Załącznik nr 3

AUTHOR'S REVIEW

OF HIS OWN SCIENTIFIC AND SCHOLARLY ACHIEVEMENTS

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1. PERSONAL DATA

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2. DIPLOMAS AND DEGREES

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2002	Plant Breeding and Acclimatization Institute (PhD in agricultural sciences in agronomy)

3. PROFESSIONAL LIFE

1.09.1995 – 31.01.1996	internship	Potato Institute in Bonin (from 1 January 1997 r. Plant Breeding and Acclimatization Institute in Bonin)
01.02.1996 – 28.10.1996	assistant	Department of Microbiology, Faculty of Biology and Earth Sciences, Nicolaus Copernicus University in Toruń.
29.10.1996 – 30.09.1998	technologist	Plant Breeding and Acclimatization Institute in Bonin
01.10.1998 – 31.10.2002	assistant	Plant Breeding and Acclimatization Institute in Bonin
01.11.2002 –	Assistant Professor	Department of Potato Protection and Seed Science in Bonin, Plant Breeding and Acclimatization Institute National Research Institute
01.01.2008 –	Head of Laboratory	Laboratory of Molecular Diagnostics and Biochemistry, Department of Potato Protection and Seed Science in Bonin, Plant Breeding and Acclimatization Institute National Research Institute

4. INDICATION OF THE SCIENTIFIC ACHIEVEMENT resulting from Article 16 sec. 2 of the Act of March 14, 2003. on academic degrees and academic titles, and on degrees and title in the field of art (Journal of Laws of 2016, item 882, as amended in Journal of Laws of 2016, item 1311):

A) TITLE OF SCIENTIFIC ACHIEVEMENT:

Molecular diagnosis of potato virus Y with simultaneous differentiation into genotypes corresponding to virus serotypes O and N

B) PUBLICATIONS COMPRISING THE SCIENTIFIC ACHIEVEMENT:

H1. Zacharzewska B., Przewodowska A., **Treder K**. 2014. The adaptation of silica capture RT-PCR for the detection of potato virus Y. Am. J. Potato. Res., 91: 525-531. $\mathbf{IF_{2014}}$=1,204; $\mathbf{MNiSW_{2014}}$ = 25 pkt. *Own contribution 75%.*

H2. Przewodowska A., Zacharzewska B., Chołuj J., **Treder K**. 2015. A one-step, real-time reverse transcription loop-mediated isothermal amplification assay to detect Potato virus Y. Am. J. Potato. Res., 92: 303-311.
$\mathbf{IF_{2015}}$ = 1,159; $\mathbf{MNiSW_{2015}}$ = 25 pkt. *Own contribution 70%.*

H3. **Treder K**., Chołuj J., Zacharzewska B., Mielczarek M. 2017. Detection of potato virus Y (PVY) by reverse-transcription loop-mediated nucleic acid amplification (RT-LAMP). Plant Breeding and Seed Science, 75: 77-85.
$\mathbf{MNiSW_{2016}}$ = 11. *Own contribution 85%.*

H4. **Treder K**., Chołuj J., Zacharzewska B., Babujee L., Mielczarek M., Burzyński A., Rakotondrafara A. 2018. Optimization of a magnetic capture RT-LAMP assay for fast and real-time detection of potato virus Y and differentiation of N and O serotypes. Archives of Virology, 163: 447-458.
$\mathbf{IF_{2017}}$ = 2,160; $\mathbf{MNiSW_{2015}}$ = 20 pkt. *Own contribution 55%.*

The total Impact Factor of the publications included in the scientific achievement according to the Journal Citation Reports (JCR) list, according to the year of publication for publications H1 and H2 and 2017 for the publication H4 (due to the lack of data for 2018) is **4,523**. Publication H3 is not on the JCR list and therefore has no IF value. The sum of points for publications included in the scientific achievement, according to the list of scientific journals according to the year of publication for publications H1 and H2 and 2016 for publications H3 and 2015 for publications H4 (due to the lack of data from later years) is **81** points.

The applicant's contributions to the works above are described in **Annex 4**. The co-authors' statements describing their contributions to each publication are included in **Appendix 6**. Copies of publications being scientific achievements are attached in **Appendix 5**.

C) REVIEW OF THE SCIENTIFIC PURPOSE AND RESULTS PRESENTED IN THE PAPERS INCLUDED IN THE SCIENTIFIC ACHIEVEMENT, AND DISCUSSION OF THEIR POSSIBLE APPLICATION.

INTRODUCTION

Potato is one of the four most important agricultural plants in the world, apart from corn, rice and wheat (Devaux et al., 2014). Its importance is constantly growing, especially in Asia and Africa, where the area of cultivation has doubled in the last decade, with China and India taking the first two places among the largest producers (FAO 2017). The growing popularity of potato in many regions of the world is associated with its highest energy value among all plants in relation to the area of cultivation and high nutritional value. Due to these values, the United Nations Food and Agriculture Organization considered the potato as a strategic plant, allowing the implementation of two (I and VII) Millennium Development Goals. The goal of I is to eliminate extreme poverty and hunger, and goal VII - to apply sustainable methods of natural resources management (FAO 2009, Devaux et al. 2014).

Poland is one of the largest potato producers in Europe and ranks seventh in the world (FAOSTAT 2017). It is also an important exporter of potato products. Potato is a crop propagated in a vegetative manner. Seed production, which is of key importance in potato production, in Poland covers less than 10% of the domestic demand for seed potatoes. The potato viruses, among which the most important are: potato virus Y (PVY), potato virus M (PVM) and potato leafroll virus (PLRV), have a major impact on the quality of seed potatoes and, consequently, the profitability of their production. In the economic context, the following viruses have a lower importance: potato virus S (PVS), potato virus X (PVX) and potato virus A (PVA). Seed production is subject to detailed legal regulations, and seed companies are officially controlled by the State Plant Health and Seed Inspection Service (PIORiN). The laboratory testing of seed potatoes for the presence of PVY, PVM and PLRV has been an element of the obligatory control scheme for years. From 2015, PVS, PVX, and PVA have been added to the list of obligatory viruses tested.

The health of seed potatoes is assessed by laboratories of Voivodship Inspectorates of Plant Health and Seed Inspection (WIORiN). Research is carried out using the so-called grow-out test, which is a combination of a biological and immunological test. The test consists in excising pieces of tubers with single eyes (meristems), chemical break of dormancy, sprouting at 21°C in the dark, and then planting in a greenhouse and performing the DAS-ELISA (double-

antibody sandwich enzyme-linked immunosorbent assay) according to Clark and Adams (1977) using sap from leaf samples taken from 4 weeks old plants. Each of the viruses is diagnosed with a specific set of antibodies. Detection of viruses by ELISA is carried out indirectly from plants and not directly from tubers, due to a significant increase in the concentration of virus particles in plants, which increases the chance of virus detection, even if the concentration in tubers was very low concentration.

The described procedure for identifying the viruses in potato tubers is well-developed, sensitive, reliable and effective. However, it is expensive and time-consuming. The results are obtained after a few weeks or even several months after sending the tubers for analysis. Every year, the PIORiN laboratories assess in Poland about 2,500 seed potato samples, it should be noted that one sample is composed of 100 or 200 tubers, depending on the seed potato class. This means that the number of tubers assessed ranges from 250,000 to 500,000 pieces per year. The test plants originating from the eyes are grown in the autumn and winter season, after the potato harvest, in greenhouses, which must be heated to the appropriate temperature and properly lit, which raises the costs of the assessment.

Therefore, for over 30 years, attempts have been made to develop a method that allows detection of viruses directly in tuber extracts. Attempts have been made to adapt the DAS-ELISA for this purpose but it was not sensitive enough and also generated false positive results (Hill and Jackson 1984). The main reasons for the problems with the detection of viruses in tuber extracts using DAS-ELISA were: lower than in other organs virus concentration, uneven distribution of viral particles both within the tuber and in different tubers originating from the same plant, high incidence of non-specific reactions within trials of healthy plants and the occurrence of enzymatic and non-enzymatic redox reactions causing darkening of juice (Hill and Jackson 1984; Tamada and Harrison 1980; Treder et al. 2009b). In the 1980s, attempts were made to use molecular methods to diagnose viruses, allowing the detection and analysis of nucleic acids from which virus genomes are built. Most plant viruses have genomes built of ribonucleic acid (RNA). Initially, methods based on nucleic acid hybridization were used.

The Polymerase Chain Reaction (PCR) method, developed in the early 1980s, revolutionized the biological sciences and the biotechnology industry, including the diagnostics of plant pathogens. You can use it to propagate specific DNA fragments. In combination with the reverse transcription of cDNA RNA (reverse transcription), as RT-PCR, it allows sensitive and specific detection of viruses in potato tissues. Already at the beginning of the 1990s, it was

shown that in contrast to DAS-ELISA, RT-PCR enables the detection of PVY (Barker et al. 1993) and PLRV (Spiegel, Martin 1993) in resting tubers. Over the past 20 years, many procedures have been developed to detect the most critical potato viruses in leaves and tubers (e.g., Singh 1999ab, Crosslin and Hamlin 2011, Hühnlein and others 2013, H1). The real-time RT-PCR (RT-qPCR) RT-PCR allows detection of viruses with higher sensitivity and enables quantitative virus analysis in the test sample (Mumford et al. 2006, Boonham et al. 2008). In this method, the fluorescence of the dye complex with the amplified cDNA or the fluorescence of the specific molecular probe is measured during the PCR reaction. Due to the easy adaptation to automation, high specificity and sensitivity, direct detection and the possibility of multiplex real-time RT-PCR detection seem to be the ideal successor of DAS ELISA in the diagnosis of potato viruses. However, due to high implementation costs, the use of this method in large-scale tests is still unprofitable.

Detection of potato viruses in tubers requires specific optimization of all stages of the test. The parameters such as the place of sampling (Treder et al. 2009), the composition of the extraction buffer and the selection of the RNA isolation method (H1) are essential. For potato varieties with a higher content of polyphenols, it is also necessary to optimize the RT and PCR reaction components (Singh 1999b). RT-PCR methods have so far not been used in large-scale diagnostics. Attempts at practical RT-PCR in real-time and RT-PCR showed that the sensitivity of PVY detection in potato tubers was lower than that based on an eye test (Barker et al. 1993, Fox et al. 2005, Bolotova et al. 2009). A large discrepancy in the sensitivity of assays described in publications reporting laboratory and field tests, can be explained among others, by the differences in the methods used for isolation of RNA from tubers and in the quality of this RNA (Boonham et al. 2008), and by the lack of standard methods of preparation of tuber RNA preparations in large-scale tests.

Despite the undoubted advantages, the disadvantage of tests based on PCR is the relatively high cost of the apparatus, especially for variants that enable real-time monitoring of reactions. RT-PCR itself is also more expensive than the PCR test, due to the cost of RNA isolation and reverse transcriptase - the enzyme "transcribing" RNA for single-stranded cDNA. The development, standardization, and implementation of the test involve high costs. Therefore, during the last decade, alternative methods of amplifying nucleic acids that do not require cyclical temperature changes have been sought. A number of methods of amplification of DNA and RNA occurring under isothermal conditions have been developed. Isothermal methods do

not require the use of expensive equipment to carry out the reaction. They are characterized by a higher rate and higher efficiency of nucleic acid amplification than PCR, allowing the test to be performed within 15-60 minutes while maintaining or even exceeding the sensitivity of the PCR test.

Among the many isothermal methods, the most promising seems to be the amplification of nucleic acids using loops (Loop-mediated isothermal amplification - LAMP) (Notomi et al. 2015). The method employs four or six primers specifically binding (hybridizing) to 6-8 DNA regions. Starters can be divided into internal, external and looping. The internal ones include FIP (Forward Inner Primer) and BIP (Backward Inner Primer). Internal primers are composed of two segments: 5'-final (F1c in FIP, B1c in BIP) and 3'-final (F2 in FIP, B2 in BIP). The 3'-terminal segment hybridizes to the complementary sequence in the template DNA (F2c, B2c) while the 5'-end segment is complementary to the region immediately after the sequence of the 3'-terminal segment of the starter in the newly formed strand (F1, B1). As a result, the resulting primary reaction product forms single-stranded loops at both ends. The F3 outer primers (Forward) and B3 (backward) primers are complementary to the DNA regions surrounding the fragment amplified by the internal primers. The external primers are shorter than internal primers, and their concentration in the reaction is lower comparing to FIP and BIP. Thus, they hybridize to the matrix more slowly than FIP and BIP. Their role is to initiate the substitution of one of the strands in a double-stranded (duplex) DNA by the daughter's strand. As a result, the DNA strands generated during the polymerization are released from the duplex and can be matrices for successive strands of progeny. The primer pairs discussed can independently promote the amplification of the target DNA region in the LAMP reaction by the corresponding polymerases. LoopF and LoopR loop starters complementary to loop regions can be additionally used to increase the speed and sensitivity of the reaction. LAMP is made at 60-65°C, and primers are designed for DNA regions that oscillate at this temperature between double-stranded and single-stranded. Therefore, the primers can anneal to the target strand without a thermal double-stranded DNA denaturation step. The formation of the daughter's strands is catalyzed by the DNA-dependent polymerase, with strand-displacement activity. The enzyme initiates the elongation process by attaching the nucleotide to the 3-terminal hydroxyl group of the primer and then adds further nucleotides thereby extending (polymerizing) the baby strand. Each introduced nucleotide is complementary to the nucleotide in the template DNA strand. The LAMP method allows fast and extremely efficient DNA amplification. The reaction under optimal conditions takes place within 5-30 minutes. In the first phase of the

amplification, the base product is created, terminated on both sides with loops. Then, longer fragments of double-stranded DNA are formed, consisting of an increasing number of repetitions of the basic product. As with PCR, the LAMP amplification product can be detected by agarose gel electrophoresis and gel staining with a dye solution that strongly fluoresces after binding to DNA. Due to the LAMP mechanism, in the gel-stained gel, there is a ladder of strips formed by differing sizes of repeats of the basic product. Because the amplified DNA during application to the gel often pollutes the laboratories, detection methods are recommended in which no reaction tubes need to be opened. The concentration of DNA produced during the LAMP reaction is so high that pyrophosphate, together with magnesium, forms the insoluble salt coming from the nucleotides incorporated into the progeny nucleotide strand. It is visible to the naked eye as turbidity in positive tests (Mori et al. 2001). As the concentration of the amplification product increases during the reaction, the level of magnesium in the solution decreases, which was used to develop a fluorescent LAMP test in which a fluorescent dye, calcein and manganese ions, which quench calcein fluorescence, are added to the reaction. Manganese, like magnesium, forms an insoluble salt with pyrophosphate during the amplification. As a result, the level of calcein fluorescence increases (Tomita et al. 2008), and can be monitored in real time. A less expensive variant of the test consists in visual observation of green fluoresce of calcein in positive tests induced by blue light after completion of the LAMP reaction. In the visible light, positive tests also show a green color, but the difference in the color of the negative and positive samples is not unambiguous. Another popular method of detection is the addition of hydroxy naphthol blue (HNB) to the tests, which together with the decrease in the concentration of magnesium ions changes from a violet or dark blue to a light blue (Goto et al. 2009). Malachite green (Lucchi et al. 2016), crystal violet (Miyamoto et al. 2015) and pH indicator dyes (Tanner et al. 2015) were also used to detect LAMP products visually.

PURPOSE OF RESEARCH

The research aimed to develop a molecular diagnostic method for the identification of potato viral pathogens, which will be widely used in large-scale tests. The model organism in the undertaken studies was the most important viral pathogen of the potato, which is currently the virus Y (PVY). The immunological and molecular diagnosis of PVY is much more difficult compared to other viruses, mostly because PVY is a more exact "quasi-species" than most viruses. The high frequency of point mutations and frequent recombination events between

genomes of different strains result in a fast rate of evolution of PVY (Glais et al 2002, Nie i Singh 2003ab, Lorenzen et al 2006ab, Schubert et al 2007, Ogawa et al 2008, Hu et al. 2009a, Karasev et al. 2011, Cuevas et al. 2012, Visser et al. 2012). Currently, at least nine strains are known, including "old" non-recombinant strains (PVY^{C}, PVY^{O}, PVY^{N}) and new recombination strains (PVY^{E}, PVY^{Z}, PVY^{NTN}, $PVY^{N:O}$, PVY^{N-Wi}, PVY^{NA-N} and PVY-NE11), differing biologically, serologically and molecularly (Karasev and Gray 2013). In the study of changes in the population structure of the Y virus in Poland, there was a change in the frequency of occurrence of strains. In the 80s of the 20th century, the PVY^{O} strain accounted for 90% of the population, which changed for six consecutive years, the PVY^{N-Wi} strain was detected in 90% of cases. The PVY^{NTN} strain was identified for the first time in Poland in 1994, and the frequency of its occurrence increased systematically (Zimnoch-Guzowska et al. 2013). Currently, the largest share in the population has two strains of the virus Y - NTN and N-Wi (Yin et al. 2012). PVY^{N-Wi} isolates can cause mild, difficult to detect symptoms on popular potato varieties or are asymptomatic.

In contrast, virus isolates from the PVY^{NTN} group often cause necrosis on tubers, contributing to the disqualification of the crop. On susceptible potato varieties, necrosis and tuber deformation may also be caused by other PVY strains under appropriate conditions. Most PVY strains inducing tuber necrosis are recognized by monoclonal antibodies specifically reactive with the PVY^{N} coat protein. Strains that do not cause or induce tuber symptoms are recognized by monoclonal antibodies specific for PVY^{O}. Therefore, in the diagnosis of the virus, it is crucial not only to detect it but also to determine its serotype.

RESULTS

As part of the presented scientific achievement, research was undertaken to develop an effective and inexpensive method of RNA isolation from potato tubers on silica (**H1**) and its simplification through the use of magnetic particles instead of silica (**H4**), development and validation of primers to identify PVY by RT-test PCR (**H1**), adaptation of the isothermal RT-LAMP test to detect PVY (**H2**), development of a repeatable and easy-to-follow procedure for PVY identification with RT-LAMP (**H3**) and development of a sensitive variant of the RT-LAMP assay for simultaneous detection and differentiation of PVY genotypes corresponding to virus serotypes O and N (**H4**). The research was carried out as part of the Biological Progress in Plant Production program, financed by the Ministry of Agriculture and Rural Development in the years 2008-2013 and 2014-2020.

Reliable detection of viruses in potato tissues by molecular methods requires obtaining high-quality RNA preparations. Potato tissues are rich in polysaccharides, polyphenols, and enzymes that catalyze redox reactions. In contact with oxygen in homogenates from potato tissues, there is an intensive release of free radicals and enzymatic and non-enzymatic oxidation of macromolecules, including RNA. Tubers are a particularly tricky source of RNA. The distribution of the virus in the tuber tissue is not even (Hill and Jackson 1984; Tamada and Harrison 1980). Therefore, some of the tuber meristems ("eyes") may be free from the virus. As a result, samples taken from these eyes will be considered healthy, regardless of whether the test will be performed by ELISA or RT-PCR. This problem can be solved using a quasi-stub portion of the tuber for virus detection because it is the place where the virus particles accumulate when flowing from the aboveground part to the tubers. Also, our studies showed that the concentration of PVY and PLRV in the post-quadruple tissue was higher than in other parts of the tuber irrespective of whether the tubers were examined immediately after harvest or after storage for several months (Treder et al. 2009b).

Due to the high content of starch in tubers the isolation of high quality RNA is not easy. The vast majority of commercial RNA isolation kits do not provide good quality RNA preparations from this organ (Treder, unpublished results). Good RNA preparations can be obtained using manual methods, including a complicated extraction procedure and DNAse digestion (Singh et al. 2002) or using reagents containing guanidine and phenol salts (Chomczyński and Sacchi 1987), commercially available, e.g., as Trizol (Invitrogen). However, it is difficult to use such methods for large-scale tests. Therefore, work was undertaken to adapt the method using silica and guanidine salts to isolate DNA / RNA from potato leaves (Boom et al. 1990, Malinowski 1997) in the PVY (H1) detection procedure. For this work, a pair of primers were designed, recognizing in silico all the PVY genotypes available in the NCBI gene bank. It has been shown that the choice of the RNA isolation method has a significant impact on the sensitivity of PVY detection. The isolation method on silica facilitated purification of RNA with a better quality to cost ratio comparing to commercial kits. The sensitivity of PVY detection by RT-PCR using RNA isolated on the silica (as a template) was higher than the sensitivity of PVY detection in RNA preparations isolated with the majority of tested commercial kits. At the same time, the workload and time of RNA isolation on silica and commercial kits were comparable (H1). Through the same method, proper quality RNA preparations were also obtained from potato bulbs (H4). The method allowed detection of PVY in tubers by RT-LAMP isothermal test and by real-time RT-PCR with the same sensitivity in

laboratory conditions (H4). The mechanism of nucleic acid binding to silica in the presence of guanidine salts consists of the formation of hydrogen bonds between the hydroxyl groups located on the silica surface and denatured nucleic acid. Hydroxyl groups are also found on the surface of magnetic particles produced by the precipitation of iron salts. To further optimize the PVY detection procedure, these magnetic particles were used to isolate RNA from potato tissues, replacing them with silica and retaining the buffer system used in the silica method. At the same time, the centrifugation steps were replaced by the collection of magnetic particles on a magnetic stand. Also, in a shortened version of RNA isolation on magnetic particles, several washes of microspheres recommended in the original silica procedure were omitted with no negative impact on the sensitivity of PVY detection. The isolation time in the short magnetic method was about 20 minutes compared to 60 minutes in the silica method (H4).

Further work carried out as part of the scientific achievement focused on the development of an isothermal RT-LAMP test for sensitive detection of PVY. Due to the lower sensitivity to amplification inhibitors (Kaneko et al. 2007), the LAMP test is particularly useful for the detection of RNA in potato tissues. Polymerases used in the LAMP can reverse transcription, so RNA detection does not require the addition of reverse transcriptase, but the presence of this enzyme in the reaction mixture shortens the time and has a positive effect on the sensitivity of the detection. This type of RT-LAMP test was developed for PSTVd (Lenarčič et al. 2012), potato leaf-rolling virus (PLRV) (Almasi et al. 2012) and potato virus Y (PVY) (No 2005, Almasi et al. 2013, Hasiów-Jaroszewska and others 2015, H2-H4). The test developed for PSTVd had ten-fold higher detection sensitivity compared to the RT-PCR and allowed detection of PSTVd in both leaves and tubers. The duration of the test was from 15 to 25 minutes, depending on the concentration of the viroid in the trials (Lenarčič et al 2012). Almasi et al. (2012) developed an immunosorbent assay variant (IC-RT-LAMP) in which PLRV particles were bound to the surface of tubes coated with virus-specific antibodies and after LAS flushing in the same tubes LAMP reaction was performed. The authors showed that the positive sample could be monitored both by the turbidity of the samples and by changing the color of different dyes (Almasi et al. 2012). The RT-LAMP variant based on the opacity of samples was also developed for PVY (No 2005). Hasiów-Jaroszewska et al. (2015) developed a visual RT-LAMP test in which the reaction product was detected by adding SybrGreen dye to the samples after amplification. The assay, which could be performed in a water bath or a thermal block, enabled the detection of various PVY strains with sensitivity higher than RT-PCR (Hasiów-Jaroszewska et al. 2015).

As part of the scientific achievement, an RT-LAMP fluorescence RT-LAMP test was developed allowing for quantitative PVY detection within 60-80 minutes (taking into account the RNA isolation time) with sensitivity ten times higher than RT-PCR and 1000-fold higher than DAS-ELISA and strip tests (H2). The effect of the continuation of the research was the development of Y4 primers, which in comparison to the method developed in H3 allowed to increase the real-time RT-LAMP sensitivity by order of magnitude (from 0.1 to 0.01 pg of total RNA in the reaction). Designed primers also made it possible to differentiate PVY isolates regarding coat protein genotypes with N or O serotype (H4). The effectiveness of detection and differentiation of PVY with these primers has been verified on several of PVY isolates from Europe, as well as, by establishing cooperation with Dr. Aurelia Rakotondrafara (Wisconsin University, Plant Pathology Department, Madison, USA), on PVY isolates from North America. The isolates tested represented different genetic strains of PVY. The complete PVY detection procedure shown in the H4 publication included rapid (20 minutes) RNA isolation from potato tissues using magnetic nanoparticles and the RT-LAMP test, allowing detection of PVY depending on its concentration in the sample over 8-20 minutes (H4). The RT-LAMP in laboratory tests has been shown to have the same sensitivity as real-time RT-PCR. In real-time field RT-PCR studies, significantly fewer PVY infected tubers were detected than by RT-LAMP. At the same time, the effectiveness of RT-LAMP in this experiment was comparable to the efficacy of loop tests (H4). The procedure for detecting PVY using the Y4 primers and the real-time RT-LAMP test has been refined and published as a detailed protocol. In the same publication the conditions for detecting PVY using a colorimetric RT-LAMP test, using a change in the color of hydroxy naphthol blue, which allows fast, visual detection of PVY, e.g., under field conditions (H3), are given.

SUMMARY

The four publications constituting my post-doctoral dissertation include the results of research on the molecular diagnostics of the potato virus Y, with simultaneous differentiation into genotypes corresponding to the serotypes O and N of the virus. The results of the research are:

- development of an effective and inexpensive method of RNA isolation from potato tubers on silica and its simplification and increase of sensitivity in comparison to commercial tests by the use of magnetic particles in place of silica (**H1, H4**),
- development and validation of primers for PVY detection using RT-PCR (**H1**),
- adaptation of the isothermal RT-LAMP test to detect PVY (**H2**),

- development of a repeatable and easy to perform PVY detection procedure using RT-LAMP (**H3**),
- development of the RT-LAMP test with increased sensitivity for simultaneous detection and differentiation of PVY genotypes corresponding to the O and N serotypes of the virus (**H4**).

The results of the research presented in this work have cognitive and practical significance. The developed methods may be useful for diagnosing potato Y viruses in seed materials.

Literature

1. Almasi M.A., Moradi A., Nasiri J., Karami S., Nasiri M. 2012. Assessment of performance ability of three diagnostic methods for detection of potato leafroll virus (PLRV) using different visualizing systems. Appl. Biochem. Biotechnol., 168: 770-84.
2. Almasi M.A., Dehabadi S.H. 2013. Colorimetric immunocapture reverse transcription Loop-mediated isothermal AmPlification assay for rapid detection of the Potato virus Y. J. Plant. Pathol. Microbiol., 4: 188-193.
3. Barker H., Webster K.D., Reavy B. 1993. Detection of potato virus Y in potato tubers: a comparison of polymerase chain reaction and enzyme-linked immunosorbent assay. Potato Res., 36: 13–20.
4. Bolotova Y. V., Karasev A. V., McIntosh C.S. 2009. Statistical analysis of the laboratory methods used to detect potato virus Y. Am. J. Pot. Res. 86: 265-271.
5. Boonham N., Glover R., Tomlinson J., Mumford R. 2008. Exploiting generic platform technologies for the detection and identification of plant pathogens. Eur. J. Plant Pathol., 121: 355-363.
6. Boom R., Sol C.J., Salimans M.M., Jansen C.L., Wertheim-van Dillen P.M., van der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. Journal Clinical Microbiology 28: 495–503.
7. Chomczynski P., Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162: 156–159.
8. Clark M.F., Adams A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475 483.
9. Crosslin J., Hamlin L. 2011. Standardized RT-PCR conditions for detection and identification of eleven viruses of potato and potato spindle tuber viroid. Am. J. Pot. Res. 88: 333-338.
10. Cuevas J.M., Delaunay A., Visser J.C., Bellstedt D.U., Jacquot E., Elena S.F. 2012. Phylogeography and molecular evolution of Potato virus Y. PLoS ONE 7(5): e37853., doi: 10.1371/journal.pone.0037853.
11. Devaux A, Kromann P., Ortiz O. 2014. Potatoes for sustainable global food security. Potato Res., 57:185–199.
12. Flegg C. L., Clark M. F. 1979. The detection of Apple chlorotic leaf spot virus by a modified procedure of enzyme-linked immunosorbent assy. Ann. Appl. Biol. 91: 61-65.
13. Fox A., Evans F., Browning I. 2005. Direct tuber testing for potato Y potyvirus by real-time RT-PCR and ELISA: reliable options for post-harvest testing? Bull. OEPP., 35: 93 97.
14. Gadkar V.J., Goldfarb D.M., Gantt S., Tilley P.A.G. 2018. Real-time detection and monitoring of loop mediated amplification (LAMP) reaction using self-quenching and de-quenching fluorogenic probes. Sci. Rep., 3;8(1):5548. doi: 10.1038/s41598-018-23930-1.
15. Glais L., Tribodet M., Kerlan C., Rheu R. 2002. Genomic variability in Potato potyvirus Y (PVY): evidence that PVY(N)W and PVY(NTN) variants are single to multiple recombinants between PVY(O) and PVY(N) isolates. Arch. Virol., 147: 363–378.
16. Goto M., Honda E., Ogura A., Nomoto A., Hanaki K. 2009. Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxynaphthol blue. Biotechniques 46:167-172.

17. Hasiów-Jaroszewska B, Stachecka J, Minicka J, Sowiński M, Borodynko N. 2015. Variability of Potato virus Y in tomato crops in Poland and development of a reverse-transcription loop-mediated isothermal amplification method for virus detection. Phytopathology, 105:1270-1276.

18. Hill S.A., Jackson E.A. 1984. An investigation of the reliability of ELISA as a practical test for detecting potato leaf roll virus and potato virus Y in tubers. Plant Pathology 33: 21 26.

19. Hu, X., Karasev, A. V., Brown, C. J., and Lorenzen, J. H. 2009a. Sequence characteristics of Potato virus Y recombinants. J. Gen. Virol. 90:3033-3041.

20. Hühnlein, A., Drechsler, N., Steinbach, P., Thieme, P., Schubert, J. 2013. Comparison of three methods for the detection of potato virus Y in seed potato certification. J. Plant Dis. Protect., 120: 57-69.

21. Kaneko H., Kawana T., Fukushima E., Suzutani T. 2007. Tolerance of loop-mediated-isothermal amplification to a culture medium and biological substances. J. Biochem. Biophys. Methods, 70: 499-501.

22. Karasev A.V., Gray S.M. 2013. Continuous and emerging challenges of Potato virus Y in potato. Annu. Rev. Phytopathol., 51: 571–586.

23. Karasev A.V., Hu X., Brown C.J., Kerlan C., Nikolaeva O.V., Crosslin J., Gray S.M. 2011. Genetic diversity of the ordinary strain of Potato virus Y (PVY) and origin of recombinant PVY strains. Phytopathology, 101: 778–785.

24. Kubota R., Alvarez A.M., Su W., Jenkins D.M. 2011. FRET-based assimilating probe for sequence-specific real-time monitoring of loop-mediated isothermal amplification (LAMP). Biol. Eng. Trans., 4: 81–100.

25. Lenarčič R, Morisset D., Mehle N., Ravnikar M. 2012. Fast real-time detection of potato spindle tuber viroid by RT-LAMP. Plant Pathology 62: 1147-1156.

26. Lorenzen J.H., Meacham T., Berger P.H., Shiel P.J., Crosslin J.M., Hamm P.B., Kopp K. 2006a. Whole genome characterization of Potato virus Y isolates collected in the western USA and their comparison to isolates from Europe and Canada. Arch. Virol., 151: 1055–1074.

27. Lorenzen J.H., Piche L.M., Gudmestad N.C., Meacham T., Schiel P. 2006b. A multiplex PCR assay to characterize Potato virus Y isolates and identify strain mixtures. Plant Dis. 90: 935–940.

28. Lucchi N.W., Ljolje D., Silva-Flannery L., Udhayakumar V. 2016. Use of malachite green-loop mediated isothermal amplification for detection of Plasmodium spp. parasites. PLoS One. 2016 Mar 11;11(3):e0151437.

29. Malinowski T. 1997. Silica capture-reverse transcription-polymerase chain reaction (SC-RT-PCR): application for the detection of several plant viruses. Diagnosis and Identification of Plant Pathogens 11: 445-448.

30. Miyamoto S., Sano S., Takahashi K., Jikihara T. 2015. Method for colorimetric detection of double-stranded nucleic acid using leuco triphenylmethane dyes. Anal. Biochem., 473: 28-33.

31. Mori Y., Nagamine K., Tomita N., Notomi T. 2001. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. BiochemBiophys Res Commun., 289: 150-154.

32. Mumford R. A., Boonham N., Tomlinson J., Barker I. 2006. Advances in molecular phytodiagnostics – new solutions for old problems. – Eur. J. Plant Pathol., 116: 1-19.

33. Nie X. 2005. Reverse transcription loop-mediated isothermal amplification of DNA for detection of Potato Virus Y. Plant Dis., 89: 605–610.

34. Nie X, Singh R.P. 2003a. Evolution of North American PVY NTN strain Tu660 from local PVY N by mutation rather than recombination. Virus Genes 26:39–47.

35. Nie X, Singh R.P. 2003b. Specific differentiation of recombinant PVYN:O and PVYNTN isolates by multiplex RT-PCR. J. Virol. Methods, 113: 69–77.

36. Notomi T., Mori Y., Tomita N., Kanda H. 2015. Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. J. Microbiol., 53: 1–5.

37. Ogawa T., Tomitaka Y., Nakagawa A., Ohshima K. 2008. Genetic structure of a population of Potato virus Y inducing potato tuber necrotic ring spot disease in Japan; comparison with North American and European populations. Virus Res., 131: 199-212.

38. Oscorbin I.P., Belousova E.A., Zakabunin A.I., Boyarskikh U.A., Filipenko M.L. 2016. Comparison of fluorescent intercalating dyes for quantitative loop-mediated isothermal amplification (qLAMP). Biotechniques, 61 :20-25.

39. Schubert J., Fomitcheva V., Sztangret-Wiśniewska J. 2007. Differentiation of Potato virus Y strains using improved sets of diagnostic PCR-primers. J. Virol. Methods, 140: 66–74.

40. Singh R. P. 1999a. A solvent-free, rapid and simple virus RNA release method for potato leafroll virus detection in aphids and plants by reverse transcription polymerase chain reaction. J. Virol. Meth., 83: 27-33.

41. Singh R. P. 1999b. Development of the molecular methods for potato virus and viroid detection and prevention. Genome 42: 592-604.

42. Singh R.P., Nie X., Singh M., Coffin R., Duplessis P. 2002. Sodium sulphite inhibition of potato and cherry polyphenolics in nucleic acid extraction for virus detection by RT-PCR. J. Virol. Methods, 99: 123– 131.

43. Spiegel S., Martin R. R. 1993. Improved detection of potato leafroll virus in dormant potato tubers and microtubers by the polymerase chain reaction and ELISA. Ann. Appl. Biol. 122: 493 500.

44. Tanner N.A., Zhang Y., Evans T.C. 2012. Simultaneous multiple target detection in real-time loop-mediated isothermal amplification. Biotechniques, 53: 81–89.

45. Tanner N.A., Zhang Y., Evans T.C. 2015. Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes. Biotechniques, 58: 59-68.

46. Tamada T., Harrison B.D. 1980. Factors affecting the detection of potato leafroll virus in potato foliage by enzyme-linked immunosorbent assay. Ann. Appl. Biol. 95: 209 219.

47. Tamada T., Harrison B.D. 1980. Application of enzyme-linked immunosorbent assay to the detection of potato leaf roll virus in potato tubers. Ann. Appl. Biol. 96: 67 78.

48. Treder K., Pilecki T., Łycuś P. 2009a. Wykrywanie wirusów ziemniaczanych bezpośrednio w ekstraktach z bulw – porównanie testu ELISA z immuno-captured RT PCR. Prog. Plant Prot./Post. Ochr. Roślin, 49: 740-745.

49. Treder K., Przewodowski W., Barnyk A. 2009b. Factors influencing detection of Potato leafroll virus and Potato virus Y in potato tuber extracts. Plant Breeding and Seed Science 59: 65-74.

50. Tomita N., Mori Y., Kanda H., Notomi T. 2008. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. Nat. Protoc., 3: 877-882.

51. Visser J.C., Bellstedt D.U., Pirie M.D. 2012. The recent recombinant evolution of a major crop pathogen, Potato virus Y. PLoS ONE: 7(11): e50631. doi:10.1371/journal.pone.0050631.

52. Van den Heuvel J. F. J. M., Peters D. 1989. Improved detection of potato leafroll virus in plant material and in aphids. Phytopathology 79: 963-967.

53. Yin Z., Chrzanowska M., Michalak K., Zagórska H., Zimnoch-Guzowska E. 2012. Recombinations of PVY strains predominate among isolates from potato crop in Poland. J. Plant Prot. Res., 52: 214-219.

54. Zacharzewska B., Treder K. 2014. Test ELISA jego modyfikacje. Ziemn. Pol. 1: 14-16.

55. Zimnoch-Guzowska E., Yin Z., Chrzanowska M., Flis B. 2013. Sources and effectiveness of potato PVY resistance in IHAR's breeding research. Am. J. Potato Res., 90: 21-27..

5. DISCUSSION OF THE REMAINING SCIENTIFIC ACHIEVEMENTS.

In the years 1994-1995 I participated, as a graduate student, in research on arylsulfatases conducted by Dr. Antoni Leźnicki in the Department of Biochemistry, Faculty of Biology and Earth Sciences, UMK in Toruń. The supervisor of my master's thesis was Prof. Jadwiga Gniot-Szulżycka, but the direct tutor was Dr. Antoni Leźnicki. As part of my master's thesis, I developed a method for the isolation of B-type arylsulfatase from rat organs using ion exchange chromatography, affinity chromatography, and gel filtration. I characterized the obtained preparations regarding their biochemical properties using polyacrylamide electrophoresis under reducing and denaturing conditions, native electrophoresis, chromatographic focusing, and isoelectric focusing. The view that the heterogeneity of the molecular forms of arylsulfatase B corresponds to the different content of sialic acid or phosphorylated mannose forms present in the sugar part of this glycoprotein. I demonstrated that this hypothesis is false because the enzyme treatment with glycosidases removing the sugar residues mentioned above did not influence the number of isozymes observed after isoelectric focusing. I described the results in my master's thesis, which I defended in July 1995.

In the period from September 1, 1995, to January 31, 1996, I worked as a trainee headed by Dr. hab. Jerzy Lewosz, Department of Molecular Diagnostics and Biochemistry (ZDMiB) in Bonin, which is part of the Potato Institute (and from October 1997 until the present Branch in Bonin of the Institute of Plant Breeding and Acclimatization - PIB). During this period, I learned how to infect plants with viruses, procedures for isolation of potato viruses from infected plants, methods for assessing the purity of virus preparations and using the obtained preparations for immunizing rabbits to produce antibodies specific for the virus. I have also learned how to collect blood collection, extract serum, isolate the antibodies (Immunoglobulins G, IgG), conjugate IgG with the enzyme - alkaline phosphatase, determine the titer and specificity of serum or antibodies. My IgGs specific to the potato leafroll virus (abbreviation: PLRV) were sent to the Laboratory of Serology in Gdańsk (Institute of Potatoes), a part of ZDMiB headed by Prof. Zbigniew Mierzwa, where they produced commercial PLRV detection kits using the DAS-ELISA test. Characterizing the obtained PLRV preparations using electrophoresis under denaturing conditions and western blotting technique, I found that in pure virus preparations, in addition to the expected proteins of 23 kDa (coat protein) and 56 kDa (so-called readthrough protein), 17 kDa protein was present, that strongly reacted with PLRV specific antibodies [1]. The results of these studies were presented at the Symposium of the Viral Section of the Polish Phytopathological Society by Dr. hab. Jerzy Lewosz.

CONFERENCE

1. Lewosz J., Treder K. 1996. Immunoreaktywność białek cząsteczki wirusa liściozwoju ziemniaka. Sympozjum Sekcji wirusologicznej P.T. Fitopatologicznego, Warszawa SGGW, 26-27. 09. 1996. Talk co-author.

From February 1, 1996, to October 31, 1996, I worked as an assistant in the Department of Microbiology at the Faculty of Biology and Earth Sciences, Nicolaus Copernicus University in Toruń. The head of the Department was Prof. Edmund Strzelczyk. During this period, I learned the methods of isolating bacteria from the environment to pure cultures, determining the concentration of bacteria in suspensions, biological and analytical methods for determining the level of vitamins and the substances beneficial for plant development produced by symbiotic microorganisms. In connection with the problems with the identification of some of the bacterial species tested, I proposed that the microbiological identification could be supplemented with protein profiles made with polyacrylamide gel electrophoresis. Initial research was done in cooperation with Dr. Henryk Różycki. Their results confirmed the usefulness of protein profiles as an additional criterion in the identification of bacteria. Due to the change of work, I did not participate in the continuation of this research.

In November 1996, I again joined the ZDMiB (Institute of Potato in Bonin) as a technologist. During this period, I continued production of rabbit antibodies to detect PLRV by DAS-ELISA. At the same time, I conducted research that became the basis of my doctoral dissertation and will be briefly discussed below.

An important issue in my doctoral thesis was the research on the reason for the appearance of an additional protein of 17 kDa in PLRV preparations [1, 2]. Using several protease inhibitors, I have shown that this protein can be produced by proteolytic modification of proteins present in the virus mantle by a plant protease inhibited by pepstatin - an aspartic protease inhibitor. Part of this work I did at the Scottish Crop Research Institute, in a virological laboratory headed by Prof. Michael Mayo. By using monoclonal antibodies specific for different regions of the p90 protein of the virus, I showed that additional proteins present in PLRV preparations originated from proteolytic modification of the p90 protein, which is a p56 protein precursor (the so-called "readthrough" protein). This protein is, next to the main coat protein (p23), a component of the virus coat responsible for the transmission of the virus by aphids. Using as a model system an extract of virus particles, aspartic protease - cathepsin D and its inhibitor - pepstatin, I showed that inhibition of protease activity changed the immunological properties of virus particles, which was less well recognized by polyclonal

antibodies. I also found that in the presence of pepstatin the yield of PLRV purification was more than three-fold higher. However, the pure virus obtained in this way was not transmitted by aphids, which proved the significant effect of aspartic protease on the biological properties of PLRV [4, 5, 7, 9].

As part of the work on improving the PLRV diagnostics, I tried to optimize the cocktail-ELISA test for detecting PLRV in leaves and adaptation to detect this virus in potato tubers. I found a higher sensitivity of this method compared to DAS ELISA in PLRV detection in tuber and leaf extracts. The sensitivity of the test additionally increased the use of a reagent system amplifying the signal generated by alkaline phosphatase. The efficacy of the cocktail-ELISA test in assessing tuber infestation was comparable to the effectiveness of eye tests [6, 11].

In order to improve the production of specific antibodies, I optimized the individual stages of this process, including both the method of obtaining a pure virus preparation and the procedure of immunizing rabbits and purifying antibodies. To improve the production of antigen for immunization of rabbits, I got the cDNA of the coat protein of the virus by RT-PCR and introduced them to the expression plasmid, which I then transformed E. coli [3, 10]. After selection of the colonies maintaining the stably introduced construct, I induced the expression of the PLRV coat protein in the suspension culture of the bacteria with isopropyl-β-D-1-thiogalactopyranoside. I have developed a procedure for the isolation of recombinant coat protein using immunochromatography. For this purpose, I activated polyacrylamide microspheres (P-100 gel, BioRad) using glutaraldehyde and bound antibodies specific for PLRV. After filling the chromatography column, the gel I used was used for one-step purification of the recombinant coat protein from the bacterial lysate. I immunized the recombinant protein with rabbits, obtaining antibodies with high titer and excellent specificity [8, 10].

The results of studies on PLRV proteolytic modification and its influence on virus properties, on the optimization of PLRV detection in leaves and tubers, and on the cloning of PLRV coat protein and its use for antibody production are detailed in my doctoral dissertation, which I defended in 2002. This work has been honored by the IHAR Scientific Council, and a year later I received the Prime Minister's award for doctoral dissertations. The results were also presented at scientific conferences. The research and training discussed above in the laboratory of Prof. Michael Mayo were funded by a two-year Ph.D. grant, awarded by the KBN to my promoter – Dr. hab. Jerzy Lewosz in 2000.

CONFERENCES

2. Treder K., Lewosz J., Sekrecka D. 1998. Isolation of 17, 23, 56 kD proteins from potato leaf roll virus particles and their employment as antigenes. [In:] The 10th EAPR Virology Section Meeting, Baden, Austria, 5-10th July, abstracts: 69-70. Talk co-author.
3. Treder K., Lewosz J. 1999. Zastosowanie techniki PCR do klonowania genu białka płaszcza wirusa liściozwoju ziemniaka (PLRV). [W:] I Krajowy Kongres Biotechnologii, Wrocław, 20-25 września, abstrakty: 20. Poster.
4. Treder K., Lewosz J. 1999. Modification of the properties of PLRV particles by endogenous plant proteinases. [In:] 14th Triennial Conference of the European Association for Potato Research, Sorrento, Italy, 2-7 May, abstracts.: 557-558. Talk.
5. Treder K., Lewosz J. 2000. Wpływ proteaz na wykrywalność wirusa liściozwoju ziemniaka w soku z bulw ziemniaka. XXXVI Zjazd PTBioch., Poznań. Poster.
6. Treder K., Lewosz J. 2000. Detection of PLRV and PVY in dormant potato tubers by cocktail and amplified ELISA assay. [W:] Report of the joint meeting of the Potato Section of EUCARPIA and the Section Breeding and varietal assessment of the EAPR, 3-7 July, 2008, Warsaw, Poland. Potato Research, 43: 424. Poster.
7. Treder K., Lewosz J. 2001. Wpływ proteaz asparaginowych na własności wirusa liściozwoju ziemniaka (PLRV). [W:] XXXVII Zjazd PTBioch., Toruń 10-14.09., Streszczenia: 337. Poster.
8. Treder K., Lewosz J. 2001. Production of antibodies against potato leafroll virus using coat protein expressed in *Escherichia coli* as an antigen. The 11th EAPR Virology Section Meeting, Havlickov Brod, Trest, Czech Republic, 7-13 October. Talk co-author.

CHAPTERS

9. Treder K., Lewosz J. 1999. Isolation of 17, 23 and 56 KD proteins from potato leafroll particles and some evidence for their proteolytic modification. [In:] Proceedings of the 10th EAPR Virology Section Meeting, Baden, Austria, 5-10th July 1998, pp 175-180.
10. Treder K., Lewosz J. 2001. Production of antibodies against potato leafroll virus using coat protein as an antigen. [In:] Proceedings of the 11th EAPR Virology Section Meeting, Havlickuv Brod – Trest, Czech Republic, 7-13th October 2001, pp 62-64.

PUBLICATION

11. Treder K., Lewosz J. 2000. Zastosowanie metod molekularnych do wykrywania wirusa liściozwoju ziemniaka (PLRV). Prog. Plant Prot./Post. Ochr. Roślin, 40: 177-187.

PROJECT

- 2000-2001. P06A 031 18. Ph. D. research project funded by KBN. "Wpływ proteolitycznej modyfikacji cząstek wirusa liściozwoju ziemniaka w roślinie na jego własności i wykrywalność". Main contractor.

In addition to the works discussed above, which were included in my doctoral thesis, I took an active part in other studies carried out by Dr. hab. Jerzy Lewosz. Some research topics initiated by him are conducted in PDMiB to this day and will be discussed below.

At the end of the nineties of the last century, Dr. hab. Jerzy Lewosz, researching *Phytophthora infestans* - the perpetrator of the potato blight, observed that some sporangia of this pathogen are breaking down releasing bacterial cells, which he identified as *Pseudomonas fluorescens*. Colonies of this bacterium and fluid from suspension cultures strongly inhibited the growth of many fungal and bacterial potato pathogens [13]. My contribution to the continuation of this research was to purify the antibiotics produced by *P. fluorescens* using

various high-performance liquid chromatography (HPLC) variants. Thanks to the kindness of Prof. Goran Sandberg (Plant Science Center, Umea, Sweden), the identification of antibiotics was made by his Ph. D. student - Mariusz Kowalczyk, whom I helped in analyzing the composition of the preparations I prepared using gas chromatography and mass spectrometry. In the preparations, 1-carboxyfenazine and hemiphiocyanine were responsible for antibiotic activity [12, 14]. The results of these studies were presented at two international and one national conference.

CONFERENCES

12. Lewosz J., Kowalczyk M., Treder K., Hołubowska M., 2001. Purification and properties of antibiotic substances produced by fluorescent strain of *Pseudomonas*. [In:] Pathology Section Meeting of EAPR, Poznań, 10-15 07. IHAR Oddz. Bonin: 43-44. Talk.
13. Lewosz J., Hołubowska M., Treder K. 2001. Antibiosis/parasitism performed by fluorescent strain of *Pseudomonas* isolated from sporangia of *Phytophthora infestans*. Konferencja IHAR-CEEM Warszawa, 9-14. 06. Poster.
14. Lewosz J., Hołubowska M., Kowalczyk M., Treder K. 2001. Charakterystyka antybiotyków wytwarzanych przez szczep *Pseudomonas* antagonistyczny wobec patogenów roślin. [W:] XXXVII Zjazd PTBioch., Toruń 10-14 09. Streszczenia: 173. Poster.

I also took an active part in the work that Dr. hab. Jerzy Lewosz conducted on the differentiation and identification of potato cultivars using biochemical methods and molecular biology techniques. Already in the seventies of the last century, he observed that the tubers of various potato varieties have a unique composition of proteins. Therefore, individual cultivars can be identified using native electrophoresis. He also found that one of the main tuber protein fractions - protease inhibitors - can be used to differentiate cultivars. My contribution to the continuation of this part of the study was the differentiation of approximately one hundred potato varieties based on the electrophoretic separation of trypsin inhibitors isolated by affinity chromatography from tubers of the tested cultivars [15, 16, 18]. The same variations were also differentiated using the SSR-PCR (Simple Sequence Repeat PCR) technique using primers with AC and AG repeats. My role in this part of the research was to optimize DNA isolation from potato tissues and PCR conditions [15]. I continued this research direction in later years. I streamlined the differentiation of potato cultivars by native electrophoresis of tuber proteins and adapted the ISAP-PCR (Inter SINE Amplified Polymorphism) using the Short Interspersed Nuclear Elements (SINE) polymorphism to differentiate potatoes [17, 19].

CONFERENCES

15. Pilecka A., Lewosz J., Treder K., 2001. Utilization of molecular markers for characterization of potato germplasm resource. [In:] Konferencja EUCARPIA, Poznań 16-20 05.2001. UAM: 47-50. Poster.

16. Przewodowski W., Lewosz J., Treder K., Pilecki T., Barnyk A. 2006, Identyfikacja odmian ziemniaka metodami biochemicznymi. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol., Kołobrzeg, 30-31.03., IHAR ZNiOZ Bonin: 96. Poster.
17. Pawłowska A., Chołuj J., Treder K. 2016. Ocena jednorodności odmian ziemniaka za pomocą elektroforezy natywnej i molekularnego testu ISAP. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 11-13.05. IHAR ZNiOZ Bonin: 47-48. Talk co-author.

PUBLICATION

18. Przewodowski W., Lewosz J., Treder K., Pilecki T., Barnyk A. 2007. Identyfikacja odmian ziemniaka metodą elektroforetyczną. Biul. IHAR 243: 151-157.
19. Treder K., Pawłowska A., Chołuj J. 2016. Identyfikacja odmian ziemniaka za pomocą elektroforezy natywnej białek i molekularnego testu ISAP. Ziemniak Polski, 4: 21-27.

In the nineties of the last century, Dr. hab. Jerzy Lewosz also worked on peroxidases from potato pulp, a waste from the starch industry. My contribution to the continuation of this research was to carry out experiments showing that the peroxidase from the pulp effectively removes several phenolic compounds from the reaction mixture. The studied phenols polymerized and the resulting polymer precipitated from the reaction in the form of a precipitate. Unfortunately, peroxidase was rapidly losing activity [20, 21]. Purification of water should be a continuous process, which can be achieved by adding fresh portions of the enzyme. Another solution is to immobilize the enzyme on the surface of the bed or microspheres. Immobilization of enzyme usually leads to its stabilization and increase durability. In the studies mentioned above, the peroxidase was isolated from the pulp with high salt concentrations, which indicates that it is strongly ion bound with the pulp. I assumed that pulp could be used to purify water, as a material with a naturally bound enzyme. I verified this hypothesis in cooperation with Dr. hab. Jarosław Tyburski (UMK in Toruń). This is one of the crucial aspects of doctoral research conducted by Katarzyna Katarzyna Kurnik (now Krzyżyńska), whose promoter is Dr. hab. Jarosław Tyburski, and I'm the auxiliary supervisor. As part of this work, it was possible to demonstrate the suitability of the pulp for removing phenols [22-24]. The biochemical characterization of peroxidase from waste [22, 23] and brewers' spent grain [24] was also made. My role in these studies was to create the concept of research, to propose the research methodology [22-24] and to identify peroxidase isoforms by native electrophoresis [22].

CONFERENCES

20. Lewosz J., K. Treder: 1999. Otrzymywanie peroksydazy z wycierki ziemniaczanej i jej wykorzystanie do usuwania związków fenolowych z wody. [W:] I Krajowy Kongres Biotechnologii, Wrocław 20-25 września, streszczenia: 264. Poster.
21. Treder K., Lewosz J.: 1999. Utilization of by-products of starch industry for biotechnological purposes. [In:] 14th Triennial Conference of the European Association for Potato Research, Sorrento, Italy, 2-7 May, abstracts.: 293-294. Talk co-author.

PUBLICATIONS

22. Kurnik K., Treder K., Skorupa-Kłaput M., Tretyn A., Tyburski J. 2015. Removal of phenol from synthetic and industrial waste water by potato pulp peroxidases. Water Air Soil Pollut., 2015; 226(8): 254.
23. Kurnik K., Treder K., Twarużek M., Grajewski A., Tretyn A., Tyburski J. 2018. Potato pulp as the peroxidase source for 2,4-dichlorophenol removal. Waste Biomass Valor., 9: 1061-1071.
24. Kurnik K., Krzyżyński M., Treder K., Tretyn A., Tyburski J. 2018. Study on utilizing solid food industry waste with brewers' spent grain and potato pulp as possible peroxidase sources. J. Food Biochem., 42: 12446.

Another issue implemented by Dr. hab. Jerzy Lewosz was research on peptides and proteins of potato tuber cell walls, which could inhibit the growth of pathogenic microorganisms. The main ingredient of the potato pulp is the cell walls of tubers. Therefore, it is possible to obtain proteins and peptides with antibiotic activity. I participated in studies aimed at confirming the presence of antibiotic peptides in cell wall extracts [28] and from extracting [25, 29] and developing a method for their purification by several chromatographic methods [25, 26]. I showed that polypeptides active against microorganisms could bind nucleic acids [28] and also had trypsin inhibitor activity [27].

CONFERENCES

25. Lewosz J., Treder K., Pilecki T. 2005. Isolation of antimicrobial peptides from potato pulp, by-product of starch industry. [In:] The 16th Triennial Conference of the EAPR, Bilbao, Spain, July 17-22, Abstracts: 894-899. Poster.
26. Barnyk A., Przewodowski W., Lewosz J., Treder K. 2008. Purification of antimicrobial peptides from potato pulp. [In:] The Congress of Biochemistry and Cell Biology, Olsztyn, Poland, September 7-11, Abstracts, Acta Biochimica Polonica, 55 (suppl.): 114. Poster.
27. Barnyk A., Przewodowski W., Treder K. 2010. Hamowanie wzrostu bakterii Cms przez ziemniaczany inhibitor trypsyny. Konf. nauk.-szkol. Darłówko, 20-21 maja. IHAR ZNiOZ Bonin: 86. Poster.

PUBLICATIONS

28. Pilecka A., Lewosz J., Treder K. 2000. Charakterystyka antymikrobiologicznej aktywności niektórych polipeptydów ścian komórkowych ziemniaka. Prog. Plant Prot./Post. Ochr. Roślin, 40: 188-194.
29. Barnyk A., Przewodowski W., Treder K. 2009. Wpływ białek ziemniaka na wzrost wybranych drobnoustrojów chorobotwórczych. Prog. Plant Prot./Post. Ochr. Roślin, 49: 890-893.

An important research direction implemented by Dr. hab. Jerzy Lewosz was work on diagnostics and methods of combating *Clavibacter sepedonicus* bacteria, the culprit of potato ring-rot disease. *C. sepedonicus* was classified in the past as a subspecies of *C. michiganensis* ssp. *sepedonicus*. It is an important potato quarantine pathogen to which the principle of zero tolerance is applied. In part of the work on the molecular diagnostics of *C. sepedonicus*, my role was to optimize the DNA isolation method for the detection of bacteria by PCR and optimization of the PCR reaction conditions. Dr. hab. Jerzy Lewosz also researched the development of new materials for concentration and immunocapturing bacterial cells by binding on various substrates, including the gold colloid he produced and on numerous types

of materials that he covered with polyaniline. My contribution consisted in comparing the sensitivity of the ELISA test performed on unmodified plates with the sensitivity of this test performed on polyaniline coated plates. An additional method used by me in these studies was the PCR test. Modification of microplates with the aniline polymer caused that the sensitivity of the ELISA test was comparable to the sensitivity of the PCR [30, 31]. One of many original ideas of Dr. hab. Jerzy Lewosz was the production of antibodies specific for *C. sepedonicus*, by using bacterial cells lacking exopolysaccharides forming the mucus part of the bacterial cell wall. Their presence in antigens used to immunize animals was the primary cause of low specificity of commercial antibodies, which mostly recognized bacterial mucus and even plant polysaccharides, resulting in a high background in immunoassays. My role in this part of the study consisted of immunizing rabbits with the preparation prepared by Dr. hab. Jerzy Lewosz, consisting of *C. sepedonicus* cells without mucus. The antibodies I obtained recognized the mucus-deficient *C. sepedonicus* cells and did not identify bacterial exopolysaccharides. The results of the works discussed above with my participation, Dr. hab. Jerzy Lewosz reported on two conferences.

CONFERENCES

30. Lewosz J., Treder K., Barnyk-Jaskot A. 2004. Detection of bacterial plant pathogens by biosensors. [In:] International Workshop "Improvement and unification of plant disease diagnostics", Skierniewice, 30 September – 1 October, Abstracts: 20. Talk co-author.
31. Lewosz J., Treder K., Pilecki T. 2005. Wykrywanie i identyfikacja mikroorganizmów z użyciem biosensorów. [W:] XLV Sesja Naukowa IOR, Poznań 3-4 luty, streszczenia: 93 94. Talk co-author.

After my doctoral thesis, I also conducted my research on the development of the method of detecting potato viruses (PLRV, PVM, PVS, PVX, and PVY) directly in tuber extracts. I adapted for this purpose the cocktail-ELISA test, previously developed by me as part of my doctoral dissertation for PLRV detection. I optimized the composition of the buffers used in the cocktail-ELISA test, complementing it among others, with hydrophilic polymers [32, 33]. In experiments, I used mainly model plants infected with the viruses mentioned above grown in the greenhouse. Examination of tubers by the cocktail-ELISA test and a grow-out test showed that both methods were equally effective in assessing the level of viral infections in the obtained tubers [32-35, 36, 37].

During this period, I continued to produce antibodies for the detection of PLRV, and after the liquidation of the Department of IHAR in Gdańsk and the Laboratory of Serology included in it, I produced together with M.Sc. Tomasz Pilecki ELISA kits for detecting the above potato viruses. The work included improving the quality of antibodies produced by developing

methods for isolating high-purity viral preparations, optimizing the composition of adjuvants added to viral antigens, methods for purifying antibodies [36], and conjugating antibodies with alkaline phosphatase. From the sale of the produced IHAR ELISA kits, it achieved a considerable financial profit, amounting to approx. PLN 300,000. PLN annually.

The research was financed from the grant KBN No. 2P06 008 26, where I was PI, and the results were presented at seven scientific conferences.

CONFERENCES

32. Treder K., Lewosz J., Pilecki T., Wróbel S. 2004. Direct tuber testing for potato viruses by cocktail-ELISA. [In:] The Third Biennial All-Iowa Virology Symposium, 22-23 October 2004, Ames, Iowa, USA, Abstract's Book: 71. Poster.
33. Treder K., Pilecki T., Lewosz J. 2004. Optimization of cocktail ELISA for detection of potato viruses in tuber sap. [In:] International Workshop "Improvement and unification of plant disease diagnostics", Skierniewice, 30 September – 1 October, Abstracts: 65. Poster.
34. Treder K., Pilecki T., Lewosz J. 2005. Detection of PVY, PVM and PLRV in the sap of the dormant tubers by cocktail ELISA. [In:] The 16th Triennial Conference of the EAPR, Bilbao, Spain, July 17-22, Abstracts: 1010 –1014. Poster.
35. Pilecki T., Lewosz J., Treder K., 2005. Optymalizacja testu koktajl-ELISA do wykrywania PLRV, PVM oraz PVY. [W:] Nasiennictwo i Ochrona Ziemniaka. Konf. nauk.-szkol., Kołobrzeg 10-11 marca 2005, IHAR ZNiOZ Bonin: 26-27. Poster.
36. Barnyk A., Lewosz J., Treder K., Pilecki T., Przewodowski W. 2006. Zastosowanie chromatografii tiofilnej w procesie produkcji zestawów diagnostycznych. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol., Kołobrzeg, 30-31.03., IHAR ZNiOZ Bonin: 95. Poster.
37. Pilecki T., Lewosz J., Treder K., Barnyk A., Przewodowski W., 2006. Wykrywanie PLRV, PVM, PVS, PVX i PVY w bulwach ziemniaka. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol., Kołobrzeg, 30-31.03., IHAR ZNiOZ Bonin: 98. Poster.
38. Pilecki T., Treder K., Lewosz J. 2007. Możliwość wykrywania wirusów PLRV, PVM i PVY w bulwach ziemniaka. [W:] 47 Sesja IOR, Poznań, 15-16 luty, streszczenia: 236. Poster.

PROJECT

- 2004-2006. 2P06 008 26. Projekt badawczy finansowany przez KBN pt.: "Opracowanie procedur wykrywania wirusów PLRV, PVY, PVY, PVM, PVS, PVX, bezpośrednio w bulwach ziemniaka. Principal Investigator.

From the end of October 2004 to the end of December 2007 and from November 2010 to July 2012, I did postdoctoral internships in a laboratory headed by prof. Wyatt Allen Miller (Plant Pathology Department, Iowa State University, Ames, Iowa, USA). My involvement in this team's research focused mainly on studying the role of the eukaryotic translational factor 4F (eIF4F) and its eIF*iso*4F isoforms in the translation of viral proteins. In plants, the eIF4F factor is composed of a smaller eIF4E subunit and a large eIF4G protein, and eIF4*iso*4F from isoforms of both of these proteins.

The vast majority of eukaryotic messenger RNAs have both a cap, i.e., 7-methylguanosine attached by a 5 ', 5'-triphosphate bond to the 5' end of the mRNA as well as a poly (A) n chain at its 3 'end. Cap is specifically recognized by the eukaryotic translation factor eIF4E, while to

poly(A)n binds the PABP (Poly(A)n Binding Protein). Both proteins interact with eIF4G, which leads to the formation of a circular structure composed of mRNA complex and proteins. The ability to create such a form by the mRNA informs the cellular translational apparatus that it is undamaged. Also, the circular mRNA is protected from degradation and increases the affinity of the eIF4G factor for the eIF3 translation initiation factor that directly binds the small ribosome subunit. Interactions of proteins with each other and with mRNA strengthen the mutual affinity of all elements and lead to the creation of a stable initiation complex. The small subunit, due to the binding of eIF4E to the cap, recognizes the 5'-end of the mRNA and linearly moves along the leader sequence until the AUG codon, located in the correct sequential context (Kozak sequence). Then a large subunit joins it, which results in the disconnection of almost all translation initiation factors from the initiation complex. The elongation phase of the peptide chain begins. Initiation is a strictly regulated step of translation and has a decisive influence on speed, and hence the efficiency of protein biosynthesis.

The question arises how many plant viruses that do not have a cap, poly(A)n or both of these structures deal with the synthesis of their proteins in eukaryotic plant cells. The team of Prof. Wyatt Allen Miller is researching the translation of this type of viruses using the Barley Yellow Dwarf Virus (BYDV) as a model, which has no cap or poly(A)n. Genomic RNA (gRNA) of this virus is at the same time a matrix for protein biosynthesis. In the 3'-end non-coding region of BYDV resides the BTE translational element (BYDV Translation Element), which is a model representative of the class of translational elements, referred to as 3'CITE (called '3' Cap-Independent Translation Element). The BTE is an RNA structure composed of four arms and three loops, simultaneously fulfilling the function of cap and poly(A)n. When I joined the team, it was known that the BTE stem-loop III facilitates the formation of a circular structure of gRNA and subgenomic RNA 1 (sgRNA1) of the virus through direct, long-distance base pairing with the loop within the 5'-UTR of both RNAs. The formation of a circular structure did not require the participation of proteins.

The separate BTE region, the stem-loop I functionally can act as a cap when placed at the 5'-end of the reporter mRNA. This activity is independent of the stem-loop III long distance base-pairing. Therefore, it was suspected, that stem-loop I was responsible for the recruitment of a small ribosomal subunit through the direct interaction of BTE with the eIF4E factor. My role was to verify this hypothesis and determine the dissociation constant for the potential interaction of BTE with eIF4E and with eIFiso4E. To accomplish this task, I prepared BTE-

labeled RNA and pure preparations of recombinant factors from wheat: eIF4E, eIFiso4E, eIF4G and eIFiso4G (to prepare control complexes - eIF4F and eIFiso4F). My research failed to confirm the interaction of BTE with cap-binding proteins (eIF4E and eIFiso4E). At the same time, the binding of BTE to eIF4F was strong, the dissociation constant (Kd) for the complex was 37 nM. The results led me to examine the interaction of BTE with the eIF4G protein. It turned out that contrary to expectations, BTE directly interacted with this protein (Kd = 177 nM). Binding of eIF4E to eIF4G strengthened its affinity for BTE. I also found that eIF4G restored translation of reporter mRNA containing BTE in the wheat germ extract deprived of wild eiF4F/iso4F by two different methods (passaging the extract by sepharose with 7-methylguanosine or blocking the factors by adding excess BTE in trans) while eIF4E had no such activity. The eIF4G deletion mutant, lacking a long N-terminal fragment including the eIF4E binding site, had the same translation activity as eIF4G. In these experiments, eIF4F had the highest ability to restore translation, confirming that the binding of eIF4G to eIF4E strengthens not only the affinity of eIF4G to BTE but also its translational activity [40].

The experimental model developed by me in the studies mentioned above included translational extract without active translation factors initiators plus pure preparations of these factors and optimized methodology for testing the binding strength of RNA elements to proteins. These tools allowed another team member, Dr. Zhouhui Wang, to identify the 3'CITE in the genome of pea enation mosaic virus (PEMV) and demonstrate that the PTE element (PEMV Translation Element), unlike BTE, directly binds the eIF4E factor without interacting with eIF4G. I took part in creating the concept of this work, proposing a methodology and discussing the results. I also prepared eIF4E mutants devoid of tryptophan residues using targeted mutagenesis, which eliminated the ability of this protein to bind cap [41].

The results mentioned above and the identification of 3'CITE elements and their mechanisms of action by other scientific teams in many unrelated viruses became the basis of the hypothesis of jumping 3'CITEs. According to this hypothesis, in a series of plant viruses, independently different RNA translational elements were formed, differing in nucleotide sequence and structure, but performing a similar function - ribosome recruitment by interacting with various initiation factors or directly with the ribosome. 3'CITEs can be exchanged between unrelated viruses, as evidenced by the presence of 3'CITE with a sequence homologous to BTE and similar spatial structure, exposing both loops responsible for two different functions of this element in genomes of some viruses evolutionally distant from BYDV. Also closely related

viruses may have 3'CITEs of a different origin, differing in sequence and mechanism of action [39]. We have also shown that the various elements of 3'CITE varied significantly in their activity to promote cap-independent translation, which may correspond to multiple mechanisms of virus adaptation to different host plants [42].

During my further work in the team led by Prof. Wyatt Allen Miller, I have found that the 97 amino acid long eIF4G region, flanked by the eIF4E binding site from N-end and by the central MIF4G domain from the C-end, was responsible for the ability of eIF4G to promote cap-independent translation. Also, eIF4G required this region for physical binding of BTE and homologous 3'CITE elements from unrelated to BYDV viruses. Within this region, I have predicted the RNA binding domain of high homology with the human eIF4G I sequence, which determines the binding of this protein to the 5'-end mRNA and is necessary for scanning the mRNA leader sequences by the small ribosome subunit. The domain I identified contains conserved residues of glycine, proline, and arginine in eIF4Gs from plants and mammals. Also, factors I have purified and my eIF4G mutants facilitated mapping the nucleotides directly involved in eIF4G binding in stem-loop I sequence. This nucleotide sequence forming eIF4G binding site is remarkably conserved in the BTE-like 3'CITEs [43].

PUBLICATIONS

39. Miller W.A., Wang Z., Treder K. 2007. The amazing diversity of cap-independent translation elements in the 3’-untranslated regions of plant viral RNAs. Biochemical Society Transactions, 35: 1629 1633.
40. Treder K., Kneller E.L.P., Allen E.M., Wang Z., Browning K.S., Miller W.A. 2008. The 3’ cap-independent translation element of Barley yellow dwarf virus binds eIF4F via the eIF4G subunit to initiate translation. RNA, 14: 134-147.
41. Wang Z., Treder K., Miller W.A. 2009. Structure of a viral cap-independent translation element that functions via high affinity binding to the eIF4E subunit of eIF4F. Journal of Biological Chemistry, 284: 14189-14202.
42. Fan Q., Treder K., Miller W.A. 2012. Untranslated regions of diverse plant viral RNAs vary greatly in translation enhancement efficiency. BMC Biotechnology, 12: 22.
43. Kraft J.J., Treder K., Peterson M.S., Miller W.A. 2013. Cation-dependent folding of 3′ cap-independent translation elements facilitates interaction of a 17-nucleotide conserved sequence with eIF4G. Nucleic Acids Research, 41: 3398-3413.

PROJECT

- 2003-2013. 5R01GM067104. Research project. National Institute of Health, USA. ”Control of cap-independent translation by a viral 3’UTR”. Contractor during 2004-2007 and 2010-2012.

After returning from the first internship in the USA, in January 2008 I took the position of the head of the Laboratory of Molecular Diagnostics and Biochemistry in the Bonin Research Center, IHAR-PIB, which I still hold. I continued the most important research directions of the Laboratory, with particular emphasis on the diagnostics of potato viruses and the *C.*

sepedonicus. I obtained funding in the framework of two research projects in the years 2008-2013 (task numbers 70 and 71) and one in the years 2014-2020 (the task no. 58) from the program Biological Progress in Plant Production (PBwPR), implemented by the Ministry of Agriculture and Rural Development. The results of the implementation of the above PBwPR projects are available on the IHAR-PIB website. I have designed budgets in both projects to cover the existing ones and to create new job positions in the Laboratory. Thanks to this, there are currently two teams in the Laboratory, one dedicated to viruses and second, focused on C. sepedonicus. Most team members are on the right track to complete their doctoral theses. Because our research included immunization of rabbits and collection of sera, I also applied to the Local Ethics Committee (LKE) for permission to conduct animal experiments, which I got in 2008 and 2013 for the LKE projects I have managed. For the continuation of research on purification of viruses, a team member - Dr. Eng. Agnieszka Barnyk (currently Przewodowska) got the grant from the National Science Center (NCN). I am also Principal Investigator in the grant funded by NCN to investigate the transport and replication of PVY in potato plants. I have also established a scientific collaboration with Dr. hab. Jarosław Tyburski, resulting in previously discussed studies on potato peroxidase [22-24]. I also collaborate with Dr. Eng. Dominika Boguszewska (IHAR-PIB, Jadwisin Research Center) on the biochemistry of drought tolerance in potatoes. These studies are funded from the PBwPR project (task 59), where Dr. Eng. Dominika Boguszewska acts as Principal Investigator. In addition to conducting research and managing the research team, I also took care to upgrade the laboratory equipment, thanks to which we now have a well-equipped biochemical and molecular laboratory. The projects that I managed or in which I served as contractors are listed below. The results of their implementation will be discussed according to their subject.

PROJEKTY

- 2008-2013. „Opracowanie procedur i wytworzenie materiałów diagnostycznych do wykrywania *Clavibacter michiganensis* ssp. *sepedonicus*". Task nr 70. Biological Progress in Plant Production, Ministry of Agriculture and Rural Development. Principal investigator during 2008-2010.
- 2008-2013. „Opracowanie procedury wykrywania infekcji wirusowych w bulwach ziemniaka bezpośrednio po zbiorze lub w stanie spoczynku". Task nr 70. Biological Progress in Plant Production, Ministry of Agriculture and Rural Development. Principal investigator during 2008-2010 and 2013.
- 2014-2020. „Opracowanie czułych metod wykrywania najważniejszych wirusów ziemniaka". Task nr 58. Biological Progress in Plant Production, Ministry of Agriculture and Rural Development. Principal Investigator.
- 2014-2020. „Badania tolerancji odmian ziemniaka na stresy abiotyczne w świetle postępujących zmian klimatycznych". Task nr 59. Biological Progress in Plant Production, Ministry of Agriculture and Rural Development. Contractor.

- 2008-2013. „Wytwarzanie przeciwciał poliklonalnych na patogeny ziemniaka”. The LKE project no. 10/2008 implemented on the basis of the permission of the Local Ethics Committee for animal experiments in Szczecin. Principal Investigator.
- 2013-2017. „Wytwarzanie przeciwciał poliklonalnych na patogeny ziemniaka i białka roślinne". The LKE project no. 10/2013 implemented on the basis of the permission of the Local Ethics Committee for animal experiments in Szczecin. Principal Investigator.
- 2011-2014. N N310 728540. "Development of a procedure for the isolation of plant viruses using ion-exchange membrane chromatography". The project funded by the National Science Center. Contractor.
- 2017-2020. UMO-2016/21/B/NZ9/03573. "The dynamics of transport and replication of the most important strains of potato virus Y in primarily and secondarily infected potato plants". The project funded by the National Science Center. Principal Investigator.

I led the PBwPR project concerning the diagnosis of *C. sepedonicus* in 2008-2010. Due to the internship to the USA in November 2010, in the years 2011-2013 the project was headed by Dr. Eng. Włodzimierz Przewodowski. My contribution to this research was to design primers for the detection of *C. sepedonicus* by PCR. I also designed primers to detect this bacteria by an isothermal LAMP test. I directed the work aimed at selecting the LAMP test conditions and determining the specificity and sensitivity of the developed test. A measurable effect of the research work was the LAMP test specific for *C. sepedonicus*, detecting all the isolates of this bacterium collected in the Laboratory [46-47].

CONFERENCES

1. Przewodowski W., Przewodowska A., Treder K. 2013. Modern diagnosis of quarantine pathogen – *Clavibacter michiganensis* ssp. *sepedonicus* based on colloidal gold as sensitive marker. [W:] Symposium „Classical and molecular approaches in plant pathogen taxonomy”. Warsaw, Poland, September 10-11, 2013. WULS-SGGW. Book of Abstracts: 28. Talk co-author.
2. Przewodowski W., Przewodowska A., Treder K. 2013. Application of colloidal metal nanoparticles in diagnosis of quarantine bacteria – *Clavibacter michiganensis* subsp. *sepedonicus*. [In:] Abstracts of the 10th International Congress of Plant Pathology, 25-30 August, Beiging, China, Acta Phytopathologica Sinica, 43 (suppl.): 370. Poster
3. Przewodowski W., Chołuj J., Przewodowska A., Treder K. 2014. Izotermiczna metoda amplifikacji kwasów nukleinowych LAMP w nowoczesnej diagnostyce sprawcy bakteriozy pierścieniowej ziemniaka. [W]: Nasiennictwo i ochrona ziemniaka. Konferencja naukowo-szkoleniowa. Dźwirzyno, 15-16 maja. IHAR ZNiOZ Bonin: 24 25. Talk co-author.
4. Przewodowski W., Chołuj J., Przewodowska A., Treder K. 2014. Detection of *Clavibacter michiganensis* ssp. *sepedonicus* using loop-mediated isothermal amplification (LAMP). [In:] XVI International Congress on Molecular Plant-Microbe Interactions. Book of abstracts. 6-10 July, Rhodos, Greece, IS-MPMI: P209. Poster.

Continuing research on improving the methods of potato virus purification, I used ion exchange membrane chromatography for this purpose using PVY as a model virus. I used ultracentrifugation at 100,000 g on a 30% sucrose cushion to assess the integrity of the virus particles, because under these conditions PVY virions form a pellet, and proteins derived from the degradation of viral particles remain in the supernatant. Using this approach, I found that

undegraded virus particles were bound on a membrane with quaternary amine groups on the surface (membrane Q). To elute the virus high ionic strength (above 0.5 M NaCl) was required. The combination of the Q membrane with ultracentrifugation facilitated a quick, two-step purification of pure PVY preps [48, 49]. These results became the basis of the NCN project led by Dr. Eng. Agnieszka Barnyk (now Przewodowska) in 2011-2014. After returning from the second internship in the USA in August 2012, I took part in the implementation of this project as a contractor. The research results were presented at five conferences [48-52] and included in one publication [53].

CONFERENCES

1. Wieczorek J., Barnyk A., Treder K. 2010. Oczyszczanie wirusa Y ziemniaka za pomocą chromatografii membranowej. [W:] XV Sympozjum Sekcji Wirusologicznej Polskiego Towarzystwa Fitopatologicznego oraz XXXVII Konferencja Grupy Roboczej Wirusów Roślin Komitetu Ochrony Roślin PAN, Poznań, 9-10 września, streszczenia: 16-17. Talk.
2. Barnyk A., Treder K., Wieczorek J. 2010. Szybkie oczyszczanie wirusa Y z materiału roślinnego za pomocą chromatografii membranowej. [W:] 50 Sesja Naukowa IOR-PIB. Poznań, 4-5 lutego, streszczenia: 246. Poster.
3. Przewodowska A., Przewodowski W., Treder K. 2013. Ion-exchange membrane chromatography – a new tool for plant virus purification. [In:] Abstracts of 10th International Congress of Plant Pathology, 25-30 August, Beiging, China, Acta Phytopathologica Sinica, 43 (suppl.): 448-449. Poster.
4. Przewodowska A., Treder K. 2012. Purification of plant viruses by membrane ion-exchange chromatography. [In:] Abstracts of the 15th European Congress on Biotechnology, 23th-26th September, Istanbul, Turkey, New Biotechnology, 29: S133. Poster.
5. Przewodowska A., Zacharzewska B., Przewodowski W., Treder K. 2013. Ion-exchange membrane chromatography – a new tool for plant virus purification. [W:] Symposium „Classical and molecular approaches in plant pathogen taxonomy". Warsaw, Poland, September 10-11, 2013. WULS-SGGW. Book of Abstracts: 28. Talk co-author.

PUBLICATION

6. Treder K., Zacharzewska B., Przewodowska A., Przewodowski W., Otulak K. 2015. Ion-exchange membrane chromatography as an alternative method of separation of potato Y virus. Plant Breeding and Seed Science 72: 56-67.

In 2008-2010 and 2013, I led the PBwPR research project (task 71) regarding virus diagnostics. At the time of my second internship in the US, I handed over the project to Dr. Eng. A. Przewodowska (2011-2012). These studies are currently being continued under the task 58 of the PBwPR. I obtained pure preparations of PLRV, PVY, and PVM, which we used to produce rabbit sera with the high titer of specific antibodies. Thanks to the use of thiophilic chromatography [86], we have optimized the isolation of antibodies from sera. We obtained high-titer of very specific anti-PLRV, PVY and PVM antibodies (titer 4-8 million). Antibodies were used in DAS and cocktail ELISA [54, 55], and to develop an immuno-fluorescent test for PVY detection [60], as well as the immuno-capture RT-PCR assay [55, 58, 59, 87]. We have

developed a method for the stable production of antibody conjugated with colloidal gold, which enabled the production of immunochromatographic (strip) tests. The tests allowed the detection of potato viruses within 5 min with the same sensitivity as the ELISA test. However, the cost of producing them was higher than the cost of commercially available strips. The cost of commercial strip tests is about 15-20 PLN for one strip and is comparable to the cost of PCR and over ten times more expensive than the ELISA test. Therefore, we investigated immuno-concentration tests based on magnetic microspheres, coated with antibodies, and an isothermal RT-LAMP for rapid detection of PVY virus [64]. Both can act as a point-of-care test completed in 5-20 minutes, and their cost is half the cost of strip tests.

We also continued work on detecting viruses in tubers [54, 55, 88-91]. Thanks to the cooperation within PBwPR projects with Polish potato breeding companies, our methods were compared in three independent laboratories investigating viral infections of potatoes grown in different regions of Poland. Tubers for testing were prepared in field experiments by cooperating companies and by us. Initially, the results confirmed the usefulness of our cocktail-ELISA procedure in detecting viruses directly in tuber extracts [88, 89]. However, in the following years, we observed the variable efficacy of the cocktail-ELISA test for PVY. Further research has shown that the cocktail-ELISA test detects PVY well in cultivars with low- and medium-resistance to PVY, while more resistant cultivars required the grow-out test for reliable PVY detection. The cocktail-ELISA was as reliable as the grow-out test for PLRV and PVM. However, the lack of credibility for PVY eliminated its suitability for routine testing of tubers, in which the same methodology is needed for all viruses. Looking for a solution to this problem, we have researched the detection of viruses in sprouts, also in cooperation with Polish breeders. The results form last six years confirmed that detection in sprouts by ELISA is cost and time-saving alternative to the grow-out test [63, 75]. As part of the project, we have been conducting research on the use of molecular methods for the detection of PVY, PVM and PLRV directly in tubers for several years. To carry out these works, I designed sets of primers for detection of PVY, PVM and PLRV viruses by RT-PCR. The detection limit facilitated by designed primers was at least an order of magnitude higher than for the tested primers described in the literature [66-68, 76, 77, 79, 81, 94]. Based on the results of a three-year study, we showed that despite the very low limit of detection in laboratory conditions, for tubers from the field, the RT PCR RT test detects fewer virus infections than grow-out test [75]. Tubers are a particularly tricky source of RNA. Therefore, we are currently working on optimizing the RNA isolation from tubers. We have also investigated the use of an isothermal RT-LAMP test for detecting viruses

in leaves and tubers [89-74, 78], the results of which have been presented in the description of my habilitation achievement. I continue my research as part of the PBwPR project. Last year, I designed primers for detecting other potato viruses and the direct RT-LAMP test, where instead of pure RNA, crude sap is added directly to the reaction [80, 82, 83]. We are also currently launching innovative research on a multiplex version of the RT-LAMP test.

The results of the studies discussed above were presented in the form of thirty-one conference reports, including twelve papers and twelve publications, including three from the A-list.

CONFERENCES

1. Pilecki T., Treder K., Lewosz J. 2008. Detection of PVY, PVM and PLRV in the sap of dormant tubers by cocktail ELISA. [In:] The Congress of Biochemistry and Cell Biology, Olsztyn, Poland, September 7th-11th, Abstracts, Acta Biochimica Polonica, 55 (suppl.): 171. Poster.
2. Pilecki T., Treder K., Lewosz J. 2008. Optymalizacja testu koktajl-ELISA do wykrywania wirusa liściozwoju (PLRV) w bulwach ziemniaka. [W:] Nasiennictwo i Ochrona Ziemniaka. Konf. nauk.-szkol., Kołobrzeg, 3-4 kwietnia. IHAR, ZniOZ Bonin: 106-108. Poster.
3. Treder K., Pilecki T. 2009. Wykrywanie wirusów ziemniaczanych bezpośrednio w ekstraktach z bulw - porównanie testu ELISA z immuno-captured RT-PCR. [W:] 49 Sesja Naukowa IOR-PIB, Poznań, 19-20 lutego, streszczenia: 269. Poster.
4. Pilecki T., Treder K. 2010. Wpływ okresu przechowywania ziemniaków na wykrywalność wirusów PLRV, PVM oraz PVY w bulwach. [W:] 50 Sesja Naukowa IOR-PIB. Poznań, 4-5 lutego, streszczenia: 256. Poster.
5. Treder K. 2010. Optymalizacja detekcji wirusa Y ziemniaka za pomocą testu ELISA oraz RT-PCR w soku z liści i bulw ziemniaka. Konferencja „Tradycja i nowoczesność w produkcji ziemniaka", Jadwisin, 7-9 lipca. Talk.
6. Treder K., Łycuś P. 2010. Optymalizacja wykrywania wirusa Y w soku z liści i bulw ziemniaka. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Darłówko, 20-21 maja. IHAR ZNiOZ Bonin: 74. Talk.
7. Treder K., Łycuś P. 2010. Szybki test immuno-chemiluminescencyjny do wykrywania wirusa Y ziemniaka (PVY), wirusa M ziemniaka (PVM) oraz wirusa liściozwoju ziemniaka (PLRV). [W:] 50 Sesja Naukowa IOR-PIB, Poznań, 4-5 lutego, streszczenia: 259. Poster.
8. Treder K., Zacharzewska B., Przewodowska P. 2013. Application of molecular and immunological methods for direct detection of viruses in dormant tubers. [W:] Symposium „Classical and molecular approaches in plant pathogen taxonomy". Warsaw, Poland, September 10-11, 2013. WULS-SGGW. Book of Abstracts: 27. Talk.
9. Przewodowska A., Treder K. 2013. Optymalizacja testu RT-PCR do wykrywania wirusa liściozwoju ziemniaka (PLRV). [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 16-17.05. IHAR ZNiOZ Bonin: 121. Poster.
10. Treder K., Przewodowska A. 2013. Wykrywanie najważniejszych wirusów ziemniaka bezpośrednio w bulwach. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 16-17.05. IHAR ZNiOZ Bonin: 122. Poster.
11. Treder K., Przewodowska A.. 2013. Application of RT-LAMP for direct detection of potato viruses in dormant tubers. [In:] Abstracts of 10th International Congress of Plant Pathology, 25-30 August, Beiging, China, Acta Phytopathologica Sinica, 43 (suppl.): 448. Poster.
12. Przewodowska A., Zacharzewska B., Treder K. 2014. Izotermiczna technika LAMP w diagnostyce wirusów ziemniaka. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 15-16.05. IHAR ZNiOZ Bonin: 22. Talk co-author.
13. Treder K., Zacharzewska B. 2014. Optymalizacja wykrywania wirusów ziemniaka za pomocą RT-PCR. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 15-16.05. IHAR ZNiOZ Bonin: 36. Talk.

14. Zacharzewska B., Przewodowska A., Treder K. 2014. Wpływ sposobu izolacji RNA na czułość wykrywania wirusa Y ziemniaka za pomocą RT-PCR. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 15-16.05. IHAR ZNiOZ Bonin: 63. Poster.
15. Zacharzewska B., Przewodowska A., Treder K. 2014. Optimization of the detection of the most important potato viruses by reverse transcription polymerase chain reaction. [In:] XVI International Congress on Molecular Plant-Microbe Interactions. Book of abstracts. 6-10 July, Rhodos, Greece, IS-MPMI: P207. Poster.
16. Chołuj J., Zacharzewska B., Treder K. 2015. Adaptacja izotermicznego testu RT-LAMP do oceny porażenia bulw wirusem Y ziemniaka. [W:] XII Ogólnopolska Konferencja Naukowa, Nauka dla Hodowli i nasiennictwa roślin uprawnych. Streszczenia referatów i posterów. Zakopane, 2 6.02. IHAR-PIB: 171-173. Poster.
17. Treder K., Zacharzewska B., Chołuj J. 2015. Zastosowanie izotermicznej amplifikacji kwasów nukleinowych w diagnostyce wirusa Y ziemniaka. [W:] XII Ogólnopolska Konferencja Naukowa, Nauka dla Hodowli i nasiennictwa roślin uprawnych. Streszczenia referatów i posterów. Zakopane, 2-6.02. IHAR-PIB: 65-68. Talk.
18. Treder K., Zacharzewska B., Chołuj J. 2015. Wykrywanie wirusa Y ziemniaka za pomocą izotermicznej amplifikacji kwasów nukleinowych. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 13-15.05. IHAR ZNiOZ Bonin: 50. Talk.
19. Zacharzewska B., Chołuj J., Treder K. 2015. Opracowanie izotermicznego testu RT LAMP do wykrywania wirusów ziemniaka. [W:] XII Ogólnopolska Konferencja Naukowa, Nauka dla Hodowli i nasiennictwa roślin uprawnych. Streszczenia referatów i posterów. Zakopane, 2 6.02. IHAR-PIB: 217-218. Poster.
20. Zacharzewska B., Chołuj J., Treder K. 2015. Opracowanie izotermicznego testu LAMP do wykrywania wirusów ziemniaka. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 13-15.05. IHAR ZNiOZ Bonin: 99. Talk.
21. Chołuj J., Zacharzewska B., Treder K. 2016. Izotermiczna amplifikacja kwasów nukleinowych jako szybki test wykrywania wirusów ziemniaka na przykładzie wirusa PVY. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 11-13.05. IHAR ZNiOZ Bonin: 46-47. Talk co-author.
22. Treder K., Chołuj J., Pawłowska A., Dybner M., 2016. Wykrywanie wirusa Y w bulwach i kiełkach ziemniaka. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 11-13.05. IHAR ZNiOZ Bonin: 45-46. Talk.
23. Zacharzewska B., Treder K. 2016. Opracowanie testu RT-PCR do wykrywania wirusa M ziemniaka [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 11 13.05. IHAR ZNiOZ Bonin: 71-72. Poster.
24. Mielczarek M., Chołuj J., Zacharzewska B., Treder K. 2017. Nowy test do wykrywania wirusa liściozwoju ziemniaka za pomocą konwencjonalnego PCR oraz real-time PCR. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 16-17 maja. IHAR ZNiOZ Bonin: 75-76. Poster.
25. Treder K., Chołuj J., Zacharzewska B., Rakotondrafara A., Babujee L. 2017. Sensitive detection of potato virus Y and differentiation of O and N types by reverse transcription loop-mediated isothermal amplification. [In:] EAPR 20th Triennial Conference, Versailles, 9-10 July. Potato facing global challenges. Keynote lectures and abstracts, P052: 223. Poster.
26. Treder K., Chołuj J., Zacharzewska B., Mielczarek M. 2017. New sensitive assay for the detection of potato leafroll virus by conventional and real-time reverse transcription polymerase chain reaction. [In:] EAPR 20th Triennial Conference, Versailles, 9-10 July. Potato facing global challenges. Keynote lectures and abstracts, P052: 227. Poster.
27. Mielczarek M., Zacharzewska B., Treder K. 2018. Wykrywanie wirusów ziemniaka bezpośrednio w ekstraktach z liści za pomocą izotermicznego testu RT-LAMP. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 6-8 czerwca. IHAR ZNiOZ Bonin: 27-29. Talk co-author.
28. Treder K., Zacharzewska B., Mielczarek M., Chołuj J. 2018. Development of multiplex RT-PCR and real-time RT-PCR assays to detect three potato viruses. Plant Biology Europe, June 18-21, Copenhagen, Denmark. Abstr. No.: 412. Poster.

29. Treder K., Mielczarek M., Zacharzewska B. 2018. Detection of potato viruses by reverse transcription loop-mediated isothermal amplification. Plant Biology Europe, June 18-21, Copenhagen, Denmark. Abstr. No.: 413. Poster.
30. Treder K., Chołuj J., Zacharzewska B., Mielczarek M. 2018. Kolorymetryczny test RT-LAMP do wizualnego wykrywania wirusa Y ziemniaka. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol. Dźwirzyno, 6-8 czerwca. IHAR ZNiOZ Bonin: 24-26. Talk.
31. Zacharzewska B., Treder K. 2018. Opracowanie izotermicznego testu molekularnego do wykrywania wirusa liściozwoju ziemniaka w oparciu o reakcję amplifikacji kwasów nukleinowych zależną od helikazy. [W:] Nasiennictwo i ochrona ziemniaka. Konf. nauk.-szkol., Dźwirzyno, 6-8 czerwca. IHAR ZNiOZ Bonin: 29-30. Talk.

PUBLICATIONS

32. Barnyk A., Lewosz J., Treder K., Przewodowski W., Pilecki T. 2008. Zastosowanie chromatografii tiofilnej do izolacji przeciwciał poliklonalnych z surowicy krwi królików. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 248: 87-95.
33. Treder K., Przewodowski W. 2009. Poprawa czułości wykrywania wirusów ziemniaka testem ELISA dzięki zwiększeniu adsorpcyjności mikropłytek poprzez mycie w proszku do prania. Prog. Plant Prot./Post. Ochr. Roślin, 49: 1767-1769.
34. Treder K., Pilecki T., Łycuś P. 2009. Wykrywanie wirusów ziemniaczanych bezpośrednio w ekstraktach z bulw – porównanie testu ELISA z immuno-captured RT-PCR. Prog. Plant Prot./Post. Ochr. Roślin, 49: 740-745.
35. Pilecki T., Barnyk A., Przewodowski W., Treder K. 2009. Wpływ inhibitorów reakcji niespecyficznej adsorpcji przeciwciał i składu buforów na wykrywalność wirusów w bulwach ziemniaka. Prog. Plant Prot./Post. Ochr. Roślin, 49: 691–695.
36. Treder K., Przewodowski W., Barnyk A. 2009. Factors influencing detection of potato leafroll virus and potato virus Y in potato tuber extracts. Plant Breeding and Seed Science, 59: 65-74.
37. Pilecki T., Treder K. 2010. Wpływ okresu przechowywania ziemniaków na wykrywalność wirusów PLRV, PVM oraz PVY w bulwach. Prog. Plant Prot./Post. Ochr. Roślin, 50: 723 727.
38. Zacharzewska B., Przewodowska A., Treder K. 2014. The adaptation of silica capture RT-PCR for the detection of potato virus Y. Am. J. Potato. Res., 91: 525-531. (**H1**).
39. Przewodowska A., Zacharzewska B., Chołuj J., Treder K. 2015. A one-step, real-time reverse transcription loop-mediated isothermal amplification assay to detect Potato virus Y. Am. J. Potato. Res., 92: 303-311. (**H2**).
40. Treder K. 2015. Metody wykrywania obecności wirusów ziemniaka. Ziemniak Polski, 4: 18-23.
41. Treder K., Zacharzewska B. 2017. Opracowanie nowych starterów do wykrywania wirusa M ziemniaka (Potato virus M, PVM) za pomocą testu RT-PCR. Ziemniak Polski, 3: 32-35.
42. Treder K., Chołuj J., Zacharzewska B., Mielczarek M. 2017. Detection of potato virus Y (PVY) by reverse-transcription loop-mediated nucleic acid amplification (RT-LAMP). Plant Breeding and Seed Science, 75: 77-85. (**H3**).
43. Treder K., Chołuj J., Zacharzewska B., Babujee L., Mielczarek M., Burzyński A., Rakotondrafara A. 2018. Optimization of a magnetic capture RT-LAMP assay for fast and real-time detection of potato virus Y and differentiation of N and O serotypes. Archives of Virology, 163: 447-458. (**H4**).
44. Treder K. 2018. Izotermiczna amplifikacja kwasów nukleinowych metodą LAMP i jej zastosowanie w diagnostyce patogenów ziemniaka. Ziemniak Polski, 2: 48-36.

6. SUMMARY OF SCIENTIFIC AND RESEARCH ACHIEVEMENTS

My postdoctoral output includes thirty-two publications, including four concerned with scientific achievements. Among them are twenty-five original scientific papers, including twelve published in journals with the impact factor (IF), highlighted in the Journal Citation

Reports (JCR). I am also the author and co-author of seven review papers, including one published in a journal with an IF. I also disseminated the results of my doctoral dissertations in the form of seventy-nine reports presented at twenty-two international conferences and eighteen domestic conferences, of which fifteen were presentations delivered by me, and in the next fifteen papers I was their co-author. The remaining forty-nine reports had the form of posters. In two international conferences, I participated thanks to travel grants awarded by the organizers based on the competition of submitted posters.

After doctoral thesis, I had the opportunity to extend my scientific experience staying in three foreign universities (Sweden, USA, and Ireland). Especially notable were two postdoctoral internships at Iowa State University (Ames, IA, USA), jointly covering five years of work in a team headed by Prof. Wyatt's Allen Miller. Participation in inspiring molecular biology studies on the initiation of translation of viral proteins was an experience that refined my scientific skills and expanded my knowledge of molecular biology. I also had an excellent opportunity to develop my capacity for international research cooperation and gained experience in scientific care for beginning team members. The acquired skills were probably the reason for my two visits to the Re-coding Laboratory (Bioscience Institute, University College Cork, Ireland) at the invitation of Prof. John F. Atkins, during which I taught his then Ph.D. student Betty Chung the methods of studying the interaction of viral proteins with host plant proteins. Ms. Betty Chung also had two visits to the Laboratory led by me, at the IHAR-PIB, Bonin Research Center in 2008 and 2009. Prof. John F. Atkins and then members of his team - Dr. Betty Chung and Dr. Andrew Firth appreciated my contribution to their research on plant viruses, thanking me in several of their publications.

Due to my employment at a research institute, I do not have much achievement in academic work. Nevertheless, under my protection, five graduate students from several Polish universities gained the laboratory work experience. Also, I prepared and delivered seventeen lectures. Two training talks on virus diagnostics I gave to employees of the State Plant Health and Seed Inspection Service (PIORiN), Potato Breeding companies and academic workers. I have prepared four lectures for employees of PIORiN as part of the official tuber assessment and field evaluation training. One talk I have presented to scientists working in Młochów Research Center (IHAR-PIB) within the framework of scientific seminars organized in this Center. Two lectures were dedicated to foreign guests visiting Bonin Research Center. I also gave a talk at National Potato Days 2018. My academic experience included three presentations

for students and employees of Plant Pathology Department, Iowa State University (Ames, IA, USA) and four for students and academics of the Faculty of Biology and Environmental Protection of the Nicolaus Copernicus University in Toruń. Relations with my alma mater - Nicolaus Copernicus University in Toruń - I also maintain through scientific collaboration with Dr. hab. Jarosław Tyburski. This resulted, among others, in my involvement in the doctoral conduct of Mrs. Katarzyna Kurnik (now Krzyżyńska), who carries out a thesis entitled: "Vegetable waste biomass as a source of peroxidases for the needs of bioremediation of waste wastes contaminated with phenolic compounds". Following Resolution No. 32/2014 of the Council of the Faculty of Biology and Environmental Protection of the Nicolaus Copernicus University in Toruń, I am the auxiliary supervisor in this council.

I carried out my research after a doctorate in eight research projects. They included a grant managed by prof. Wyatt Allen Miller, funded by the National Institute of Health (NIH). I was the contractor of this project during both stays in the USA. I was also a contractor in a grant funded by the National Science Center and headed by Dr. Eng. Agnieszka Przewodowska. I am currently a contractor and manager of a research task included in the project led by dr inż. Dominika Boguszewska-Mańkowska, which is funded by the Ministry of Agriculture and Rural Development from the Biological Progress in Plant Production program (PBwPR). I managed one project funded by the Scientific Research Committee (KBN) and two PBWPR projects. I am currently the PI of a grant funded by the National Science Center and a research project funded by the Ministry of Agriculture and Rural Development from PBWPR.

I made twelve paid expert reports for enterprises, police and individual farmers. Nine related to the identification of potato varieties using protein and molecular markers, two- detection of pathogenic bacteria and viruses by molecular methods and one determination of the effectiveness of commercial ELISA kits for detecting in human blood antibodies specific for allergens present in food.

My knowledge of issues related to molecular biology and biochemistry of the interaction of viruses with plants and the immunological and molecular diagnostics of plant pathogens has been appreciated by the editorial offices of scientific journals for which I have made twenty-one reviews of manuscripts. They included twelve reviews that I made for journals with an IF impact factor. For the quality of three reviews for the Journal of Virological Methods, I was awarded this year with the certificate "Certificate of outstanding contribution in reviewing" by

the editors of this journal. This year I also became a member of the editorial committee of the Progress in Plant Protection, in which I am the editor of the Virology and Bacteriology section.

The data in numbers for the achievements mentioned above are summarized below in Tables 1 and 2.

Table 1. Numerical list of achievements before and after obtaining the doctoral degree.

SPECIFICATION	Number		
	Before the doctorate	After the doctorate	Total
Original scientific publications in journals (including publications that create an achievement)			
with the IF impact factor, highlighted in JCR (list A MNiSW)	0	12	12
with no IF impact factor (list B MNiSW)	2	13	15
Review papers in journals:			
with the IF impact factor, highlighted in JCR (list A MNiSW)	0	1	1
with no IF impact factor (list B MNiSW)	0	6	6
Chapters in collective works	3	0	3
Teaching materials	3	0	3
Total	**8**	**32**	**40**
Points for publications by MNiSW	**4**	**402**	**406**
The total impact factor (IF) of publications according to JCR		**35,422**	
Number of cites according to WoS (without self-citations)		**213**	
Hirsh Index according to WoS		**7**	
Research works presented at national and international conferences			
Talks	5	15	20
Talk co-autorship	8	15	23
Poster author/co-author	8	49	57
Total	**21**	**79**	**100**
Academic lectures	**1**	**17**	**18**
Participation in research projects (as a manager and / or contractor) financed with funds			
Foreign	0	1	1
MNiSW (KBN, NCN)	1	3	4
MRiRW (PBwPR)	0	4	4
Total	**1**	**8**	**9**
Made expertises	**0**	**12**	**12**
Reviews for magazines			
with the IF impact factor, highlighted in JCR (list A MNiSW)	0	12	12
with no IF impact factor (list B MNiSW)	0	9	9
Total	**0**	**21**	**21**

Table 2. Scoring of published works.

Lp	Journal name	IF	Points MNiSW
Scientific achivement			
1	American Journal of Potato Research	1,204	25
2	American Journal of Potato Research	1,159	25
3	Plant Breeding and Seed Science	-	11
4	Archives of Virology	2,160	20
Achivemnt Total		**4,523**	**81**
with the IF impact factor, highlighted in JCR (list A MNiSW)			
5	Journal of Food Biochemistry	1,552	20
6	Waste and Biomass Valorization	1,874	20
7	American Journal of Potato Research	1,156	25
8	Water Air And Soil Pollution	1,551	25
9	Nucleic Acid Research	8,808	40
10	BMC Biotechnology	2,165	30
11	Journal of Biological Chemistry	5,328	24
12	RNA	5,018	24
13	Biochemical Society Transactions	3,447	30
with no IF impact factor (list B MNiSW)			
14	Plant Breeding and Seed Science	-	11
15	Plant Breeding and Seed Science	-	11
16	Plant Breeding and Seed Science	-	4
17	Ziemniak Polski	-	5
18	Ziemniak Polski	-	5
19	Ziemniak Polski	-	5
20	Ziemniak Polski	-	5
21	Ziemniak Polski	-	5
22	Ziemniak Polski	-	0
23	Ziemniak Polski	-	0
24	Ziemniak Polski	-	2
25	Ziemniak Polski	-	2
26	Prog. Plant Prot./Post. Ochr. Roślin	-	6
27	Prog. Plant Prot./Post. Ochr. Roślin	-	4
28	Prog. Plant Prot./Post. Ochr. Roślin	-	4
29	Prog. Plant Prot./Post. Ochr. Roślin	-	4
30	Prog. Plant Prot./Post. Ochr. Roślin	-	4
31	Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin	-	4
32	Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin	-	2
Total		**35,422**	**402**

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Table 2. Scoring of published works.

Lp	Journal name	IF	Points MNiSW
Scientific achivement			
1	American Journal of Potato Research	1,204	25
2	American Journal of Potato Research	1,159	25
3	Plant Breeding and Seed Science	-	11
4	Archives of Virology	2,160	20
Achivemnt Total		**4,523**	**81**
with the IF impact factor, highlighted in JCR (list A MNiSW)			
5	Journal of Food Biochemistry	1,552	20
6	Waste and Biomass Valorization	1,874	20
7	American Journal of Potato Research	1,156	25
8	Water Air And Soil Pollution	1,551	25
9	Nucleic Acid Research	8,808	40
10	BMC Biotechnology	2,165	30
11	Journal of Biological Chemistry	5,328	24
12	RNA	5,018	24
13	Biochemical Society Transactions	3,447	30
with no IF impact factor (list B MNiSW)			
14	Plant Breeding and Seed Science	-	11
15	Plant Breeding and Seed Science	-	11
16	Plant Breeding and Seed Science	-	4
17	Ziemniak Polski	-	5
18	Ziemniak Polski	-	5
19	Ziemniak Polski	-	5
20	Ziemniak Polski	-	5
21	Ziemniak Polski	-	5
22	Ziemniak Polski	-	0
23	Ziemniak Polski	-	0
24	Ziemniak Polski	-	2
25	Ziemniak Polski	-	2
26	Prog. Plant Prot./Post. Ochr. Roślin	-	6
27	Prog. Plant Prot./Post. Ochr. Roślin	-	4
28	Prog. Plant Prot./Post. Ochr. Roślin	-	4
29	Prog. Plant Prot./Post. Ochr. Roślin	-	4
30	Prog. Plant Prot./Post. Ochr. Roślin	-	4
31	Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin	-	4
32	Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin	-	2
Total		**35,422**	**402**

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