum aestivum* L. seedlings. Plant Growth Reg 79:287-297.

Gietler M, Nykiel M, Orzechowski S, Fettke J, Zagdańska B. 2016b. Proteomic analysis of S-nitrosylated and S-glutathionylated proteins in wheat seedlings with different dehydration tolerances. Plant Physiol Biochem 108:507-518.

Gietler M, Nykiel M, Orzechowski S, Fettke J, Zagdańska B. 2017. Protein carbonylation linked to wheat seedling tolerance to water deficiency. Envir Exp Bot. 137C: 84-95.

Goltsev VN, Kalaji HM, Paunov M, Bąba W, Horaczek T, Mojski J, Kociel H, Allakhverdiev SI. 2016. Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. Russ J Plant Physiol 63:869-893.

Grudkowska M, Zagdańska B. 2004. Multifunctional role of plant cysteine proteinases. Acta Biochim Pol 51:609-624. http://www.actabp.pl/pdf/3_2004/609.pdf.

Grudkowska M., Zagdanska B. (2010) Acclimation to frost alters proteolytic response of wheat seedlings to drought. J Plant Physiol 167:1321-1327.

Gururani MA, Upadhyaya CP, Strasser RJ, Woong YJ, Park SW. 2012. Physiological and biochemical responses of transgenic potato plants with altered expression of PSII manganese stabilizing protein. Plant Physiol Biochem 58:182-194.

Jamieson PD, Semenov M., Brooking I., Fracois G. 1998. Sirius: a mechanistic model of wheat response to environmental variation. Eur J Agron 8:161-179.

Janik E, Maksymiec W, Mazur R, Garstka M, Gruszecki WI. 2010. Structural and functional modifications of the major light-harvesting complex II in cadmium-or copper-treated Secale cereale. Plant Cell Physiol 51:1330-1340.

Kacperska A. 2004. Sensor types in signal transduction pathways in plant cells responding to abiotic stressors: do they depend on stress intensity? Physiol Plant 122:159-168.

Kalaji HM, Goltsev V, Bosa K, Allakhverdiev SI, Strasser RJ, Govindjee. 2012. Experimental *in vivo* measurements of light emission in plants: A perspective dedicated to David Walker. Photosynth Rese 114:69-96.

Kholova J, Sairam R, Meena R. 2010. Osmolytes and metal ions accumulation, oxidative stress and antioxidant enzymes activity as determinants of salinity stress tolerance in maize genotypes. Acta Physiol Plant 32:477-486.

Kholová J, Hash CT, Kocová M, Vadez V. 2010. Does a terminal drought tolerance QTL contribute to differences in ROS scavenging enzymes and photosynthetic pigments in pearl millet exposed to drought? Envir Exp Bot 71:99-106.

Kholová J, Murugesan T, Kaliamoorthy S, Malayee S, Baddam R, Hammer GL, McLean G, Deshpande S, Hash CT, Craufurd PQ, Vadez V. 2014. Modelling the effect of plant water use traits on yield and stay-green expression in sorghum. Funct Plant Biol 41:1019-1034.

Lázaro JJ, Jiménez A, Camejo D, Iglesias-Baena I, Martí MC, Lázaro-Payo A, Barranco-Medina S, Sevilla F. 2013. Dissecting the integrative antioxidant and redox systems in plant mitochondria. Effect of stress and S-nitrosylation. Front Plant Sci 4:460.

Levitt J. 1985. Relationship of dehydration rate to drought avoidance, dehydration tolerance and dehydration avoidance of cabbage leaves, and to their acclimation during drought-induced water stress. Plant Cell Envir 8:287-296.

Malferrari M, Mezzetti A, Francia F, Venturoli G. 2013. Effects of dehydration on light-induced conformational changes in bacterial photosynthetic reaction centers probed by optical and differential FTIR spectroscopy. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1827:328-339.

Miazek A, Nykiel M, Rybka K. 2017. Drought tolerance depends on the age of the spring wheat seedlings and differentiates patterns of proteinases. Rus J Plant Physiol 64:333-340.

Oukarroum A, Schansker G, Strasser RJ. 2009. Drought stress effects on photosystem i content and photosystem II thermotolerance analyzed using Chl *a* fluorescence kinetics in barley varieties differing in their drought tolerance. Physiol Plant 137:188-189.

Paunov M, Koleva L, Vassilev A, Vangronsveld J, Goltsev V. 2018. Effects of Different Metals on Photosynthesis: Cadmium and Zinc Affect Chlorophyll Fluorescence in Durum Wheat. Int J Mol Sci 19:787.

Perdomo JA, Capo-Bauca S, Carmo-Silva E, Galmes J. 2017. Rubisco and Rubisco Activase Play an Important Role in the Biochemical Limitations of Photosynthesis in Rice, Wheat, and Maize under High Temperature and Water Deficit. Front Plant Sci 8:490.

Pinheiro C, Chaves MM. 2011. Photosynthesis and drought: can we make metabolic connections from available data? J Exp Bot 62:869-882.

Porter JR, Semenov MA. 2005. Crop responses to climatic variation. Philos Trans R Soc Lond B Biol Sci 360:2021-2035.

Prokopiuk K. 2016. The effect of growing season extension on the quality of football pitches sward (in Polish). Ph. D. Thesis, Plant Breeding and Acclimatization Institute-PIB, Radzikow, Poland, pp. 161 (in Polish).

Rapacz M, Sasal M, Kalaji HM, Kościelniak J. 2015. Is the OJIP Test a Reliable Indicator of Winter Hardiness and Freezing Tolerance of Common Wheat and Triticale under Variable Winter Environments? PLoS ONE 10:e0134820.

Rucińska-Sobkowiak R. 2016. Water relations in plants subjected to heavy metal stresses. Acta Physiol Plant 38:257.

Rumak I, Mazur R, Gieczewska K, Kozioł-Lipińska J, Kierdaszuk B, Michalski WP, Shiell BJ, Venema JH, Vredenberg WJ, Mostowska A, Garstka M. 2012. Correlation between spatial (3D) structure of pea and bean thylakoid membranes and arrangement of chlorophyll-protein complexes. BMC Plant Biology 12:72.

KvR

Rybka K. 2017. Fenotypowanie roślin. Konferencja EPPN 2020 w Tartu/ Estonia. Biul IHAR 282:161-174.
Rybka K, Oleksiak T. 2016. Czasowe niedobory wody a postęp biologiczny w hodowli zbóż. *W:* Dembek W et al. (Ed.), Innowacyjne metody gospodarowania zasobami wodnymi w rolnictwie. CDR, Brwinów: 261-271.
Semenov MA, Stratonovitch P. 2013. Designing high-yielding wheat ideotypes for a changing climate. Food Energy Security 2:185-196.
Semenov MA, Stratonovitch P, Alghabari ., Gooding MJ. 2014. Adapting wheat in Europe for climate change. Journal of Cereal Science 59:245-256.
Sobkowiak A, Jończyk M, Adamczyk J, Szczepanik J, Solecka D, Kuciara I, Hetmańczyk K, Trzcinska-Danielewicz J, Grzybowski M, Skoneczny M, Fronk J, Sowiński P. 2016. Molecular foundations of chilling-tolerance of modern maize. BMC Genomics 17:125.
Srivastava A, Guissé B, Greppin H, Strasser RJ. 1997. Regulation of antenna structure and electron transport in Photosystem II of Pisum sativum under elevated temperature probed by the fast polyphasic chlorophyll a fluorescence transient: OKJIP. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1320:95-106.
Stirbet A, Govindjee.2012. Chlorophyll *a* fluorescence induction: A personal perspective of the thermal phase, the J-I-P rise. Photosynthesis Res 113:15-61.
Stirbet A, Riznichenko GY, Rubin AB, Govindjee. 2014. Modeling chlorophyll *a* fluorescence transient: Relation to photosynthesis. Biochemistry-Moscow 79:291-323.
Stirbet A., Lazár D., Kromdijk J., Govindjee. 2018. Chlorophyll a fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses? Photosynthetica 56:86–104.
Strasser R.J., Srivastava A., Govindjee. 1995. Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61:32-42.
Strasser RJ, Tsimilli-Michael M, Srivastava A. 2004. Analysis of the Chlorophyll *a* Fluorescence Transient. *W:* Papageorgiou G. and Govindjee (Ed.), Chlorophyll *a* Fluorescence: A Signature of Photosynthesis, Springer Science+Business Media B.V. pp. 321-362.
Sulkiewicz M., Ciereszko I. 2016. Fluorescencja chlorofilu *a*- historia odkrycia i zastosowanie w badaniach roślin. Kosmos 65:103–115.
Tóth SZ, Schansker G, Garab G. 2013. The physiological roles and metabolism of ascorbate in chloroplasts. Physiol Plant 148:161-175.
Tóth SZ, Schansker G, Garab G, Strasser RJ. 2007. Photosynthetic electron transport activity in heat-treated barley leaves: The role of internal alternative electron donors to photosystem II. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1767:295-305.
Tsimilli-Michael M, Strasser RJ. 2013. The energy flux theory 35 years later: formulations and applications. Photosynthesis Res 117:289-320.
Vadez V, Kholova J, Zaman-Allah M, Belko N. 2013a. Water: the most important 'molecular' component of water stress tolerance research. Funct Plant Biol 40:1310-1322.
Vadez V, Kholová ., Yadav R, Hash C. 2013b. Small temporal differences in water uptake among varieties of pearl millet (*Pennisetum glaucum* (L.) R. Br.) are critical for grain yield under terminal drought. Plant Soil 371:447-462
Vadez V, Kholova J, Medina S, Kakkera A, Anderberg H. 2014. Transpiration efficiency: new insights into an old story. J Exp Bot 65:6141-6153
Vadez V, Rao S, Kholova J, Krishnamurthy L, Kashiwagi J, Ratnakumar P, Sharma K, Bhatnagar-Mathur P, Basu P. 2008. Root research for drought tolerance in legumes: *Quo vadis*? J Food Legumes 21:77-85.
Wolf J, Evans L, Semenov M, Eckersten H, Iglesias A. 1996. Comparison of wheat simulation models under climate change. I. Model calibration and sensitivity analyses. Climate Res 7:253-279.
Yadav KNS, Semchonok DA, Nosek L, Kouřil R, Fucile G, Boekema EJ, Eichacker LA. 2017. Supercomplexes of plant photosystem I with cytochrome b6f, light-harvesting complex II and NDH. Biochim Biophys Acta (BBA) - Bioenergetics 1858:12-20.
Zagdańska B. 1995. Energy metabolism in plants under water deficits. Acta Biochim Polon 42:281-289.

Krystyna Rybka

dr Krystyna Rybka

5. Presentation of other scientific achievements

Grant NCBiR PBS3/B8/19/2015 entitled: „Development and implementation of the method of shortening the breeding cycles by optimizing the light conditions in the cereal breeding process"
From 2015.06.01 to 2018.05.31
edited publications, till 22.01.2019: [Zał 3 Supl #4, #6, #7]
The grant was implemented by the IHAR-PIB & HR-Strzelce consortium
Detailed results are beeing prepared for publication. They will be a part of the doctoral dissertations of 2 people. Therefore, I am presenting only the specification of the results:

- Two types of lighting modules have been selected with an optimal spectrum for crop growing in a greenhouse and a low current consumption indicator
- Monochromatic light was chosen to shorten the vernalization time of winter wheat
- A suitable light was selected to increase the yield of DH regenerants fron androgenesis (barley)
- Light suitable for plant growth (complex spectrum) and drying (monochromatic spectrum), and germination (monochromatic spectrum) of immature seeds (on the example of wheat) were selected to raise the germination index. Results better than grain conditioning in cold could not be obtained
- Proteomic analysis has shown that optimal LED lamps do not induce stress in wheat leaves

Preharvest sprouting (PHS) of triticale
publictions [Attachement 3: #16 , #50, #51]

Research on traits determining preharvest sprouting (PHS) resistance of cultivars, hybrid materials and DH lines of triticale included physiological and molecular studies. The assessment of PHS resistance of triticale cultivars and breeding materials was carried out by determination of seed germination index (GI) from the equation: $GI[\%] = \sum_{n=1}^{n=9}[(\text{Number of germinated seeds/n\_day}) * (100/30)]$. A correlation coefficient (R = -0.81) was found between GI of seeds with a moisture content of ≤37% and grain sprouting in the ears on the 7th day of the PHS provocation test. The sprouting index was determined in 169 varieties, breeding materials and DH lines. The varieties Algoso, Atletico and Leonito were characterized by a low GI, and therefore a potentially higher tolerance to PHS, whereas the varieties Grenado, Moderato and Pigmej were characterized by a high GI, i.e. susceptibility to PHS. Among the materials tested, 32 lines were described as tolerant, while 60 lines were determined to be susceptible, as compared to commercial triticale varieties. Molecular studies on PHS resistance were carried out based on the genetic map of triticale. The QTLs of PHS resistance, expressed as germination index (GI), were mapped basing on three-year studies. There were found 11 loci of GI positively coupled with PHS on chromosomes: 1A (two loci), 3B, 4A, 5A (two loci), 5B, 6A, 6B, 7R (two loci) and 5 loci negatively coupled with PHS, on chromosomes: 2A (two loci), 4R, 5A and 7B. The differential sequences obtained by cDNA-AFLP technique were assigned *in silico* to the chromosomal groups of wheat based on data from the NCBI and GrainGenes databases. A clone 14-47-4 was located on chromosome 1A; on chromosome 2A - clone 30-71-1 was located and additionally on chromosomes 4A, 3B and 5A; on chromosome 3B - clone 1-2-2; on 6A and 6 B - clone 9-18-4; on 7A - clones 24-59-1 and 23-58-1; on 7B - clones 13-43-1, 31-75-1 and 23-58-1 were located. As part of the project, 2 master's theses were done and defended with a very good result by students from SGGW: 1) "Modification of germination processes of triticale seeds by sugars" and 2) "Modification of germination processes of triticale seeds by abscisic acid".

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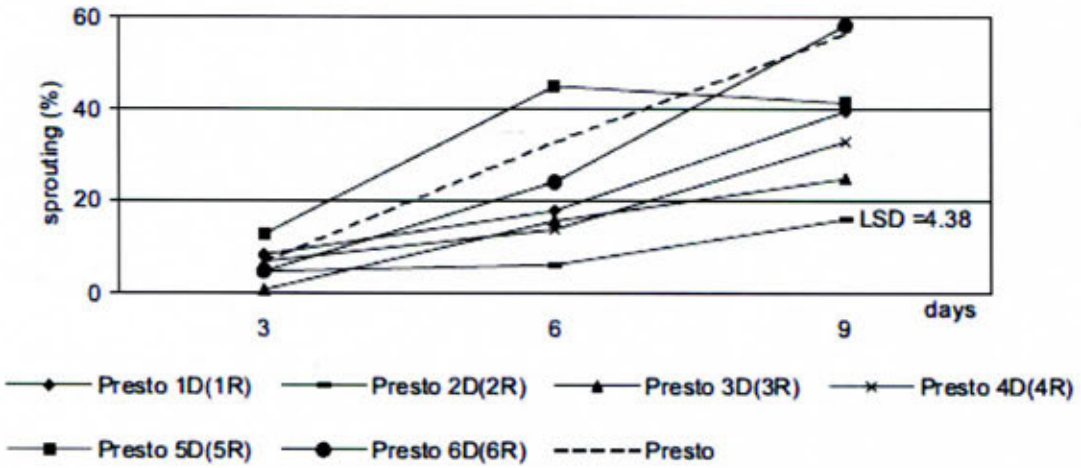
As a result of the studies run using the substitution lines of Presto, rye chromosomes that reduced PHS tolerance were identified; data were published in the Journal of Applied Genetics.



Post-doctoral internships, three-years long each

- **Japan- post doctoral fellow at National Institute of Agrobiological Resources-Tsukuba**
  positional cloning of rice resistance genes Pi-ta2 and Pib
  publications [Attachement 3: #17 - #19]
- **US- post doctoral fellow at University of California, Riverside**
  genetic and physical mapping of translocations in the wheat genome
  publications [Attachement 3: #11, #12, #14, #15]

When planning a post-doctoral internship I was looking for a laboratory working on the cell wall polymers of the kernels. I received a Japanese government scholarship and my host, Dr. Shinji Kawasaki, six months before my departure from Poland, proposed me to join the project "Positional cloning of rice blast resistance genes". It was a great challenge and a total change in the laboratory protocols I've worked with. Before leaving for Japan, I completed a quarter internship in the laboratory of prof. Andrzej Jerzmanowski (University of Warsaw) in the field of basic techniques of molecular biology. Working in Japan, I have established knowledge of molecular biology methods: from basic ones such as extraction and cleaning of DNA and RNA, then all techniques necessary for markering as well as construction and screening of genomic libraries and cDNA libraries, construction of genetic and physical maps (digestion, identification and isolation of DNA fragments, preparation of vectors, ligation, bacterial transformations, electrophoresis, sequencing, blotting, PCR techniques, hybridizations).

As a team, we first searched for molecular markers (RFLP and RAPD-PCR) of rice blast resistance genes located on the 2nd and 12th chromosomes, using the near izogenic lines of the Japonica species, in which chromosomal fragments were substituted with homeologic fragments of Indica species. We determined the physical distances between markers by conducting hybridization of probes to genomic DNA libraries in BAC and Cosmid vectors and contigs arrangement. I finished my internship in Japan when clones from the area designated by flanking markers were identified. The results of our research were described in 4 articles published in indexed journals; We presented partial results in numerous scientific conferences in Japan and in three international conferences. While working in Japan, I also trained personnel and students, mainly in PCR techniques. Today, I am surprised that I have fulfilled this obligation, despite the enormous language and cultural barriers.

The most interesting result of my work in Japan was the statement that the proportions of distances on genetic and physical chromosomal maps vary depending on the location of the mapped fragment relative to the centromere. The 1cM in distal region of the genetic map of rice was covered by c.a. 100 kbp BAC contig. In the proximal regions the distance increased over ten times. It was an innovative

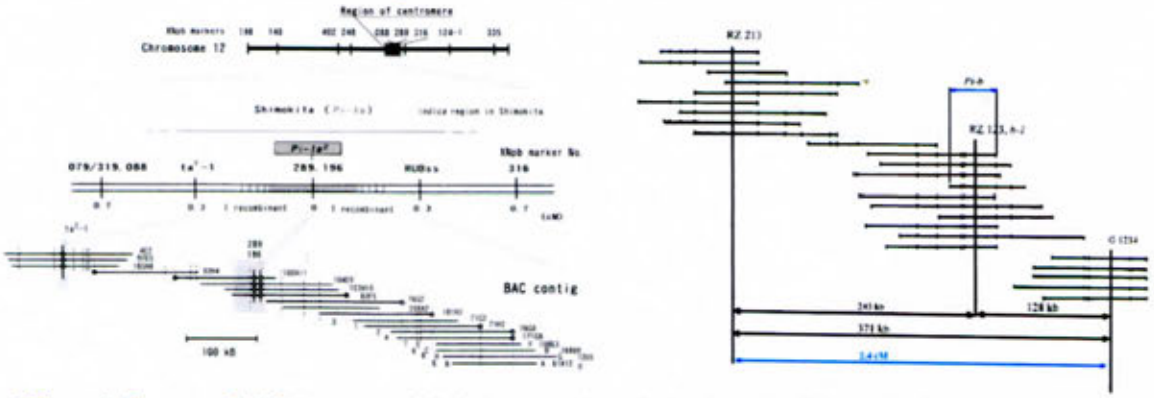
observation in the mid-nineties. I confirmed this thesis in later years by constructing genetic maps of hexaploid wheat.

Leaving for an internship in Japan, I left a technician implementing experiments in the field of biochemical bases of the resistance of triticale to the pathogen *Stagonospora nodorum*, studies in frames of experiments finaced by IHAR, leaded by prof. dr hab. Edward Arseniuk. The work was summarized by a conference report and an article published in Acta Physiologiae Plantarum.

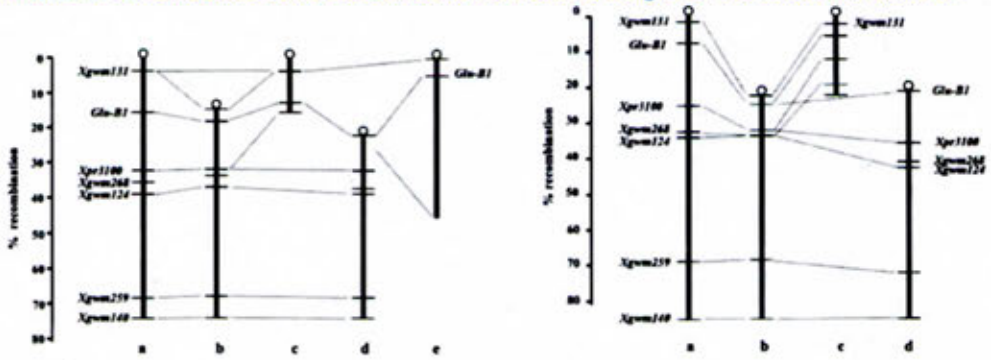
My interest in the apparent change in the length of chromosomes depending on the distance to the centromere, expressed as a question for lecturer at the IHAR, prof. Adam Łukaszewski (Polish cytogenetics working as a professor at the University of California), caused that the Professor invited me to cooperate in his laboratory. While working at the University of California, I used my knowledge of molecular biology to construct the genetic map of chromosomes 1B and 2B based on wheat lines with chromosome modified by defficiencies or deletions. My technique skills were being enhanced with *in situ* hybridization techniques, which protocols I elaborated and simplified. From the ground up, I organized a molecular laboratory, I trained 3 students and few senior visiting researchers from Poland in techniques of molecular biology. The work was summarized in 4 articles and 2 reports on conferences.

Common idea of postdoc internships: relative genetic distances

Rice: Pi-ta2 locus at centromeric region of 2nd chromosome: 0.3 cM > 1 MB
PiB locus at telomeric region of 12th chromosome: 2.4 cM = 371 kB



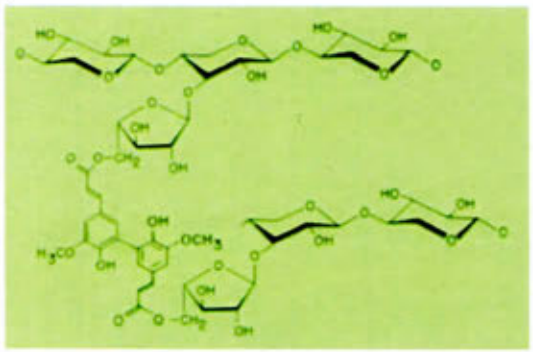
Wheat: influence of deficiences and deletions on the relative length of the 1BL chromosome



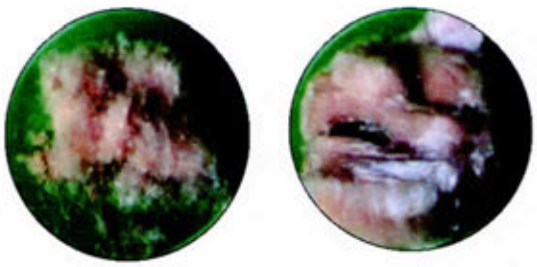
I present figures from my postdoc publications in one scheme. On chromosome maps of rice it can be seen that the relative length of the chromosome expressed in centimorgans has a different physical length depending on the location on the chromosome: 1 cM of the distal part of the chromosome is physically shorter than proximal. In contrast, the 1BL wheat chromosome maps show that, depending on the distance from telomeres, the genetic distance decreases between two the same markers on a chromosome modified by deficientions or deletions.

PhD Thesis: „The relationship between the properties of non-starch polysaccharides and the digestibility of protein in the grain of rye inbred lines",
Supervisor: prof. dr hab. Konstancja Raczyńska-Bojanowska

Graduating from the Faculty of Chemistry of the University of Warsaw, I was determined to enter the scientific career path, but I was looking for a field more related to nature than electrochemistry in a non-aqueous environment, although my master's thesis data were published in 1987 in Polish Journal of Chemistry. Fortunately, prof. Konstancja Raczyńska-Bojanowska, the head of the Department of Biochemistry at Plant Breeding and Acclimatization Institute in Radzików was looking for a chemist to start work on the biochemical bases of the nutritional value of rye grain, in frames of collaboration with prof. dr hab. Maria Rakowska and Dr. Lucjan Madej. Shortly after starting the work I implemented a technique for determination of the content of dietary fiber in cereal grains, according to modified Asp method, which was studied by (currently prof. dr hab.) Danuta Boros during her internship in Denmark. Working together in an interdisciplinary team, we found that both: the content of soluble non-starch polysaccharides and the viscosity of the aqueous extract of the meal are negatively correlated with the nutritional value of the grain of inbred rye lines tested on laboratory animals. This result was the main result of my doctoral dissertation, in which there were also stated that: • protein is an integral component of indigestible fractions (both soluble and insoluble); • the content of *in vitro* soluble fraction, in contrast to the insoluble fraction, correlates with the protein digestibility *in vivo* and can be used as a test in selection of inbred rye lines towards better fodder nutritional quality; • it is possible to determine this fraction by the use of near-infrared spectrophotometry (NIT), • there is a genetic variability of the content of this fraction in the grain of inbred rye, despite significant variability in years.



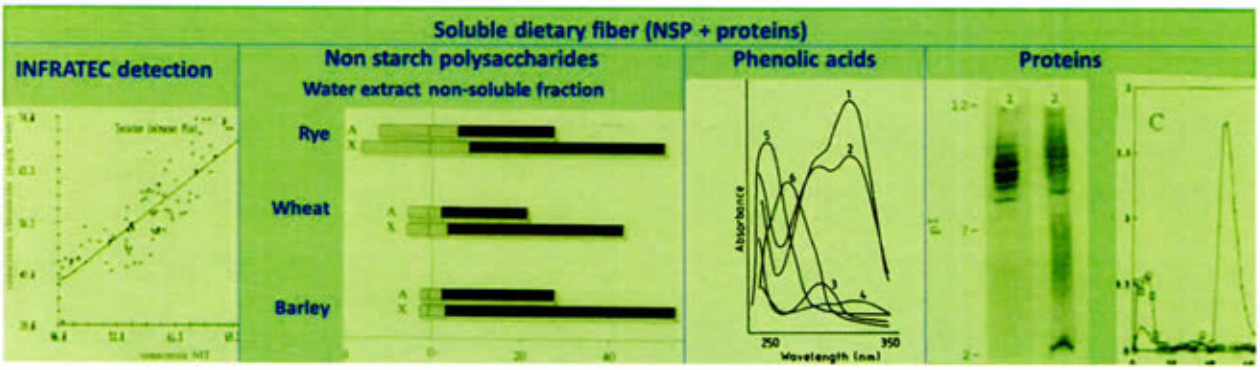
Diferulic bridge in the molecule of arabinoxylan



Lyophilisates of soluble dietary fiber:
wheat grain rye grain

We also put forward the thesis that the solubility and biological properties of arabinoxylans are determined not only by the degree of branching of xylan chains and their cross-linking by ferulic acid, but also by differences in molecular mass and molecular conformation conditioned by the distribution of arabinose substituents. Experiments on ferulic acid were published in Cereal Chemistry, to this day, my most cited publication. I follow the research on cell wall polymers, as I have been convinced for years (and experimental results increasingly confirm) that changes in conformation of wall polymers induced by bio-chemical and physical stimuli (eg. hydration) are an important factor in the regulation of plant responses to negative environmental stimuli. The content of non-starch polysaccharides in the grain and viscosity of the aqueous extract are parameters that have been consistently used in the assessment of grain quality, although the original procedures have undergone significant changes. My work on the nutritional value of rye grain ended after the defense of doctoral dissertation. The results of the entire team's work were summarized by prof. Raczyńska-Bojanowska in the monograph "Rye toward better quality",

published as a volume of Plant Breeding and Seed Science in 1994. In this monograph I am the co-author of 7 chapters.



Ackonowledgements

I would like to thank my Superiors and Colleagues with whom I am working and I have worked in the Department of Biochemistry and Plant Physiology, IHAR-PIB in Radzików as well as in other institutions and especially prof. dr hab. Konstancja Raczyńska-Bojanowska for grounding in the conviction that there is a need to explore interesting topics without running away from what is difficult.
I would also like to thank my Teachers, both academic and from lower levels of education, not only for a solid base of knowledge, but also for instilling the habit of caring about the way of thoughts expression.
I would like to express also my deepest gratitude to my family and friends.

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