<u>Załącznik 3:</u> AUTO-PRESENTATION

Study of *Potato virus Y* interaction with potato and tobacco

AUTO-PRESENTATION

1. **Name:** Zhimin Yin

2. **Obtained diplomas/scientific degrees:**

- 1983-1987 Bachelor of Science in agronomy with honours.
  Hebei Agricultural University, Department of Agronomy, Baoding, P.R. China.
  Title of thesis: "Analysis of the agronomic traits of wheat F1 generation" (in Chinese)
  Supervision by Professor Zongzhi Li.
- 1997-1998 Master of Science in horticulture (extramural).
  Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture, Warsaw Agricultural University – SGGW, Warsaw, Poland.
  Title of thesis: "Transformation of cucumber (*Cucumis stativus* L.) with *PR-2d/uidA* reporter gene constructs" (in English)
  Supervision by Professor Stefan Malepszy.
- 1998-2002 Doctor (PhD) of agricultural sciences with specialization in horticulture.
  Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture and Landscape Architecture, Warsaw Agricultural University – SGGW, Warsaw, Poland.
  Title of thesis: "Analysis of transgenic cucumber plants containing *PR-2d uidA* and *p35S CaMV* thaumatin constructs" (in English)
  Supervision by Professor Stefan Malepszy

3. **Information on employment in scientific organizations**

- 1987-1998 Cotton Institute, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang, P. R. China, assistant.
- 2003-2008 Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland, adjunct.
- 2008 – until now, Plant Breeding and Acclimatization Institute – National Research Institute, Młochów Research Center, Młochów, Poland, adjunct.

4. **Scientific achievement according to –** art 179 ustawy z 3 lipca 2018 r. – *Przepisy wprowadzające ustawę – Prawo o szkolnictwie wyższym i nauce* (*Dz. U. z 30.08.2018 r., poz. 1669)* - **z art. 16 ust. 2 ustawy z dnia 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki** (Dz. U. z 2017 r., poz. 1789)"

   a) Title of the achievement:

   Study of *Potato virus Y* interaction with potato and tobacco.

   b) The achievement consists of the following single-subject publications:

H1. Yin Z, Chrzanowska M, Michalak K, Zagórska H, Zimnoch-Guzowska E (2012) Recombinants of PVY strains predominate among isolates from potato crop in Poland. J. Plant Prot. Res. 52: 214-219.
(punkty $MNiSzW_{2012}$: **9)**
My contribution was: developing research concept, designing and conducting experiments, analyzing and interpreting results, as well as preparing manuscript. I rate my contribution to the publication at 60%.

H2. 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.
(IF_{2013} **0,951**; punkty $MNiSzW_{2013}$: **30**)
My contribution was: planning studies on the structure of the PVY population and assessing the resistance of cultivars to PVY and preparing the manuscript of subsections: Structure of PVY Population in Poland and Evaluation of Potato Cultivars registered in Poland. I rate my contribution to the publication at 40%.

H3. **Yin Z**, Xie F, Michalak K, Pawełkowicz M, Zhang B, Murawska Z, Lebecka R, Zimnoch-Guzowska E (2017) Potato cultivar Etola exhibits hypersensitive resistance to PVY^{NTN} and partial resistance to PVY^{Z}-NTN and PVY^{N-Wi} strains and strain-specific alterations of certain host miRNAs might correlate with symptom severity. Plant Pathol. 66: 539-550.
(IF_{2017} **2,303;** punkty $MNiSzW_{2017}$: **35**)
My contribution was: developing the research concept and designing the experiments. In addition, I had a leading role in carrying out molecular and biological research, analysis and interpretation of results and preparation of the manuscript. I was the leader of the project covering the research described in this work. I rate my contribution to the publication at 50%.

H4. Yin Z, Murawska Z, Xie F, Pawełkowicz M, Michalak K, Zhang B, Lebecka R **(2018)** microRNA response in potato virus Y infected tobacco shows strain-specificity depending on host and symptom severity. *Virus Res.* https://doi.org/10.1016/j.virusres.2018.11.002. (*Virus Res.* 2019. 260: 20-32)
(IF_{2017} **2,484;** punkty $MNiSzW_{2017}$: **25**)
Impact Factor from 2017, when appropriate data was not available.
My contribution was: developing the research concept, designing and conducting experiments, analyzing and interpreting results, and preparing manuscript. I was the leader of the project covering the research described in this work. I rate my contribution to the publication at 50%.

H5. Yin Z (2018) Host miRNAs and virus-derived small RNAs in plants infected with certain potyviruses. In Plant Viruses: Diversity, Interaction and Management, eds. RK Gaur, SMP Khurana, and Y Dorokhov. Boca Raton, FL: CRC Press. Chapter 17, pp 279-299. (in English).
(punkty $MNiSzW_{2018}$: **5)**
My contribution was: creating the concept of this chapter and preparing the manuscript. My contribution was 100%.

c) Scientific goal and results of the above works and the possibilities of their application

A brief introduction

Potato (*Solanum tuberosum* L.) is the fourth most important crop cultivated in the world after wheat, rice and maize. Poland is the first biggest potato producer in Central and Eastern Europe, with potato yield about 8 mln t per year (Dzwonkowski 2018). An average Pole ate 75.2 kg of potatoes in 2018 indicates a high consumption of potato in Poland (Dzwonkowski et al. 2018). Potato is a vegetatively propagated crop, thus prone to virus infections. In Poland, the most problematic virus in potato production is *Potato virus Y* (PVY) (Kostiw 2011). Tuber yield losses due to PVY infection are substantial, and quality is sometimes impaired by potato tuber necrotic ringspot disease (PTNRD) (Kehoe and Jones 2011). This virus is transmitted in a non-persistent way to the plant, a few hours after virus is acquired by aphids (Hussain et al. 2016). This way of transmission can't be controlled by using chemicals against aphids, because they are not acting fast enough and in proper time. Breeding for resistance to virus diseases is the best control mean. There are two types of resistance, i.e., extreme resistance (ER) and hypersensitive resistance (HR), to PVY in potato (see review Valkonen 2015). ER suppresses virus multiplication in the initially infected cells. HR restricts the virus in the necrotic tissue at the site of infection and prevents virus spreading to other parts of the plants. The genes designated as *Ry* confer ER against all strains of PVY, whereas the HR (*N*) genes are strain-specific.

PVY is among the top 10 plant viruses in molecular plant pathology (Scholthof et al. 2011). *Potato virus Y* is the type member of the genus *Potyvirus*. It possesses a positive – sense single-stranded RNA (ssRNA) genome ca. 9700 nucleotides (nt) in length. In potato, basically, PVY is classified into five strains based on its ability to elicit HR in differential potato cultivars (cvs.) possessing specific HR (*N*) genes, e.g., potato cv. King Edward (*Nc:ny:nz*), cv. Désirée (*nc:Ny:nz*) and cv. Pentland Ivory (*Nc:Ny:Nz*) (Singh et al. 2008). The PVY strains that elicit HR (*N*) genes *Ny*, *Nc* and *Nz* are classified as PVY^{O}, PVY^{C} and PVY^{Z} strains, respectively. The PVY strains that overcome all these three HR genes are classified as PVY^{N} if they cause veinal necrosis (VN) in tobacco, or PVY^{E} if they do not induce VN in tobacco. The strains PVY^{C}, PVY^{O} and PVY^{Z} do not cause VN in tobacco. The strain variants $PVY^{N\text{-}Wi}$ and PVY^{NTN} belong to the PVY^{N} strain. $PVY^{N\text{-}Wi}$ and PVY^{NTN} possess the recombinant genome between PVY^{N} and PVY^{O}. PVY^{Z}-NTN belongs to PVY^{Z} strain. On RNA sequence level, PVY^{Z}-NTN strain is aligned with PVY^{NTN} strain. Both PVY^{NTN} and PVY^{Z}-NTN strains cause PTNRD in sensitive potato cultivars and decrease the tuber quality.

The knowledge about mechanisms of plant defense against biotic stresses, among others, virus infection, is widened following the discovery of genes encoding microRNA (miRNA). They were discovered in all eukaryotes tested so far. miRNAs in different organisms are highly conserved. Plant miRNAs are endogenous non-coding RNAs of 20-24 nt in length that post-transcriptionally regulate eukaryotic gene expression by targeting specific messenger RNAs (mRNAs) for cleavage or translational inhibition (Bartel 2004; Voinnet 2009). They play essential roles in plant development and in biotic and abiotic stress responses including virus infection, among others (Jones-Rhoades et al. 2006; Khraiwesh et al. 2012; Jin et al. 2013; **Yin et al. 2014 c see Załącznik 4**). Certain miRNAs are involved in antiviral immunity by regulating plant disease resistance genes (*R* genes) that encode proteins containing nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains (NBS-LRR) (Li et al. 2012; Shivaprasad et al. 2012).

A book chapter written by me (**H5**, point 4.b of the auto-presentation) summarized, among others, the experimentally validated host miRNAs and their mRNA target expression in different plant species in response to PVY (Table 17.2 in **H5**).

Scientific goal

The original research papers that form my habilitation achievement have the following scientific goals:

(1) to monitor the population changes of PVY in potato crop in Poland and to characterize detected strains of this virus (**H1** and **H2**, point 4.b of the auto-presentation);

(2) to evaluate resistance and reaction of potato cultivars to different PVY isolates (**H2**);

(3) in PVY-host interaction, to study the expression patterns of a set of stress-responsive host miRNAs in PVY-potato (**H3**) and PVY-tobacco (**H4**) pathosystems.

Results of the above works

Paper **H1**, in which I am the first author and co-corresponding author, described biological, serological characterization and strain-typing of 282 PVY isolates.

Paper **H2**, in which I am the co-author, described changings of PVY population and resistance reaction of 113 registered potato cultivars to four PVY isolates.

The results of papers **H1** and **H2** were derived in part from the project 3-6-00-0-01 (2008-2013) and/or the project 3-1-06-0-01 (2008-2013), financed by Ministry of Agriculture and Rural Development of Poland, in which I was the main investigator worked on monitoring of potato viruses and collection of potato viral pathogens (see **Załącznik 4).**

Papers **H3** and **H4**, in which I am the first author and the corresponding author, described the expression pattern of the stress-responsive host miRNAs in PVY-potato and PVY-tobacco interaction, the whole genome sequencing of three PVY isolates and their strain classification using the potato differential cultivars.

The results of papers **H3** and **H4** were derived from two projects, in both of them I was the project leader, i.e., project NN310 304439 (2010-2013) financed by the National Science Center (NCN) of Poland and project Temat 95 (2015-2017) financed by the Ministry of Agriculture and Rural development of Poland, respectively (see **Załącznik 4**).

I. Study of the pathogen – PVY

I.1 Changing of PVY population and characterization of strains occurring in Poland

Recent study indicated that the recombinant PVY variants are found to be prevalent worldwide in potato-growing area. In the UK, a survey of the molecular diversity of PVY indicated that 80-90% belong to the recombinant European PVY^{NTN} group (Davie et al. 2017). In the United States, there is a rise in the recombinant PVY^{N-Wi} strain incidence, from less than 27% in 2011 to 53% in 2015 (Funke et al. 2017). Historically, in Poland, the first PVY^{N-Wi} isolate (named Wi) was identified on the potato cv. Wilga collected from western Poland in 1984 (Chrzanowska 1991) and the first PVY^{NTN} isolate (named 12/94) was detected on a tobacco bait plant grown in a potato field at Młochów in 1994 (Chrzanowska and Doroszewska 1997). In 2018, based on tobacco bait plant assay in the potato field at Młochów, the strains PVY^{N-Wi} and PVY^{NTN} composed 59% and 17% of the PVY population respectively (Yin et al. unpublished).

In this study, the PVY population at Młochów (central Poland) has been monitored continuously on tobacco (cv. Samsun) bait plants, which were placed in the potato fields, mostly every second year since 1980 to 2010 (**H2**). Moreover, a total of 282 PVY isolates, which were collected from potato crops in northern and central Poland from 1995 to 2009, were characterized by serological and biological assays (**H1**). Out of these 282 PVY isolates, 112 isolates collected from 2006 to 2009 were additionally analyzed by one-step triplex Reverse Transcription-Polymerase Chain Reaction (RT-PCR) (**H1**).

Based on Enzyme-Linked Immunosorbent Assay (ELISA) with strain specific monoclonal antibodies (mAbs) and non-specific antibody (Ab) and on symptoms on tobacco, the isolates were classified into PVY^{O} (PVY^{O} serotype, vein clearing, i.e., VCl on tobacco), PVY^{N} (PVY^{N} serotype, veinal necrosis, i.e. VN on tobacco) and $PVY^{N\text{-}Wi}$ (PVY^{O} serotype, VN on tobacco) strain groups. Selected isolates showing positive reaction to PVY^{N} mAb were tested on sensitive potato cv. Vital, Igor and Nicola. The isolates invoking necrosis on tubers were classified as PVY^{NTN} strain. All tested PVY^{N} isolates induced PTNRD, indicating a PVY^{NTN} strain type.

As shown in **H2**, in early 1980s PVY^{O} strain was up to 85-90 % of the PVY population in Poland and it had dropped below 10% since 1986. From 1984 to 2004 $PVY^{N\text{-}Wi}$ strain had dominated in the PVY population and then decreased to 32 % in 2008. However, in 2010, $PVY^{N\text{-}Wi}$ became the dominant form again (62 %). PVY^{NTN} strain had gradually increased in the PVY population since 1994; since 2004, a significant increase of PVY^{NTN} frequency has been noted. It reached 66 % of the population in 2008. Among the isolates tested in 2010, 14 % reacted positively to both PVY^{N} and PVY^{O} specific mAb, what might be indication of mixed infection.

As shown in **H1**, of the 282 PVY isolates tested, 144 isolates were belonging to $PVY^{N\text{-}Wi}$ strain, 126 were PVY^{NTN} strain, and 12 were PVY^{O} strain. Serological and biological assays of 144 isolates of $PVY^{N\text{-}Wi}$ strain showed that 100 isolates were the expected PVY^{O} serotype with VN symptoms on tobacco (cv. Samsun). However, 10 $PVY^{N\text{-}Wi}$ isolates exhibited VCl on tobacco. All the isolates of $PVY^{N\text{-}Wi}$ strain induced severe local lesions (LL) on *Chenopodium amaranticolor*. Out of 126 isolates of PVY^{NTN} strain tested, 76 were typical PVY^{N} serotype with VN on tobacco, but their reactions on *C. amaranticolor* were different: 13 isolates did not show symptoms, 23 isolates induced weak, and 40 isolates induced severe LL. Out of 12 PVY^{O} isolates tested, 4 isolates showed expected PVY^{O} serotype with VCl on tobacco. The remaining isolates of $PVY^{N\text{-}Wi}$, PVY^{NTN} or PVY^{O} were serologically PVY^{N} and PVY^{O} positive or exhibited unpredictable serological and biological reactions.

As shown in **H1, o**ut of the 112 isolates tested by triplex RT-PCR (Rigotti and Gugerli 2007), 71 isolates were identified as $PVY^{N\text{-}Wi}$ strain, 29 isolates were identified as PVY^{NTN} strain, and 12 isolates were identified as the PVY^{O} strain. PVY^{C} strains were not found.

In our continued survey conducted in 2012 and 2014 on PVY population study using tobacco bait plants in Młochów, similar results were obtained, i.e., the dominant strains infecting Polish potato crop were $PVY^{N\text{-}Wi}$ and PVY^{NTN} (**Yin 2017** see **Załącznik 4**). Moreover, similar trend was obtained based on the survey conducted from 2001 to 2012 using potato tuber samples collected from ware potatoes in Northern and Central Poland (**Yin 2017** see **Załącznik 4**).

To sum up, based on our survey (**H1**; **H2**; **Yin 2017** see **Załącznik 4), the recombinant strains PVY$^{N\text{-}Wi}$ and PVY^{NTN} were the predominant forms among the isolates infecting potato in Poland;** the strains PVY^{O}, PVY^{NTN}, PVY$^{N\text{-}Wi}$ and PVY^{Z}-NTN (**H1; H3**), as well as PVY^{E} (Yin et al. unpublished), were identified, but the PVY^{C} strain was not detected, in the population of Polish PVY isolates.

The worldwide spread of PVY^{NTN} and PVY$^{N\text{-}Wi}$ has been explained by their selective advantage over the parent strains (Kerlan 2003/4). The recombinant strains, PVY$^{N:O}$ (PVY$^{N:O}$ is also known as PVY$^{N\text{-}Wi}$ according to Singh et al. 2008) and PVY^{NTN}, were transmitted more efficiently than PVY^{O} by *Myzus persicae* (Mondal and Gray 2017). In Poland, transmissibility by *M. persicae* of PVY^{NTN} and PVY$^{N\text{-}Wi}$ isolates has been found to be higher than that of PVY^{O} and PVY^{N} isolates (Kaliciak and Syller 2009) and PVY^{NTN} was more effectively transmitted than PVY$^{N\text{-}Wi}$ (Kostiw and Trojanowska 2011). In a cultivar exhibiting HR to PVY^{O}, aphid transmission was significantly reduced (Carroll et al. 2016). Moreover, PVY^{NTN} and PVY$^{N\text{-}Wi}$ can frequently escape detection by visual inspection in seed potato certification schemes because of mild symptoms on potato plants (Kerlan 2003/4). This is especially true for Polish isolates of PVY$^{N\text{-}Wi}$ which seem to be more infective to most potato cultivars and causes mild mosaic symptoms, making negative selection in growing seed crops difficult (Chrzanowska 1994).

I.2 Characterization of three PVY isolates in potato indicator cultivars, in tobacco test plant and whole genome sequencing

Three isolates named PVY-3202, PVY-3303 and PVY-3411 were selected to test in potato indicators (**H3**). The three PVY isolates did not induce a HR, i.e. a necrotic reaction, in inoculated leaves of potato cultivars King Edward, Désirée and Pentland Ivory, with the exception of PVY-3303 which induced HR in inoculated leaves and necrosis in the noninoculated upper leaves, i.e., systemic hypersensitive resistance (SHR), in cv. Pentland Ivory. The three isolates caused severe mosaic and leaf deformation in cv. King Edward, mild mosaic in cv. Désirée and severe mosaic in cv. Pentland Ivory in the noninoculated upper leaves. ELISA tests confirmed the systemic infection of the three cultivars by isolates PVY-3202, PVY-3303 and PVY-3411, indicating they overcame the resistance genes *Ny*, *Nc* and *Nz*.

Tobacco (*Nicotiana tabacum* L.) is a commonly used test plant for diagnosis of viruses and is a host of PVY. The three PVY isolates were also tested in tobacco cv. Samsun. The isolates named PVY-3202, PVY-3411 and PVY-3303 caused severe VN (veinal necrosis), mild VN, and milder VCl (vein clearing) (i.e., loss of VN) in tobacco, respectively (**H3; H4**).

Based on multiplex RT-PCR assay, PVY-3202, PVY-3303 and PVY-3411 belong to PVY^{NTN} (B type), PVY^{NTN} (A type) and PVY$^{N\text{-}Wi}$ (B type), respectively (Lorenzen et al. 2006; Chikh-Ali et al. 2010) (**H3**).

The three isolates, i.e., PVY-3202, PVY-3303 and PVY-3411, were sequenced (**H3**). The whole genome phylogenetic tree, consisting of the three isolates sequenced in this study and another 68 PVY isolates selected from the NCBI GenBank, suggests that PVY-3202, PVY-3303 and PVY-3411 were clustered with PVY^{NTN} (B type), PVY^{NTN} (A type) and PVY$^{N:O}$/PVY$^{N\text{-}Wi}$ isolates, respectively. The NCBI GenBank accession numbers for PVY-3202, PVY-3303 and PVY-3411 are KX356068, KX356069 and KX356070, respectively.

To sum up, the strain classification of the tested three isolates is based on the combined biological and sequence features. The isolates PVY-3202 and PVY-3411, which overcame the *Ny*, *Nc* and *Nz* HR genes in potato indicators and induced VN in tobacco, were classified as PVY^{N} strain according to Singh et al. (2008). Multiplex RT-PCR and whole genome sequencing further classified the isolates PVY-3202 and PVY-3411 as the recombinant PVY^{NTN} (PVY^{NTN} B type) and $PVY^{N\text{-}Wi}$ ($PVY^{N\text{-}Wi}$ B type) strain, respectively. Both PVY^{NTN} and $PVY^{N\text{-}Wi}$ are the variants of PVY^{N} strain (Singh et al. 2008). The isolate PVY-3303 clearly induced HR in cv. Pentland Ivory possessing the *Nz* gene (although the resistance was not strong enough to localize the virus at the infection sites), and it did not induce VN (induced VCl only) in tobacco, therefore the isolate PVY-3303 is classified as PVY^{Z} strain according to Singh et al. (2008). Additionally, PVY-3303 induced PTNRD in potato cv. Etola, it is further classified as PVY^{Z}-NTN strain. Furthermore, multiplex RT-PCR and whole genome sequencing confirmed that, on RNA level, the isolate PVY-3303 is classified as PVY^{NTN} (PVY^{NTN} A type) strain.

I.3 Comparison of HC-Pro in the three PVY isolates

The helper component proteinase (HC-Pro) from the *Potyviridae* family including PVY, is an essential and multifunctional protein, involving in virus infection (plant to plant transmission, long distance viral movement and development of disease symptoms in the host), plant defense responses and RNA silencing suppression, among others (Valli et al. 2018).

Therefore, we compared the amino acid (aa) sequence and the predicted three-dimensional (3D) structure of the HC-Pro among the three PVY isolates PVY-3202, PVY-3303 and PVY-3411 (**H4)**. Numbering of the PVY HC-Pro residues is based on the P1/HC-Pro cleavage site determined by a comparison of a large number of potyviral genomes (Adams et al., 2005) which is nine residues downstream from the original position used for the numbering of residues by Tribodet et al. (2005) and Hu et al. (2009) and is comparable to the numbering used by Tian and Valkonen (2013).

Five aa residues were different among HC-Pro in the three isolates (**H4**).

(1) H_{73} is present in all the three isolates, however the H_{73} in PVY-3303 is encoded by CAY (nt 1228-1230, Y=C+T) and both CAC and CAT encode aa residue H. H_{73} in PVY-3303 represents an example of synonymous single nucleotide polymorphism (SNP). In this case, on RNA level, single nucleotide change occurred in some of the RNA molecules only, resulted in two types of RNA molecules. The two nucleotides, i.e., C or T which were presented at the position nt 1230, did not cause changes in the encoded aa on protein level. Finally, these two types of RNA molecules were translated into the same protein.

(2) Another example of synonymous SNPs is S_{434} in PVY-3411.

(3) X_{252} in PVY-3303 represents two aa residues I_{252} and V_{252}, which is differed than I_{252} in PVY-3202 and PVY-3411. The X_{252} (I_{252} and V_{252}) at the aa position 252 is encoded by RTT (nt 1765 -1767, R=A+G), and the genetic codes ATT and GTT encode for aa residues I and V respectively. $I_{252}V$ represent an example of single amino acid polymorphism (SAP), also known as non-synonymous single nucleotide polymorphism (nsSNP) (Huang et al. 2012). In such case, on RNA level, single nucleotide change occurred in some of the RNA molecules only, resulted in two types of RNA molecules. The two nucleotides, i.e., A and G which were presented at the position nt 1765, indeed caused changes in the encoded aa and resulted in two aa residues. Finally the two types of RNA molecules were translated into two types of proteins. Of these two aa residues, I_{252} is a PVY^{N}-specific signature that overcomes the potato HR gene

Ny recognizing the PVY^O strains, while V_{252} is a PVY^O-specific signature that induces the HR gene *Ny* in potato (Tian and Valkonen 2013).

(4) R_{412} in PVY-3303 is differed than Q_{412} in PVY-3202 and PVY-3411. A single nucleotide change (A_{2242} to G_{2246}) resulted in the single aa change (Q_{412} to R_{412}).

(5) N_{263} in PVY-3411 is differed than K_{263} in PVY-3202 and PVY-3303. A single nucleotide change (G_{1796} to T_{1796}) resulted in the single aa change (K_{263} to N_{263}).

Some other conserved motifs were present in the HC-Pro in all the three isolates (**H4**), including:

- The six PVY^N-like aa residues needed for the induction of VN in tobacco (Tribodet et al. 2005; Faurez et al. 2012; Tian and Valkonen 2015)
- The FRNK motif (aa 179-182), a probable point of contact with small interfering RNA (siRNA) and miRNA duplexes (Shiboleth et al. 2007)
- The RNA binding motifs RNP-2 (IGN) (aa 246-251) and RNP-1 (aa 282-289) and the CCCT motif (aa 290-293) related to long-distance viral movement (Cronin et al. 1995; Maia et al. 1996; Urcuqui-Inchima et al. 2000)
- The eight PVY^N-specific aa "signatures", which overcome the potato HR gene Ny_{tbr} recognizing the PVY^O strains (Tian and Valkonen 2013), except the SAPs $I_{252}V$ in PVY-3303

Modeling of the 3D structure of HC-Pro in the three isolates was carried out using the web server I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) (**H4**).

The five aa residues that are different among the HC-Pro in the three isolates, i.e., H_{73} SNP, $I_{252}V$ SAP, $R_{412}Q$ and $N_{263}K$ substitutions and S_{434} SNP, did not cause changes in the 3D structure of the corresponding proteins (**H4**). For PVY-3303 HC-Pro, two variants were analyzed, HC-Pro I_{252} and HC-Pro V_{252}. The predicted 3D structure of PVY-3303 HC-Pro I_{252} and HC-Pro V_{252} was the same.

The conserved motifs RNP-1, CCCT and the IGN in RNP-2 (LAIGNL) were located in the same structure in the HC-Pro in PVY-3202, PVY-3303 and PVY-3411.

Differences in the 3D structure were only detected in HC-Pro in the isolate PVY-3411 (**H4**). For the PVY-3411 HC-Pro, the aa residues M_{17} was located in a coil structure, L_{238} was in a strand and T_{243} in coil structure; whereas those for PVY-3202 and PVY-3303 were located in a helix, coil and strand structure respectively.

To sum up, **the PVY^Z-NTN isolate named PVY-3303 identified in this study is the first described of its type in Europe** (**H3**) and this study demonstrated the possible new genetic elements related to loss of VN in tobacco (**H4**). Previously, a PVY^Z-NTN isolates named L26 infecting potato in USA was characterized (Hu et al. 2009; Kerlan et al. 2011). Multiple alignment of the whole genome sequences of L26 and other PVY^{NTN} isolates that cause VN in tobacco, suggests that a single aa change (D_{196} to G_{196}) in the HC-Pro in L26 correlates with the loss of VN in tobacco (Hu et al. 2009). However, in HC-Pro in PVY-3303 identified in this study (**H4**), the D_{196} is present, indicating that the VN genetic determinant of PVY in tobacco is complex, and includes other elements (the identified possible other elements are described in details in point 4c ***III.8*** of the auto-presentation „***Possible involvement of PVY HC-Pro in symptom development and defense response***".

In addition, to my knowledge, although SNPs have been used as the genotypic markers for discrimination between PVY strains (PVY^{O}, PVY^{N}, PVY^{NTN} and PVY^{N-Wi}) (Jacqcot et al. 2005; Rolland et al. 2008; Rupar et al. 2013), SAPs found within a single isolate have not been published previously. The quasispecies nature of RNA viruses is well known, and it refers to the fact that a single virus isolate does not contain the same nucleotide sequence, but consists of mutant spectra (Domingo et al. 2012).

I.4 Summary of the PVY research

1. PVY^{N-Wi} (since 1984) and PVY^{NTN} (since 1994) were the predominant strains infecting ware potato in Poland. (**H1; H2**)
2. The whole genomes of three isolates representing PVY^{NTN}, PVY^{N-Wi} and PVY^{Z}-NTN were sequenced. (**H3**)
3. The unique PVY^{Z}-NTN strain is the first described of its type in Europe. (**H3**)
4. Mutations, e.g., SNPs, SAPs, amino acid substitutions and differences in the predicted 3D structure, were found in the RNA silencing suppressor (RSS) PVY HC-Pro in the three isolates sequenced in **H3**. (**H4**)
5. Putative new genetic elements in HC-Pro which might be related to VN in tobacco were found. (**H4**)

II. Research on the host – potato and tobacco

II.1 Evaluation resistance and reaction of potato cultivars to PVY isolates

Out of 133 potato cultivars registered in Poland in 2009, 113 cultivars were evaluated for resistance and reaction to PVY (**H2**). For these 113 cultivars, the level of resistance to PVY was assessed in field resistance tests in a 1 to 9 grade scale, where 1 means susceptible and 9 extremely resistant. In addition, the reaction of these cultivars to PVY was examined by mechanical inoculation in greenhouse conditions using four isolates. The four PVY isolates used were: the isolate LW (PVY^{O} strain, collected in 1970, NCBI GenBank accession number AJ890349), the isolate Ny (PVY^{N} strain, collected in 1974, FJ666337), the isolate Wi (PVY^{N-Wi} strain, collected in 1984, EF558545) and the isolate 12/94 (PVY^{NTN} strain, collected in 1994, AJ889866). According to percentage of infected plants, infection symptoms and ELISA readings, the examined potato cultivars were classified as: (1) ER; (2) HR; (3) relatively resistant (R); (4) moderately resistant (mR); (5) susceptible (S). Out of 113 cultivars tested, 45 cultivars showed resistance (i.e., 23 ER, 11 HR and 11 R or mR) to all isolates and 24 cultivars were susceptible to all isolates, 44 cultivars were resistant (R or mR) to some isolates but susceptible to others. Cultivars varying in sensitivity to the PVY isolates, in majority, were more sensitive to isolates Wi and 12/94, than to older isolates LW and Ny.

II.2 Strain-specific resistance to PVY of potato cultivar Etola based on HR

Polish potato cultivar Etola was released in 2009, with a PVY resistance score of 5-6 in 9 grade scale, where 9 means ER. In a few years of evaluation of resistance to PVY under greenhouse conditions conducted by us, cv. Etola showed HR to a PVY^{NTN} isolate but was only partially resistant or susceptible to some other isolates. Therefore, the reaction type of cv. Etola

to the three newly sequenced isolates, i.e., PVY-3411 (PVY^{N-Wi}), PVY-3303 (PVY^{Z}-NTN) and PVY-3202 (PVY^{NTN}), was further investigated (**H3**).

Cv. Etola showed HR to the isolate PVY-3202 (PVY^{NTN}) (**H3**). PVY-3202 caused HR, i.e., necrotic reactions, in the inoculated leaves. However, the noninoculated upper leaves showed no symptoms and lacked infection based on ELISA results. Moreover, no accumulation of PVY *HC-Pro* RNA was detected in the noninoculated upper leaves, which further confirmed no systemic viral multiplication in Etola plants inoculated with PVY-3202.

Cv. Etola showed different levels of partial HR to the PVY^{N-Wi} and PVY^{Z}-NTN isolates (**H3**). The isolates PVY-3411 (PVY^{N-Wi}) and PVY-3303 (PVY^{Z}-NTN) caused HR and/or VN in the inoculated leaves but to a lesser extent than did PVY-3202. However, the resistance was not strong enough to localize the virus at the infection sites. The virus spread to the noninoculated upper leaves, caused SHR, i.e., necrotic reactions in the upper noninoculated leaves, and induced symptoms, e.g., severe SHR, severe mosaic and severe VN by PVY-3411, mild SHR and mild VN by PVY-3303 (**H3**). The isolate PVY-3411 spread and accumulated faster, with a higher abundance of viral coat protein (CP) and *HC-Pro* RNA detected, relative to the isolate PVY-3303.

To date, the HR (*N*) genes Ny_{tbr} for the PVY^{O} strain, Nc_{tbr} and Nc_{spl} for the PVY^{C} strain, Nz_{tbr} for the PVY^{Z} strain and *Nd* for a newly defined PVY^{D} strain have been identified (Singh et al. 2008; Moury et al. 2011; Tian and Valkonen 2013; Chikh-Ali et al. 2014; Kehoe and Jones 2016). Rowley et al. (2015) demonstrated the presence of three new putative HR (*N*) genes, i.e., *Nw* resistance against PVY^{N-Wi}, *Nne* resistance against PVY-NE11 and *Ne* resistance against PVY^{E}, that may confer resistance to multiple strains of PVY in cv. Yukon Gem in North America.

In our study, cv. **Etola represents a new source of multiple strain-specific resistance genes against three strains of PVY** (**H3**). We concluded that **cv. Etola has *Nz* gene**, which is elicited by the newly identified PVY^{Z}-NTN strain (the isolate PVY-3303), in its background, **providing complete resistance against the PVY^{NTN} recombinant and different levels of partial resistance against PVY^{Z}-NTN and PVY^{N-Wi} recombinants.**

II.3 Summary of the research on PVY hosts

1. Potato cultivars vary in resistance reaction to PVY. (**H2**)
2. Potato cultivar Etola, demonstrated in this study, represents the new source of resistance available in potato. (**H3**)
3. Cv. Etola has, among others, *Nz* gene providing HR against multiple strains (**H3**):
   HR to PVY^{NTN}, no symptoms
   Partial HR to PVY^{N-Wi}, severe symptoms
   Partial HR to PVY^{Z}-NTN, mild symptoms
4. Tobacco cv. Samsun shows strain-specific symptoms after infection with different strains of PVY (**H4**) (see point 4.c ***I.2*** of the auto-presentation):
   Severe VN to PVY^{NTN}
   Mild VN to PVY^{N-Wi}
   Milder VCl to PVY^{Z}-NTN

III. Study on the PVY-host interaction

III.1 PVY-host interaction in relation to miRNA – a hypothesis

Our findings in this study suggest that the same host can exhibit different reactions to different PVY strains. Therefore, my further research is focused on PVY-host strain-specific interaction and the selected subject is miRNA. The hypothesis is that in a host having the same genetic background, different PVY strains would cause changes in the studied molecular aspects, e.g., miRNA expression; and the miRNA alteration patterns would be related to the specific strains.

Two hosts, i.e., potato cv. Etola showing strain-specific HR and tobacco cv. Samsun showing strain-specific symptoms to PVY, and three PVY strains, i.e., PVY^{NTN} strain represented by the isolate PVY-3202, PVY^{N-Wi} strain represented by the isolate PVY-3411 and PVY^{Z}-NTN strain represented by the isolate PVY-3303, were used. We analyzed a group of stress-responsive miRNAs: 10 miRNAs and 14 mRNA targets in PVY-potato interaction, 26 miRNAs and 23 mRNA targets in PVY-tobacco interaction.

III.2 Strain-specific alteration of miRNAs and targets in PVY-inoculated potato cv. Etola

In PVY-potato interaction, the tested miRNAs and targets showed strain-specific alteration in HR and partial HR reaction and were related to symptom severity (see section 4.c ***III.7*** of the auto-presentation) and *HC-Pro* RNA level (see point 4.c ***III.6*** of the auto-presentation) (**H3;** Table 1).

In the non-inoculated upper leaves, the tested miRNAs and their mRNA targets were altered only in the Etola plants infected with the isolate PVY-3411 (PVY^{N-Wi}) that caused severe symptoms in leaves (**H3**). However, in the plants infected with the isolate PVY-3303 (PVY^{Z}-NTN) showing mild symptoms and in the plants inoculated with the isolate PVY-3202 (PVY^{NTN}) that were resistant to PVY-3202 (PVY^{NTN}) (i.e., no infection) and showing no symptoms, the same set of miRNAs and their mRNA targets remained unchanged. The parallel increase of stu-miR162, stu-miR168a and miR172e, together with their targets, i.e., *AGO1-2*, *DCL1* and *TOE3* respectively, in PVY-3411-infected plants correlated with high abundance of *HC-Pro* RNA encoding an RSS and might be linked with the severe symptoms in leaves. Moreover, PVY-3411 caused parallel increase in two members of stu-miR482 and their mRNA targets *Gpa2* and *CC-NBS-LRR* that are involved in defense response (**H3**).

The full description for the names of genes *AGO1-2*, *DCL1*, *TOE3*, *Gpa2* and *CC-NBS-LRR* are referred to the section "**Abbreviations**" of the auto-presentation.

Very recently, Križnik et al. (2017) studied the role of small RNAs, including miRNA, in tolerance response of potato cv. Désirée to PVY^{NTN} in the inoculated local leaves 3 dpi, before virus could be detected. Down-regulation of gibberellin signaling by miR167, regulation of immune receptor transcripts by miR6022 as well as up-regulation of miR164, miR167, miR169, miR171, miR319, miR390, and miR393 in tolerant cv. Désirée were observed (Križnik et al. 2017). However, our study provided **the first example of strain-specific alteration of a set of host miRNAs and their targets in the potato-PVY resistance interaction (H3**).

Table 1
Alteration of host miRNAs and targets in PVY-potato and PVY-tobacco interaction (**H3; H4**)

Host	PVY strain[a]	Severe or mild strain for the respective host	Host defense response	Host symptoms	HC-Pro RNA level (REL)	miRNAs / targets alteration	
						Number of genes	Expression change
Potato cv. Etola	PVY^{NTN}	No infection	HR	no symptoms	0	0	nc
	PVY^{N-Wi}	Severe strain	Partial HR	severe SHR severe mosaic severe VN	5.18	13	↑1.4-5.7
	PVY^{Z}-NTN	Mild strain	Partial HR	mild SHR mild VN	0.13	1	↑3.0
Tobacco cv. Samsun	PVY^{NTN}	Severe strain	Inefficient HR	Severe VN	85	31	↑ 1.4-38 (76[b]) ↓ 0.09-0.6
	PVY^{N-Wi}	Severe strain	Inefficient HR	mild VN	94	29	↑ 1.5-24 (71[b]) ↓ 0.18-0.7
	PVY^{Z}-NTN	Mild strain	Unknown	Loss of VN (milder VCl)	40	9	↑ 1.7-3.4 (39[b]) ↓ 0.3-0.7

[a]The PVY^{NTN}, PVY^{N-Wi} and PVY^{Z}-NTN strains were represented by the isolates PVY-3202, PVY-3411 and PVY-3303 respectively.
[b]The highest induction of a miRNA, miR482.
cv.: cultivar.
Expression change: the fold of the relative expression level of a given gene in the PVY-infected plants to that of the mock-inoculated ones.
HR: hypersensitive resistance, i.e., the necrotic reaction in the inoculated leaves.
nc: no change.
REL: relative expression level of a given gene, which were normalized to the reference gene.
SHR: systemic HR, necrosis in the noninoculated upper leaves.
VCl: vein clearing.
VN: veinal necrosis.
↑: up-regulation.
↓: down-regulation.

III.3 Strain-specific alteration of miRNAs and targets in PVY-infected tobacco cv. Samsun

Paper H4 provided **the first information on how different PVY strains affected the miRNA balance in tobacco** cv. Samsun **(H4)**. The observed strain-specific alteration of miRNAs and their targets corresponded to the symptom severity (see point 4.c ***III.7***) and the viral *HC-Pro* RNA levels (see point 4.c ***III.6*** of the auto-presentation) (**H4;** Table 1).

In PVY-tobacco interaction, the strains PVY^{NTN} (PVY-3202) and PVY^{N-Wi} (PVY-3411) caused severe and mild VN in the upper non-inoculated leaves of tobacco respectively, whereas the PVY^{Z}-NTN strain (PVY-3303) induced milder VCl (**H4**). The abundance of 18 out of the 26 tested miRNAs increased upon infection by the severe strains PVY^{NTN} and PVY^{N-Wi}. Expression of a group of defense related transcripts were increased, while the down-regulated mRNAs were related to regulation of transcription, protein phosphorylation and cell differentiation (**H4**). The mild PVY^{Z}-NTN strain caused increase in 3 tested miRNAs only. For the same set of miRNAs and targets being altered by PVY^{NTN} and PVY^{N-Wi} strains or by all the three strains, the most severe PVY^{NTN} strain caused greater changes in their levels than did the severe one PVY^{N-Wi} or the mild PVY^{Z}-NTN.

Plant miRNAs perfectly or near perfectly complement their mRNA targets, leading to target cleavage and degradation (Bartel 2004). Thus, the miRNAs and their targets essentially show mutually antagonistic expression levels in a virus-infected plant (Naqvi et al 2010). However, a parallel increase in expression of target mRNAs and the corresponding miRNAs was also frequently observed in virus-infected plants, particularly at the later stage of infection. In the later stage of infection, the viral proteins, e.g., RSS, may inhibit miRNA activity. For example, the potyvirus P1/HC-Pro, an RSS, which was expressed in transgenic *Arabidopsis* plants, clearly inhibited miRNA* turnover, suggesting that miRNA/miRNA* unwinding and RNA-induced silencing complex (RISC) assembly was suppressed (Chapman et al 2004). The *Turnip mosaic virus* (TuMV)-encoded RSS P1/HC-Pro interfered with the activity of *Arabidopsis* miR171, which directs cleavage of several mRNAs coding for Scarecrow-like transcription factors, by inhibiting its nucleolytic function (Kasschau et al 2003).

The set of PVY responsive tobacco miRNAs identified in this study are among those described as biotic and abiotic stress-responsive ones, e.g., in *Tobacco mosaic virus* (TMV)-infected tobacco (Bazzini et al. 2011; Khraiwesh et al. 2012). The data obtained in this study (**H4**) confirmed the recent findings on the up-regulation of the members of nta-miR159, nta-miR319 and nta-miR166 in PVY^{N} infected tobacco plants (Guo et al. 2017).

In contrast, although the enhanced expression of the members of nta-miR172, nta-miR390, nta-miR6025 and nta-miR6164 in PVY^{NTN} and PVY^{N-Wi} infected tobacco was observed in this study (**H4**), the expression of the same set of miRNAs was down-regulated in PVY^{N} infected tobacco as demonstrated by Guo et al. (2017), which might indicate strain-specificity in host miRNA expression. Similarly, the expression of nta-miR396b was up-regulated and that of nta-miR164 was down-regulated in PVY^{N} infected tobacco (Guo et al. 2017), their expression level remain unchanged in PVY^{NTN}, PVY^{N-Wi} or PVY^{Z}-NTN infected tobacco as shown in this study (**H4**).

III.4 The strain-specific alteration of miRNAs is host dependent

This study provided additional evidence that the strain-specific alteration of miRNAs is host dependent (**H3; H4**).

The same PVY strain that in one host caused severe symptoms and changes in miRNA levels, in another host neither induced symptoms nor altered miRNA expression, e.g., PVY^{NTN}-infected tobacco showing inefficient HR, severe VN and alteration in miRNAs or PVY^{NTN}-inoculated potato cv. Etola showing complete HR, no symptoms, and no altered miRNA expression (**H3; H4;** Table 1).

On the other hand, different or the same strains, which caused severe symptoms in different hosts, would lead to the alteration of miRNAs, e.g., in PVY^{N-Wi}-infected potato cv. Etola showing partial HR with severe mosaic, severe VN and severe SHR, and in PVY^{NTN}- or PVY^{N-Wi}-infected tobacco showing inefficient HR with severe VN (**H3; H4;** Table 1).

III.5 The strain-specific alteration of miRNAs is related to defense response

In PVY-Etola interaction, the strain-specific altered miRNAs are related to the partial HR with severe symptoms in PVY^{N-Wi} infected potato cv. Etola (**H3**).

In PVY-tobacco interaction, the strain-specific altered miRNAs are observed in PVY^{NTN}- and PVY^{N-Wi}-infected tobacco showing VN (**H4**). Recent findings by Michel et al. (2018) suggested that the PVY^{N}-induced VN in tobacco likely represents an inefficient defense response with HR-like characteristics.

Based on the data obtained in **H3** and **H4,** it might be inferred that the strain-specific alteration of the tested host miRNAs in both PVY-potato and PVY-tobacco interaction is involving an HR response.

III.6 The strain-specific alteration of miRNAs and targets is related to the levels of PVY RSS HC-Pro

Previously, strain-specific alteration of host miRNAs has been observed in *Cucumber mosaic virus* (CMV)-infected tomato and *Arabidopsis* plants (Cillo et al. 2009; Du et al. 2014). The 2b protein, an RSS derived from the severe strain CMV-Fny but not from the mild strains CMV-LS or CMV-Q, has been shown to block Argonaute 1 (AGO1) activity and impair proper miRNA-guided mRNA cleavage in *Arabidopsis* (Chapman et al. 2004; Zhang et al. 2006; Lewsey et al. 2007). The potyvirus multifunctional HC-Pro is an RSS and may interfere with plant development and miRNA function (Kasschau et al. 2003).

In this study, in PVY-potato interaction, the levels of *HC-Pro* RNA in severe isolate PVY-3411 (PVY^{N-Wi})-infected potato cv. Etola plants was much higher (40-fold) than that in mild isolate PVY-3303 (PVY^{Z}-NTN)-infected plants, and no *HC-Pro* RNA was detected in PVY-3202 (PVY^{NTN})-inoculated Etola that was resistant to PVY-3202 (**H3**). In PVY-tobacco interaction, higher levels of PVY *HC-Pro* RNA were detected in the severe isolates PVY-3202- and PVY-3411-infected tobacco plants, and lower levels in the mild PVY-3303 infected ones (**H4**).

Therefore, in both host, higher extent alteration of miRNAs and targets was observed in the PVY^{N-Wi}-infected potato and in PVY^{NTN}-or PVY^{N-Wi}- infected tobacco, which showed a higher levels of *HC-Pro* RNA accumulation, compared to that in the PVY^{Z}-NTN-infected potato and tobacco showing lower levels of *HC-Pro* RNA (**H3; H4**; Table 1).

III.7 The strain-specific alteration of miRNAs is related to symptom severity

Previous studies indicated that severity of symptoms, caused by either DNA or RNA viruses, is correlated with miRNA accumulation (Bazzini et al. 2007; Naqvi et al. 2010). miR159/319 and miR172 might be linked to with tomato leaf curl disease caused by infection with *Tomato leaf curl New Delhi virus* (ToLCNDV) (Naqvi et al. 2010). Du et al. (2014) suggested that the severe CMV-Fny strain contributes directly to pathogenicity by perturbing miR159 activity, and severe disease symptoms resulted from the deregulation of the miR159 targets *MYB33* and *MYB65*.

In this study, it can be proposed that certain altered miRNAs in the PVY^{N-Wi} infected potato cv. Etola plants, e.g., stu-miR168a, stu-miR162 and stu-miR172e, might be linked with the severe symptoms in leaves (**H3**).

Moreover, our results confirmed the recent findings by Michel et al. (2018) that the PVY^{N} induced VN in tobacco is likely an inefficient HR response and the tobacco *NBS-LRR NtTPN1* (*R*) gene and signal transduction is required. In this study, a group of defense related miRNAs and the targeted transcripts were indeed up-regulated in tobacco plants infected with the severe strains PVY^{NTN} and PVY^{N-Wi} showing VN, but remained unchanged in the mild strain PVY^{Z}-NTN infected ones showing VCl (**H4**). Among them, three *TMV N* transcripts targeted by nta-miR6020a-5p and nta-miR6164a/b are belonging to the *TIR-NBS-LRR* resistance gene family and are involved in the signal transduction based on a gene ontology (GO) analysis (**H4**). Therefore, it can be inferred that the up-regulation of **nta-miR6020a-5p and nta-miR6164a/b might correlate with the PVY^{NTN} and PVY^{N-Wi} induced VN in tobacco** observed in this study (**H4**).

The full description for the genes *MYB33, MYB65, NtTPN1, TMV N and TIR-NBS-LRR* are referred to the section "**Abbreviations**" of the auto-presentation.

III.8 Possible involvement of PVY HC-Pro in symptom development and defense response

Previous study demonstrated six PVY^{N}-like aa residues needed for the induction of VN in tobacco, i.e., N_{330}, K_{391} and E_{410} (Tribodet et al. 2005; Faurez et al. 2012) and N_{330}, R_{338}, F_{341} and I_{346} (Tian and Valkonen 2015). All these PVY^{N}-like aa residues are present in the HC-Pro in the three isolates, i.e., PVY-3202 (PVY^{NTN}) (severe VN in tobacco), PVY-3303 (PVY^{Z}-NTN) (milder VCl in tobacco) and PVY-3411 (PVY^{N-Wi}) (VN in tobacco), used in this study (**H4**), indicating involving of other elements in PVY-induced symptoms in tobacco.

By comparing the HC-Pro among the three isolates, for the first time, this study demonstrated that **additional elements**, i.e., **the SAP $I_{252}V$ and the Q_{412} to R_{412} substitution**, in the HC-Pro in the PVY^{Z}-NTN (isolate PVY-3303) **might be related to the loss of VN in tobacco** compared to that of PVY-3202 causing severe VN in tobacco (**H4**). The different 3D structure predicted for **M_{17}** (coil), **L_{238}** (strand) and **T_{243}** (coil) and the **K_{263} to N_{263} substitution**, in the HC-Pro in the PVY^{N-Wi} (isolate PVY-3411) **might be related to** the mild VN (i.e., **reduced severity of VN**) in tobacco compared to that of PVY-3202 causing severe VN (**H4**).

The eight PVY^{N}-specific aa "signatures", i.e., N_{236}, L_{238}, A_{247}, I_{252}, R_{262}, K_{269}, R_{270}, and V_{301}, which overcome the potato HR gene *Ny_{tbr}* recognizing the PVY^{O} strains (Tian and Valkonen 2013), are present in the HC-Pro in the three isolates, i.e., PVY-3202, PVY-3303 and PVY-3411, used in this study, except the SAP $I_{252}V$ in PVY-3303.

In this study, the **V_{252}**, **a PVY^{O} specific signature** which induces *Ny* HR gene in potato, in the $I_{252}V$ SAP in the PVY^{Z}-NTN strain (PVY-3303) **might be related to the partial HR with mild symptom in potato** cv. Etola (**H3; H4**). The different confirmation of **L_{238}**, **a PVY^{N} specific signature** to overcome the HR gene *Ny_{tbr}* in potato, in a strand structure in PVY^{N-Wi} strain (PVY-3411) **might be related to the partial HR with severe symptoms in cv. Etola** (**H3; H4**).

III.9 Summary of the study on PVY-host interaction

1. This study provides the first example of strain-specific alteration of a set of host miRNAs and their targets in PVY-potato and PVY-tobacco interaction. (**H3; H4**)
2. Majority of tested miRNAs are up-regulated only in plants infected with strains causing severe symptoms - (**H3; H4**)
   in PVY^{N-Wi} infected potato cv. Etola
   in PVY^{NTN} and PVY^{N-Wi} infected tobacco
   but not in the mild strain PVY^{Z}-NTN infected hosts
3. A set of defense-related *NBS-LRR* (*R*) gene targets are up-regulated in both hosts. (**H3; H4**)
4. Two miRNAs, miR6020a-5p and miR6164a/b targeting the *TIR-NBS-LRR TMV N* (*R*) genes, which involve in signal transduction, might correlate with the PVY^{NTN} and PVY^{N-Wi} induced VN in tobacco. (**H4**)
5. A SAP ($I_{252}V$, the V_{252}) and a Q_{412} to R_{412} substitutions in the HC-Pro of the PVY^{Z}-NTN strain might be related to the loss of VN in tobacco. (**H4**)
6. The different 3D secondary structure predicted for M_{17} (coil), L_{238} (strand) and T_{243} (coil) and the K_{263} to N_{263} substitution, in the HC-Pro of the PVY^{N-Wi} strain might be related to the mild VN (i.e., reduced severity of VN) in tobacco. (**H4**)
7. The V_{252}, a PVY^{O} specific signature which induces HR gene *Ny* in potato, in the $I_{252}V$ SAPs in the HC-Pro of PVY^{Z}-NTN strain might be related to the partial HR with mild symptom in potato cv. Etola. (**H4; H3**)
8. The different confirmation of L_{238}, a PVY^{N} specific signature to overcome the HR gene *Ny* in potato, reside in a strand structure in the HC-Pro of PVY^{N-Wi} strain might relate to the partial HR with severe symptoms in cv. Etola. (**H4; H3**)

III.10 Conclusion on miRNA study

The PVY-responsive miRNAs and their targets are:
Strain-specific
Host-dependent
Defense response related
Symptom severity related
PVY RSS HC-Pro related

The possibilities of an application of studies on PVY, its host and pathogen-host interaction

The results of virological and molecular research presented here have both basic and applied character.

1. The identified new source of PVY resistance in potato, i.e., *Nz* gene in cv. Etola, is important in potato breeding and production, providing resistance to multiple strains of this virus.

2. The identified PVY-responsive or HR-related miRNAs from potato or tobacco and the mutations found in PVY multifunctional HC-Pro provided new elements, and may lay foundation for study on mechanism(s) involved in PVY-host interaction.

Abbreviations

3D structure: Three-dimensional structure.
aa: Amino acid.
Ab: Antibody.
AGO1: Argonaute 1.
AGO1-2: Isoform 2 of Argonaute 1.
CC-NBS-LRR: *R* genes encoding the proteins with the coiled-coil/nucleotide-binding site/leucine-rich repeat domains.
CMV: *Cucumber mosaic virus*.
CP: Coat protein.
cv.: Cultivar.
DCL1: Endoribonuclease Dicer homologue 1.
ER: Extreme resistance.
ELISA: Enzyme-Linked Immunosorbent Assay.
GO: Gene ontology.
Gpa2: Disease resistance protein Gpa2.
HC-Pro: Helper component proteinase.
HR: Hypersensitive resistance.
ITEs: Independent transformation events.
LL: Local lesions.
mAbs: Monoclonal antibodies.
miRNA: microRNA.
mRNAs: messenger RNAs.
MYB33: MYB domain protein 33.
MYB65: MYB domain protein 65.
NBS-LRR: *R* genes encoding proteins with nucleotide binding site and leucine-rich repeat domains.
nc: no change.
nsSNP: Non-synonymous single nucleotide polymorphism.
nt : Nucleotides.
NtTPN1: *R* gene encoding *Nicotiana tabacum* Tolerance to PVY-induced Necrosis 1 protein.
PMTV: *Potato mop-top virus*.
PTNRD: Potato tuber necrotic ringspot disease.
PVY: *Potato virus Y*.
R genes: Plant disease resistance genes.
REL: relative expression level.
RISC: RNA-induced silencing complex.
RSS: RNA silencing suppressor.
RT-PCR: Reverse Transcription-Polymerase Chain Reaction.
SAP: Single amino acid polymorphism.
SHR: Systemic hypersensitive resistance.
siRNA: small interfering RNA.
SNP: Single nucleotide polymorphism.
ssRNA: single - stranded RNA.

TIR-NBS-LRR: *R* genes encoding the proteins with the Toll-interleukin-1 receptor/nucleotide-binding site/leucine-rich repeat domains.
TMV: *Tobacco mosaic virus*.
TMV N: The tobacco *N* gene conferring resistance to *Tobacco mosaic virus*.
TOE3: Apetala 2-like ethylene-responsive transcription factor TOE3-like.
ToLCNDV: *Tomato leaf curl New Delhi virus.*
TRV: *Tobacco rattle virus*.
TuMV: *Turnip mosaic virus.*
VN: Veinal necrosis.
VCl: Vein clearing.

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5. Other research and development achievements

The subject of my Bachelor of Science study in Hebei Agricultural University in China was breeding for wheat cultivars resistant to fungal pathogen *Blumeria graminis f. sp. tritici* and *Puccinia recondita f. sp. tritici*, which causes powdery mildew of wheat and leaf rust of wheat, respectively. The F_1 generation was characterized in respect to agronomic traits, resistance index and grain quality.

After graduation with a Bachelor of Science degree in agronomy in 1987, I was employed at the Cotton Institute, Hebei Academy of Agricultural and Forestry Sciences in China. As an assistant, I have been working on breeding new cotton cultivars resistant to fungal pathogens *Fusarium vasinfectun* and *Verticillium alboatrum*, which cause Fusarium wilt of cotton and Verticillium wilt of cotton, respectively. I participated in different stages of hybridization breeding, including, selection of the parental lines, hybrid pollination, selection of the breeding lines based on resistance, agronomic and quality traits, selfpollination for producing the homozygous lines and the regional tests of cotton cultivars. I worked on characterization and regional tests of the breeding line named 492, which was bred through distant crossing among upland cotton (*Gossypium hirsutum* L.), sea-island cotton (*Gossypium barbadense* L.) and wild cotton species. The line 492 was later officially released as the cultivar Jimian 20.

In 1997, I received a one-year scholarship from the Ministry of Education of China, to come to Poland and to study at SGGW as a visiting scientist supervised by Professor Stefan Malepszy. During this visit, I have also conducted my Master of Science study (extramural). I have learned the *Agrobacterium*-mediated plant transformation method. I introduced a reporter gene (*uidA*) driving by a *PR-2d* promoter, which was derived from a tobacco pathogenesis related gene, into the cucumber (*Cucumis sativus* L.) genome. Seven fertile independent transformation events (ITEs) were obtained. Integration of the transgene was confirmed by PCR of *uidA* and *nptII* gene in T_0 plants, while the detection of GUS activity in the floral organs indicating the expression of *uidA* gene on the protein level. In 1998, I received Master of Science degree in horticulture.

From 1998 to 2002 I continued my doctoral study supervised by Professor Stefan Malepszy and financed by SGGW. The subject continued as *Agrobacterium*-mediated leaf microexplant transformation of cucumber as a model system. During my doctoral study, the transformation method was improved in the following aspects – (1) carefully selection of the explants; (2) using phenolic compound acetosyringone during inoculation and co-cultivation period for the *vir* gene induction; (3) selection only the tissue with high regeneration potential. The improved method was reliable and highly reproducible. Four different transgene constructs were introduced into a highly inbred line of *Cucumis sativus* L. cv. Borszczagowski, and in total 43 ITEs were produced. Two constructs were specially transformed into the cucumber genome on the request by the Max-Planck-Institute for Plant Molecular Physiology in Golm, Germany. In addition, 48 transgenic lines (of them 14 homozygous lines) from T1 to T3 generations for the *PR-2d* construct, and 165 transgenic lines (of them 13 homozygous lines) from T1 to T5 generations for *thaumatin* construct, were produced. For my PhD dissertation, the results chosen from the transgenic lines with pPR-2d::*uidA* construct, together with pCaMV35S::*thaumatin* lines, were used and these transgenic plants were characterized for integration, inheritance and expression of the transgenes. I was awarded bilaterally from SGGW of Poland in 2002 and from the Ministry of Education of China in 2004 as the outstanding doctoral student.

The results obtained during my PhD study were published later on as original research papers in peer-reviewed scientific journals **(Yin et al. 2004 a, 2004 c, 2006 a; Filipecki et al. 2005, 2006; Burza et al. 2006; Tagashira et al. 2005** in **Załącznik 4)** and the research related to the transformation was awarded by the Rector of SGGW in 2007.

After my PhD study, from 2003 to April 2008, I worked at the Institute of Plant Genetics, Polish Academy of Science in Poznań, as adjunct, at the laboratory of prof. Professor Tadeusz Rorat. During this period, I have been working on analysis the role of dehydrin genes in the cold tolerance in two *Solanum* species *S. sogarandinum* and *S. tuberosum* using transgenic approach. I have involved in two KBN (the State Committee for Scientific Research) projects as the main investigator (Grant no. 2 PO6A 030 27, 2004-2006) and as the project leader (Grant no. 2 PO6 023 29, 2005-2008 in **Załącznik 4**). Transgenic cucumber and potato lines containing *S. sogarandinum* pGT::*Dhn10* and pGT::*Dhn24* constructs were produced (in total 38 ITEs) and were analyzed on DNA, RNA, protein and phenotypical level.

The results derived from the above two projects were published as original research papers in peer-reviewed scientific journals **(Yin et al. 2004 b, 2006 b; Rorat et al. 2006; Glodek et al. 2008** in **Załącznik 4)** and the research on „Isolation and identification of genes whose expression is related to the tolerance of cultivars of potato and wild species *Solanum sogarandinum* to stress caused by cold, drought and salinity" was awarded by the Faculty of Agricultural, Veterinary and Forestry Sciences, Polish Academy of Sciences in 2007.

From May 2008, I was employed as adjunct at IHAR-PIB/Młochów and my research was shifted to plant molecular virology – study on potato viral pathogens and their interaction with the hosts.

On arriving at Młochów in 2008, I immediately involved in two EU projects coordinated by Professor Ewa Zimnoch-Guzowska.

The first one: ResistVir – research on genetic resistance to control plant pathogenic viruses and their vectors in European crops (EU project, VI Framework 2004-2008 in **Załącznik 4**). Within ResistVir, I had a chance to attend the first PVYwide meeting in Paris and to pay a short visit to the Science and Advice for Scottish Agriculture (SASA) UK to learn the standard operating procedures for virus testing, organized by Dr. Colin Jeffries.

Within the second project: Enhanced control of *Potato mop-top virus* (PMTV) in the Nordic and Baltic Sea region (2004-2008), I have been working on monitoring and diagnostics of PMTV in potato tuber samples collected from Poland and the results were published together with other European scientists **(Santala et al. 2010** in **Załącznik 4)**.

At the same time, since 2008, I have been involving in a multiple year project financed by the Ministry of Agriculture and Rural Development Poland (MRiRW projects 3-6-00-0-01 2008-2013, 3-1-06-0-01 2008-2013 and PW zad. 3.1 2015-2020). I am engaged in monitoring, characterization and collection of viral pathogens infecting potato crops in Poland, including PVY and *Tobacco rattle virus* (TRV), among others. In 2010 and in 2015, I obtained research grants in which I was the project leader: (1) project no. NN310 304439, 2010-2013, financed by the National Science Center (NCN) of Poland; (2) project no. 95, 2015-2017, financed by the Ministry of Agriculture and Rural development of Poland. It opened my research on miRNA study. Within these two projects, I had good cooperation with Professor Baohong Zhang from East Carolina University USA on microRNA study and Dr Magdalena Pawełkowicz from SGGW in Warsaw on bioinformatics analysis. The results derived from the above projects on PVY study (**H1; H2**), evaluation the resistance reaction of potato cultivars to PVY **(H2),** and miRNA study in PVY-potato **(H3)** and in PVY-tobacco **(H4)** interaction formed **my habilitation achievement described in details in the point 4.c of the auto-presentation.**

Besides PVY, my virology research is also extended to other aspects of potato viruses, e.g., TRV study (**Yin et al. 2014 a and b; Yin and Michalak 2014; Chrzanowska et al. 2014**), collection of viral pathogens (**Gawińska-Urbanowicz et al. 2014; Yin et al. 2017 b**), diagnosis of the presence of viruses in potato and in soil (**Yin and Michalak 2017**) (see **Załącznik 4**).

YIN ZHIMIN

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The Applicant's Signature