SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

„Expansion and specialization of ABCG transporters in legumes”

Joanna Banasiak, PhD
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I. Name

Joanna Banasiak

II. Diplomas and Degrees

2013 **PhD (Chemistry, specialization Biochemistry),** Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan; PhD thesis title: "Identification of full-size ABCG transporters in *Medicago truncatula* and functional analysis of selected genes involved in biotic stress response"
Supervisor: dr hab. Michał Jasiński, prof. IBCh
(PhD thesis was awarded by the Scientific Board of the IBCh PAS, Director's Award of ICHB PAN for one of the best doctoral dissertations in 2013)

2007 **MSci (Biology, specialization Molecular biology),** Faculty of Biology, Adam Mickiewicz University, Poznan, and Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan; Master's thesis title: „Identification and obtaining of a full-length cDNA of selected ABC genes from *Medicago truncatula* by RT-PCR and RACE approaches".
Supervisor: Prof. dr hab. Marek Figlerowicz
(studies graduated with distinction)

2005 **Bachelor's degree in Biology,** Faculty of Biology, Adam Mickiewicz University in Poznań; Bachelor's
Supervisor: dr Mirosława Dabert

III. Employment in research institutions and completed research internships

07. 2014 –	Assistant professor: Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan, Department of Natural Products Biochemistry, Department of Plant Molecular Physiology (since 2017)
07. 2022 – 01. 2023	Department of Biochemistry and Biotechnology, Poznań University of Life Sciences– research internship
3.11 – 2.12. 2016	Department of Plant and Microbial Biology, University of Zurich (Prof. Enrico Martinoia), research conducted as part of a grant SONATA (PI - Dr. Joanna Banasiak)

7-14.05. 2016 r.	Department of Plant and Microbial Biology, University of Zurich (Prof. Enrico Martinoia), research conducted as part of a grant HARMONIA (PI - Prof. Michał Jasiński)
08. 2013 – 06. 2014	Assistant: Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan, Department of Natural Products Biochemistry
09. 2012 – 07. 2013	Biologist: Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan, Laboratory of Molecular and Systems Biology (including maternity leave January-June 2013)

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

1. Title of the scientific achievement

"Expansion and specialization of ABCG transporters in legumes"

2. List of publications included as the scientific achievement *(the name of the candidate is in bold; * corresponding author; #authors contributed equally; bibliographic data are given according to Web of Science Core Collection)*

H1. Banasiak J, Jasiński M*.

***corresponding author**

Defence, symbiosis and ABCG transporters. Geisler M. (ed.), *Plant ABC Transporters*, Signaling and Communication in Plants 22, Springer-Verlag Berlin Heidelberg , František Baluška and Jorge Vivanco (eds), (2014) pp 163-184.

- Citations: 12

My contributions to the publication involved: formulating the concept of the book chapter in collaboration with the corresponding author; literature study, identifying ABCG transporters from various plant species; performing phylogenetic analyses; conducting in silico analyses of available transcriptomic data; and preparing the manuscript in cooperation with the corresponding author.

H2. Biala W#, **Banasiak J#**, Jarzyniak K, Pawela A, Jasinski M*.

***corresponding author**
#these authors contributed equally

Medicago truncatula ABCG10 is a transporter of 4-coumarate and liquiritigenin in the medicarpin biosynthetic pathway. Journal of Experimental Botany, (2017) Jun;68(12):3231-3241

- doi: 10.1093/jxb/erx059
- **$IF_{(2016)}$ = 5.830; $IF_{(5\ Year)}$ = 6.538**
- MNiSW $_{(2016)}$ = 45

- Plant Science Q1
- Citations: 43
- Director's Award of ICHB PAN for the best experimental work in 2017

My contribution to the publication involved: designing and conducting part of the experiments (gene expression analysis – ddPCR, promoter activity analysis, subcellular localization – microscopic analyses); formulating some of the hypotheses; analyzing and interpretating the obtained data together with the co-authors; writing the first version of the manuscript, preparing figures, and writing responses to reviewers, along with the co-first author and the corresponding author; editing the final version of the manuscript in collaboration with the other co-authors.

H3. Pakuła K, Sequeiros-Borja C, Biała-Leonhard W, Pawela A, **Banasiak J**, Bailly A, Radom M, Geisler M, Brezovsky J, Jasiński M*.

***corresponding author**

Restriction of access to the central cavity is a major contributor to substrate selectivity in plant ABCG transporters. Cellular and Molecular Life Sciences, (2023) Mar 23;80(4):105

- doi: 10.1007/s00018-023-04751-6
- **$IF_{(2022)}$ = 8; $IF_{(5\ Year)}$ = 8.7**
- $MEiN_{(2022)}$ = 140
- Biochemistry and Molecular Biology Q1
- Citations: 7

My contribution to the publication involved: participating in the identification and selection of ABCG proteins obtained from the 1KP project; conducting microscopic observations, and editing the final version of the manuscript in collaboration with the other co-authors.

H4. Jarzyniak K, **Banasiak J**, Jamruszka T, Pawela A, Di Donato M, Novák O, Geisler M, Jasiński M*.

***corresponding author**

Early stages of legume–rhizobia symbiosis are controlled by ABCG-mediated transport of active cytokinins. *Nature Plants* (2021) 7, 428–436.

- doi: 10.1038/s41477-021-00873-6
- **$IF_{(2020)}$ = 15.793; $IF_{(5\ Year)}$ = 17.349**
- $MEiN_{(2020)}$ = 200
- Plant Science Q1
- Citations: 31
- Award of the Director of IBCh PAS for an outstanding experimental article published in 2021
- Distinction in the Professor Kazimierz Bessalik competition awarded by the Committee on Molecular Biology of the Cell, Polish Academy of Sciences for the article "Early stages of legume–rhizobia symbiosis are controlled by ABCG-mediated transport of active cytokinins" published in the journal Nature Plants 2021
- Award of the Division II of Biological and Agricultural Sciences of the Polish Academy of Sciences for outstanding research achievement titled: "Understanding

the mechanism of cytokinin distribution in the process of biological nitrogen fixation in leguminous plants"

My contribution to the publication included participation in: conducting preliminary analyses that led to the selection of MtABCG56 as the research object, performing part of the experiments (expression pattern analysis of the studied genes – MtABCG56 and its homologs, MtRR4, staining, preparation of microscopic sections, microscopic observations, mutant line selection, preparation of the silencing construct, phenotypic analyses of infection threads, and subcellular localization observations in protopllast). Additionally, I contributed to writing a section of the manuscript, as well as preparing figures, editing the manuscript, and preparing responses to reviewers with other co-authors.

H5. Pawela A[#], **Banasiak J[#]**, Biała W, Martinoia E, Jasinski M*.

***corresponding author**
[#]these authors contributed equally

MtABCG20 is an ABA exporter influencing root morphology and seed germination of *Medicago truncatula*. *Plant Journal* (2019) 98(3), 511-523.

- doi: 10.1111/tpj.14234
- **IF(2018) = 5.726; IF(5 Year) = 6.467**
- MNiSW (2018) = 140
- Plant Science Q1
- Citations: 45
- Award of the Director of IBCh PAS for an outstanding experimental article published in 2019

My contribution to the publication involved: designing and conducting part of the experiments (mutant selection, phenotypic analyses, gene expression analyses, and microscopic observations); interpreting the results together with the co-authors; preparing the first draft of the manuscript, figures, and responses to reviewers along with the co-first author and the corresponding author; and editing the final version of the manuscript in collaboration with the other co-authors.

H6. **Banasiak J***, Borghi L, Stec N, Martinoia E, Jasiński M

***corresponding author**

The Full-Size ABCG Transporter of *Medicago truncatula* Is Involved in Strigolactone Secretion, Affecting Arbuscular Mycorrhiza. *Frontiers in Plant Science* (2020) 7;11:18,

- doi: 10.3389/fpls.2020.00018
- **IF(2019) = 4.402; IF(5 Year) = 5.207**
- MNiSW (2019) = 100
- Plant Science Q1
- Citations: 47
- The publication is the result of the implementation of an own grant (Sonata)
- The article was chosen to feature in Frontiers in Plant Science 2020 highlights e-book collection as one of the most viewed and downloaded articles of the year

My contribution to the publication involved: formulating the research problem and concept of the study; designing and conducting the majority of the experiments; interpreting the results together with the co-authors; preparing the first draft of the manuscript and figures; preparing responses to reviewers and editing the final version of the manuscript in collaboration with the other co-authors; serving as the corresponding author; and securing funding for the research.

H7. Banasiak J[#], Jamruszka T[#], Murray J, Jasiński M*.

***corresponding author**
[#]these authors contributed equally

A roadmap of plant membrane transporters in the arbuscular mycorrhizal and legume-rhizobium symbioses. *Plant Physiology* (2021) 187(4):2071-2091.

- doi: 10.1093/plphys/kiab280
- **IF(2020) = 8.340; IF(5 Year) = 8.972**
- MNiSW (2020) = 140
- Citations: 38
- The publication is the result of the implementation of an own grant (Sonata)

My contribution to the publication involved: formulating the article concept together with the corresponding author; collecting and analyzing source materials with the co-first author; preparing the figures; preparing the first version of the manuscript together with the co-first author and the corresponding author; and editing the final version of the manuscript and responses to reviewers in collaboration with the other co-authors.

H8. **Banasiak J***, Jasiński M*

***corresponding author**

ATP-binding cassette transporters in nonmodel plants. *New Phytologist* (2022) 233: 1597-1612.

- doi: 10.1111/nph.17779
- **IF(2021) = 10.323; IF(5 Year) =10.768**
- MEiN(2021) = 140
- Plant Science Q1
- Citations: 33
- Tansley review article type, widely recognized across the plant science community
- The publication is the result of the implementation of an own grant (Sonata)

My contribution to the publication involved: formulating the article concept together with the second corresponding author; collecting and analyzing source materials; preparing figures and a table; identifying ABCG transporters from various plant species and conducting phylogenetic analyses; and preparing the manuscript and responses to reviewers together with the second corresponding author; serving as the corresponding author.

Total scientific metrics of scientific achievement:

- Number of publications according to Web of Science Core Collection: **8**
- h-index **7**
- Combined Impact Factor (IF/IF 5 Year) of the scientific achievement: **58.414/64.001**
- Total citations according to Web of Science Core Collection (with/without self-citations) as of 28.08.2025: **256/241**
- Copies of the above publications are included as Annex **6B**
- Co-authors' statements regarding the author's contributions are in Annex **7B**

Total scientific metrics:

- Number of publications according to Web of Science Core Collection: **13**
- h-index **11**
- Combined Impact Factor (IF/IF 5 Year): **89.503/96.217**
- Total citations according to Web of Science Core Collection (with/without self-citations) as of 28.08.2025: **419/391**

3. Presentation of scientific goals and results of the achievement

3.1 Introduction and Goals

The ATP-Binding Cassette (ABC) proteins are evolutionarily ancient, widely distributed, and versatile membrane transporters present in all living organisms. These proteins utilize ATP hydrolysis as a source of energy to translocate molecules across biological membranes. Moreover, they share common structural features, including two types of modules: i) the transmembrane domain (TMD) and ii) the nucleotide-binding domain (NBD). Depending on the number and arrangement of domains within a single polypeptide, ABC proteins can be categorized as either half-size or full-size, with forward or reverse orientation (Fig. 1). ABC proteins are classified into nine subfamilies (ABC**A**-ABC**I**) based on domain organization, phylogenetic relationships, and sequence homology. Notably, ABCH members have not been identified in plants (Verrier et al., 2008).

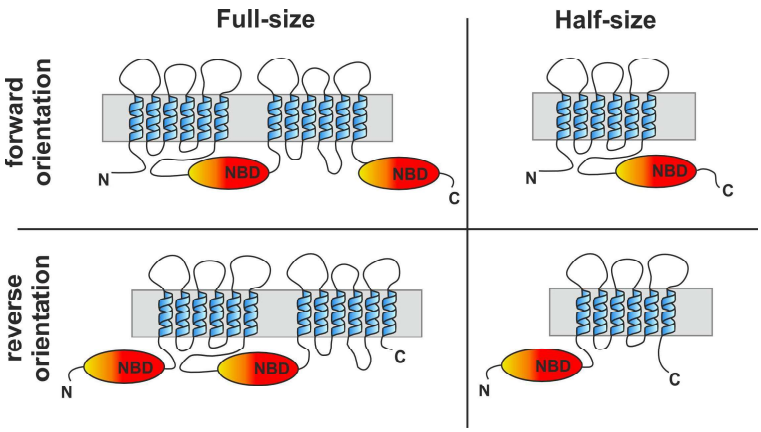

Figure 1. Topology of ABC transporters. Full-size ABC proteins are composed of two TMD and two NBD domains, half-size ABC proteins are composed of one TMD and one NBD domain. They can take the forward or reverse orientation with TMD or NBD domain at the N-terminus of the protein, respectively.

Plant ABC transporters are responsible for translocating structurally and functionally unrelated compounds such as lipids, carbohydrates, heavy metals, products of chlorophyll catabolism, and plant hormones. Initial work on these proteins focused on their role in detoxification and defense against pathogens. Later studies revealed that ABC transporters play a crucial role in proper plant growth and development, contribute to responses to abiotic stresses, and participate in a wide range of plant-microbe interactions (Do et al., 2018; Do et al., 2021). Importantly, the catalog of recognized biological processes involving plant ABC proteins continues to expand, opening new research directions into their function and significance in plant physiology.

It is worth emphasizing that ABC transporters in plants are particularly abundant and diverse compared to other taxa. The need for an efficient and extensive transport system in plants may result from: (i) the remarkable adaptive plasticity of plants, forced by their sessile lifestyle, (ii) "chemodiversity," resulting from the evolution of specialized metabolism, and (iii) the ability for post-embryonic development, largely controlled by plant hormones. Notably, the crucial role of ABC proteins in plant adaptation to changing environmental conditions is

reflected in the duplication of ABC-encoding genes that occurred before the emergence of land plants (One Thousand Plant Transcriptomes, 2019). This is especially pronounced for the largest ABCG subfamily, whose members are involved in the proper formation of the cuticle, which protects against excessive water loss, establishment of arbuscular mycorrhiza, as well as the transport of secondary/specialized metabolites and plant hormones, thus fundamental innovations that enabled the successful colonization of land (Hwang et al., 2016).

This raises the question of whether ABCG transporters could contribute to the evolution of distinct plant lineages within flowering plants, e.g., the agriculturally and economically important Fabaceae family. Fabaceae plants (legumes) form a unique symbiosis with nitrogen-fixing bacteria, known as the legume-rhizobium symbiosis (LRS), and an evolutionarily older arbuscular mycorrhizal symbiosis (AMS) (Larrainzar and Wienkoop, 2017). Furthermore, they produce a specific class of specialized metabolites, such as 5-deoxy(iso)flavonoids (Shimada et al., 2003).

Considering the role of ABCG proteins in transporting biologically active compounds and their involvement in various plant-microbe interactions, exploring their significance in legume biology is an intriguing research area. This became the main focus of my studies after completing my Ph.D.

Notably, the knowledge of plant ABC proteins beyond the canonical model *Arabidopsis thaliana* (a representative of the Brassicaceae family) remains limited. Consequently, the role of ABC proteins in processes unique to specific taxa, such as the symbiosis between legumes and nitrogen-fixing bacteria, is still poorly understood. Therefore, my research focused on ABCG transporters from *Medicago truncatula*, a model organism for legume plants (Fig. 2). This first required developing or adapting protocols dedicated to *M. truncatula*. Additionally, it was important to create or adapt tools that would enable comprehensive functional analyses of ABCG transporters. I was actively involved in these efforts as Professor Michał Jasiński established his research team. Ultimately, these works facilitated the effective testing of hypotheses regarding the function of ABCG proteins in *M. truncatula*. It is worth emphasizing that my research development was significantly enhanced by internships at one of the leading scientific centers specializing in ABC transporter studies, the Department of Plant and Microbial Biology at the University of Zurich, led by Prof. Enrico Martinoia, an undisputed authority in this field. The experience I gained was applied during the implementation of research grants. As an investigator, I was involved in the execution of two grants focusing on ABCG proteins and their role in interactions with symbiotic bacteria. Additionally, I had the opportunity to develop my own research directions. Within the SONATA project, which I led, I expanded the research scope of the Department of Molecular Plant Physiology at IBCH PAN to include another type of interaction between legume plants and microorganisms – arbuscular mycorrhiza. Furthermore, as a member of the management committee and vice-leader of a working group, I'm involved in the European COST Action CA22142 "Beneficial root-

associated microorganisms for sustainable agriculture (ROOT-BENEFIT)," which focuses on the use of beneficial soil microorganisms to promote sustainable agriculture.

All of this contributed to my scientific development and resulted in a series of publications that form a significant part of my scientific achievement, in which the expansion and specialization of ABCG proteins in functions related to the biology of legume plants were demonstrated, including: (i) adaptation to a specialized metabolism **(H1, H2, H3, H8)**, (ii) establishment of specific symbiotic interactions **(H1, H4, H5, H7, H8)**, and (iii) adaptation to root anatomical structure **(H6)**. The results obtained have broadened and deepened our understanding of ABCG transporters concerning their role in interactions between legume plants and microorganisms, as well as in the translocation of secondary metabolites and plant hormones. Beyond their scientific significance, these findings may also contribute to the development of more sustainable agricultural practices by promoting the use of legumes, thereby reducing the reliance on chemical inputs in the environment.

Figure 2. ***Medicago truncatula***, different types of plant material used in the study of the role of ABCG proteins in the legume-associated functions.

3.2 Selection of future research objects (H1)

The initial stage of my research involved selecting future study objects, including ABCG proteins potentially involved in specific biological processes. The starting point for their selection was a comprehensive analysis of available transcriptomic data concerning changes in the gene expression profile of *M. truncatula* in the context of LRS and AM. As a source of information, I used data from Affymetrix Medicago GeneChip and RNA-seq obtained by various research groups. This allowed for the selection of *ABC* genes whose expression changes in specific root cells/tissues at different stages of LRS and AM. Additionally, based on the genomic sequence, I identified genes encoding full-size and half-size ABCG transporters in *M. truncatula*. I also participated in analyzing their expression across Medicago organs and under conditions that promote symbiosis, such as nitrogen or phosphorus deficiency, inoculation with symbiotic bacteria, as well as studying changes in their mRNA levels in response to plant hormones and specialized metabolites.

I was also responsible for identifying and systematically updating the list of ABC genes from *M. truncatula* and other plant species across various taxonomic groups. The expertise I gained enabled me, in collaboration with Prof. M. Jasiński and bioinformatician Dr. Marcin Radom, to develop the ABCReader program. This tool allows for the automatic identification, verification, and classification of ABC protein sequences into specific subfamilies. ABCReader is specifically designed for transcriptomic data generated within the 1KP initiative (1000 Plant Transcriptomes Initiative), which collected the transcriptomes of over 1,000 plant species within Archaeplastida (glaucophytes, red algae, green algae, and land plants) (One Thousand Plant Transcriptomes, 2019). The obtained plant ABC protein sequences were used to construct phylogenetic trees, providing insights into the evolutionary relationships among the studied proteins and constituting a starting point for formulating research hypotheses.

Notably, the integration of transcriptomic data with phylogenetic analyses, while considering the functions of characterized ABCG proteins from various plant species, enabled the identification of the most promising candidates for in-depth functional studies. Examples include the proteins MtABCG56 **(H4)**, MtABCG20 **(H5)**, and MtABCG59 **(H6)**. The phylogenetic analyses of both half-size and full-size ABCG proteins, with a particular focus on those from legume plants, as well as the interpretation of transcriptomic data, were partially described in the book chapter (**H1)**, of which I am the first author.

3.3 Determining the role of the ABCG10 transporter in the biosynthetic pathway of legume specific 5-deoxy(iso)flavonoids and their derivatives (H2, H3, H8)

Initial studies on plant ABCG transporters suggested their involvement in the secretion of secondary metabolite end-products related to defense responses. As mentioned earlier, legume plants are particularly rich in secondary metabolites belonging to the flavonoid class, among which 5-deoxy(isoflavonoids) are found almost exclusively in this group of plants (Shimada et al., 2003). In *M. truncatula,* 5-deoxy(iso)flavonoids are precursors in the biosynthesis of the phenylpropanoid-derived phytoalexin medicarpin, which exhibits antifungal and antibacterial properties (Fig. 3) (Naoumkina et al., 2007). The production and secretion of this pterocarpan play a crucial role in the plant's basal defense response against a wide range of pathogens. Interestingly, the medicarpin may also contribute to enhancing the specificity of symbiosis. It has been proposed that the microsymbiont of *M. truncatula* - *Ensifer meliloti* (formerly *Sinorhizobium meliloti*) is resistant to medicarpin, in contrast to many other rhizobia (Liu and Murray, 2016). As part of my doctoral research, I demonstrated that silencing *MtABCG10* expression under biotic stress conditions reduces *de novo* medicarpin production in the roots and decreases its secretion into the rhizosphere, ultimately increasing susceptibility to fungal infections. Notably, these disruptions occur at a very early stage of the medicarpin biosynthetic pathway, suggesting that MtABCG10 plays a role in the transport of precursor(s) rather than the final product (publication **N4**). To verify this hypothesis, we aimed to identify the molecules

transported by MtABCG10. Additionally, we proposed a rationale for the significance of translocating intermediates in the phenylpropanoid pathway (PP) within root tissues, referring to the concept of metabolon described in the literature. This work is detailed in **H2**, for which I am the co-first author, and is discussed further below.

In the case of ABCG proteins, the exogenous application of transported molecules often leads to an increase in the expression levels of their genes. Therefore, as a first step, a quantitative (ddPCR) analysis was performed to assess changes in *MtABCG10* transcript accumulation after treating Medicago roots with various compounds from the phenylpropanoid (PP) pathway, which are intermediates in medicarpin production (*p*-coumaric acid, isoliquiritigenin, liquiritigenin, and formononetin) (Fig. 3). Among these, *p*-coumaric acid had the strongest effect on *MtABCG10* mRNA accumulation. However, complementation experiments in plants with silenced *MtABCG10* expression, using compounds from the PP pathway, suggested the involvement of the MtABCG10 transporter in the translocation of more than one substrate. Finally, transport assays performed in a heterologous system using tobacco BY2 cells overexpressing *MtABCG10* showed that MtABCG10 selectively transports *p*-coumaric acid and liquiritigenin. Transport and complementation studies, using high-throughput liquid chromatography coupled with mass spectrometry (HPLC/MS) as a detection tool, were performed by Wanda Biała as part of her PhD thesis, for which I was an auxiliary supervisor at the Poznań University of Life Sciences.

It is worth noting that the ABCG transporters described so far were thought to be engaged in the translocation of the end products of specialized metabolic pathways rather than in the transport of intermediates. The presented data provide evidence for the involvement of this full-size ABCG protein in the translocation of compounds from the early stages of the PP pathway. This offers a broader perspective on the role of ABCG transporters, not only in the relocation of specialized metabolites but also in the modulation of their biosynthesis. Both compounds transported by MtABCG10 are located at key branch points of the PP pathway (Fig. 3). *P*-coumaric acid is a precursor in the biosynthesis of monolignols, coumarins, and (iso)flavonoids. Liquiritigenin serves as a substrate for the biosynthesis of 5-deoxyflavonoids, which in Medicago are involved in signaling during symbiotic interactions, as well as 5-deoxyisoflavonoids associated with defense responses (Fig. 3).

This raises the question of how the transport of transient/intermediate compounds (common to several branches of the pathway) determines the formation of a specific end-product in a given biological scenario, in this case, medicarpin biosynthesis in response to biotic stress. To address this question, enzymes from the PP pathway that could potentially form temporary functional enzyme complexes, known as metabolons, were examined more closely. These complexes are responsible for coordinating the reactions in different branches of the pathway that utilize the same precursors. Notably, specific isoforms of enzymes from the early stages of the PP pathway may be involved in the production of distinct classes of phenylpropanoids, and their genes can be regulated differently depending on the plant's developmental stage, tissue type, or environmental stimuli. It is also proposed that all stages of

the biosynthesis of certain phenolic compounds take place in a single cell and are coordinated by a specific metabolon (Nakayama et al., 2019). However, this is in contrast to the observed metabolic phenotype of *MtABCG10*-silenced plants, as well as complementation and transport experiments, which suggest the involvement of MtABCG10 in the translocation of medicarpin intermediates between cells. To verify the potential spatial separation of the stages of medicarpin biosynthesis, the genes *PALs* (*phenylalanine ammonia-lyase*) and *IFSs* (*isoflavone synthase*), involved in *de novo* medicarpin synthesis, were identified, and their spatial expression patterns were analyzed. To assess the promoter activity of the analyzed genes, two reporter systems were used: i) *uidA* (encoding GUS, β-glucuronidase) and ii) nls-gfp (encoding NLS-GFP, nuclear localization signal fused to a green fluorescence protein). The obtained results suggest a spatial separation of medicarpin biosynthesis steps between different root tissues and the involvement of *MtABCG10* in redirecting precursors. This finding provides new insights into the organization and function of metabolons. It cannot be excluded that ABCG transporters form operational units with isoforms of enzymes from the PP pathway, thereby "redirecting" precursor/intermediate molecules into specific branches of the pathway, depending on the plant's needs.

Since MtABCG10 (currently referred to as MtABCG46) is characterized by high transport selectivity towards *p*-coumaric acid and liquiritigenin, subsequent studies aimed to determine the molecular determinants underlying this selectivity **H3**. The use of various methods, including phylogenetic and biochemical analyses, structure prediction using AlphaFold2, molecular dynamics simulations, and mutagenesis, allowed us to define a transient substrate access path to the central pocket of MtABCG46 and identify a key amino acid (F562) that influences its architecture and, consequently, the transporter's selectivity. My participation in this research involved preparing a set of full-size ABCG protein sequences from various plant species, which were used for comparative sequence analyses, and the selection of amino acids within the central pocket of MtABCG46 that might be involved in substrate recognition or translocation. Additionally, I conducted microscopic observations of BY2 cells transformed with genetic constructs in which various variants of *MtABCG46* (WT, F562Y, F562L, F562I, F562A) were fused with the sequence encoding GFP. The introduced point mutations did not affect the subcellular localization of the different MtABCG46 variants, demonstrating that the differences observed in biochemical tests (transport experiments) were not due to incorrect localization of the protein in the plasma membrane.

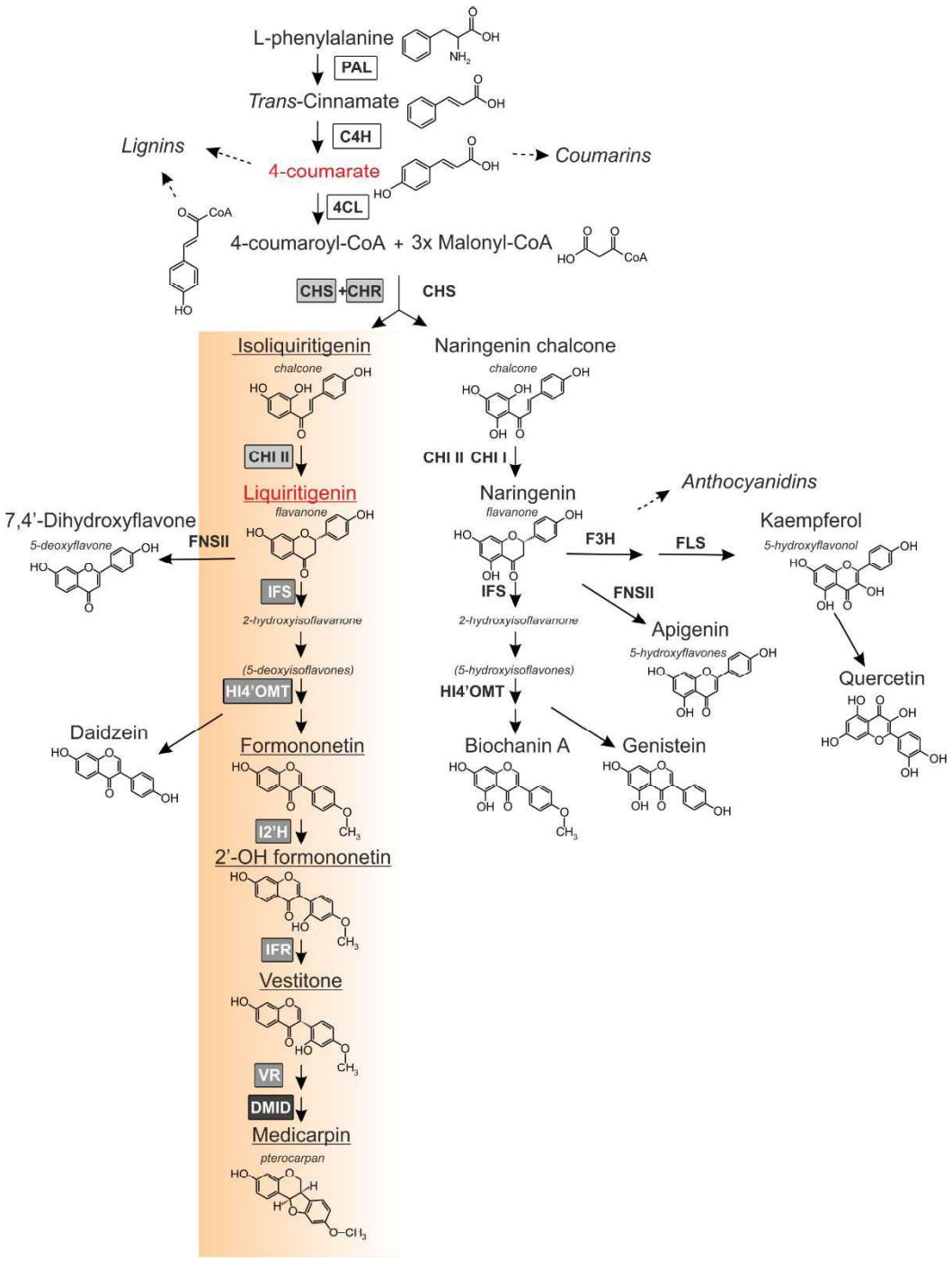

Figure 3. Scheme of the phenylpropanoid biosynthetic pathway in *Medicago truncatula*. The underlined compounds were affected by *MtABCG10* silencing. Compounds transported by MtABCG10 are marked in red. PAL, phenylalanine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; CHS, chalcone synthase; CHR, chalcone reductase; CHI, chalcone isomerase; IFS, isoflavone synthase; HI4'OMT, isoflavone 4'-O-methyltransferase; I2'H, isoflavone 2'-hydroxylase; IFR, isoflavone reductase; VR, vestitone reductase; DMID, 4'-methoxyisoflavanol dehydratase; F3H, flavanone 3 hydroxylase; FNSII, flavanone synthase II; FLS, flavonol synthase. (Adopted from publication **H2**).

Notably, the phylogenetic tree reveals the expansion of ABCG proteins in legumes within the subclade to which MtABCG10/46 belongs. This may be the result of adaptation to specialized metabolism in response to the specific requirements of a given group of plants or species. A similar increase in the number of ABCG genes has been observed in the Solanaceae

and Brassicaceae families, involving ABCG proteins responsible for the transport of terpenoids and glucosinolates, respectively. The concept of *ABCG* gene expansion in relation to the "chemodiversity" of angiosperms is addressed in the review article **H8** and is further described in the following section of this summary of professional accomplishments.

To sum up, the findings presented in publications **N4, H2, H3**, and **H8** link the function of the MtABCG10/46 transporter to the translocation of secondary metabolites associated with the legume-specific branching of the PP pathway. This suggests that MtABCG10/46 is specialized in functions related to the biology of this plant group, particularly in defense responses based on the biosynthesis of compounds with antifungal and antibacterial properties. A promising area of research in this field would be the identification and characterization of yet unknown transporters of iso(flavonoids), which play a role in partner recognition during the pre-symbiotic stage of LRS or contribute to root nodule formation. The previously mentioned expansion of MtABCG10/46 homologs in legumes may suggest their potential role not only in defense mechanisms but also in symbiotic interactions involving iso(flavonoid) transport, as discussed in the review articles **H7** and **H8**.

3.4 Determining the role of the ABCG56 transporter in the distribution of cytokinins during the establishment of *M. truncatula* symbiosis with nitrogen-fixing bacteria (H1, H4, H8)

Alongside secondary metabolites, phytohormones also play a key role in symbiotic interactions. Notably, legumes are the most numerous group within the so-called nitrogen-fixing root nodule (NFN) clade (Griesmann et al., 2018; van Velzen et al., 2019), and the symbiosis of legume plants with rhizobia (LRS) represents one of the most extensively studied non-antagonistic interspecies interactions. This prominence arises from the crucial role of symbiotic nitrogen fixation in developing sustainable agriculture, which aims to ensure crop productivity while minimizing environmental damage. Numerous laboratories around the world are working to understand the mechanisms underlying LRS. One of the key issues and debates for years has been the recognition of cytokinins (CKs) as a potential mobile signal orchestrating events between the rhizodermis/epidermis, where bacterial infection occurs, and the primary cortex, the site of root nodule formation. The findings presented in publication **H4** contribute to this ongoing discussion, providing further evidence for the specific role of CKs in nodulation. In publication H4, of which I am the second coauthor, it was shown that CKs are transported by MtABCG56 from the epidermis to the cortex at the early stages of symbiosis, influencing nodulation efficiency. Article H4 results from the implementation of the OPUS grant, titled "The role of membrane transporters in the cytokinin signaling upon Legume – Rhizobia Symbiosis", led by Prof. M. Jasiński, of which I was one of the investigators. A large part of the results presented in publication **H4** was obtained by Karolina Jarzyniak as part of her PhD thesis, for which I served as an auxiliary supervisor at the Poznań University of Life Sciences.

The phylogenetic analysis of full-size ABCG proteins, published in the book chapter **H1** and later expanded in publication **H8**, revealed the existence of a subclade specific to legume plants. The expansion of ABCG genes within this subclade suggests an adaptation to legume-specific functions. This observation prompted us to search for ABCG transporters potentially involved in LRS. Preliminary studies on the expression of *M. truncatula ABCG* genes under LRS-related conditions, such as *Ensifer meliloti* treatment, exogenous application of cytokinin (*trans*-zeatin), and the analysis of available microarray data, led to the selection of MtABCG56 as a promising candidate for further research.

More detailed analyses revealed that *MtABCG56* mRNA specifically accumulates in roots and root nodules, and its expression following *E. meliloti* inoculation is Nod factor-dependent and is restricted to the infection zone. Furthermore, under non-symbiotic conditions, the expression pattern within root tissues is localized to the stele and apical meristem, but upon *E. meliloti* treatment, it expands to the epidermis and cortex. Transport experiments conducted in collaboration with an international partner (Markus Geisler, Department of Biology, University of Fribourg, Switzerland) demonstrated that MtABCG56 functions as a transporter of bioactive CKs. To determine the biological role of MtABCG56, a series of phenotypic analyses was performed on *mtabcg56* mutant and *MtABCG56* knockdown lines. In the latter, tissue-specific promoters (epidermal and cortical) were additionally used to drive the expression of a silencing construct in specific root tissues. Analyses of the cortical cytokinin response, based on the expression of the marker gene *MtRR4*, indicated that MtABCG56 plays a role in CK translocation from the epidermis to the cortex. Furthermore, the absence of functional MtABCG56 in the epidermis led to CK accumulation in this tissue, which in turn abolished the induction of the marker gene *MtNOD11* in response to *E. meliloti* and reduced the number of infection threads. This observation aligns with the previously described negative role of epidermal CKs in regulating LRS. Moreover, both *mtabcg56* mutant lines and *MtABCG56* RNAi lines exhibited reduced nodulation, clearly highlighting the essential role of MtABCG56 in this process.

In publication **H4**, a model is proposed in which MtABCG56 transports bioactive CKs from the epidermis to the cortex, thereby mitigating the negative effects of CK on the infection process. Additionally, MtABCG56 plays a crucial role in a positive feedback loop that amplifies the cellular response to CK in the inner cortical layers. This, in turn, stimulates cell divisions leading to the formation of root nodule primordia (Fig. 4). MtABCG56 may represent an example of an evolutionarily ancient transport system adapted to a new function – regulating LRS. Its presence within a legume-specific subclade further supports the hypothesis that this transporter has acquired a specialized role in nodulation control **(H1, H8)**. Notably, MtABCG56's close homolog, MtABCG40, which belongs to the same subclade, is responsible for xylem-derived CK translocation toward the pericycle. It negatively regulates the initiation of both lateral root and nodule primordia as a part of a shared developmental program (unpublished data). The surprising opposing effects of CK on nodule primordium formation observed in studies on MtABCG56 and MtABCG40 may depend on: i) the source of CKs

(xylem vs. epidermis), ii) the transport mechanism (MtABCG40 vs. MtABCG56), and iii) the timing and location of CK perception (pericycle vs. inner cortical layers). These findings provide new insights into the role of hormone transport in the morphogenetic processes associated with nodulation.

The importance of the findings reported in article **H4** is highlighted by their publication in Nature Plants. Moreover, this work has received several awards, including the prestigious Award of the Division II of Biological and Agricultural Sciences of the Polish Academy of Sciences for outstanding research achievement titled: "Understanding the mechanism of cytokinin distribution in the process of biological nitrogen fixation in leguminous plants".

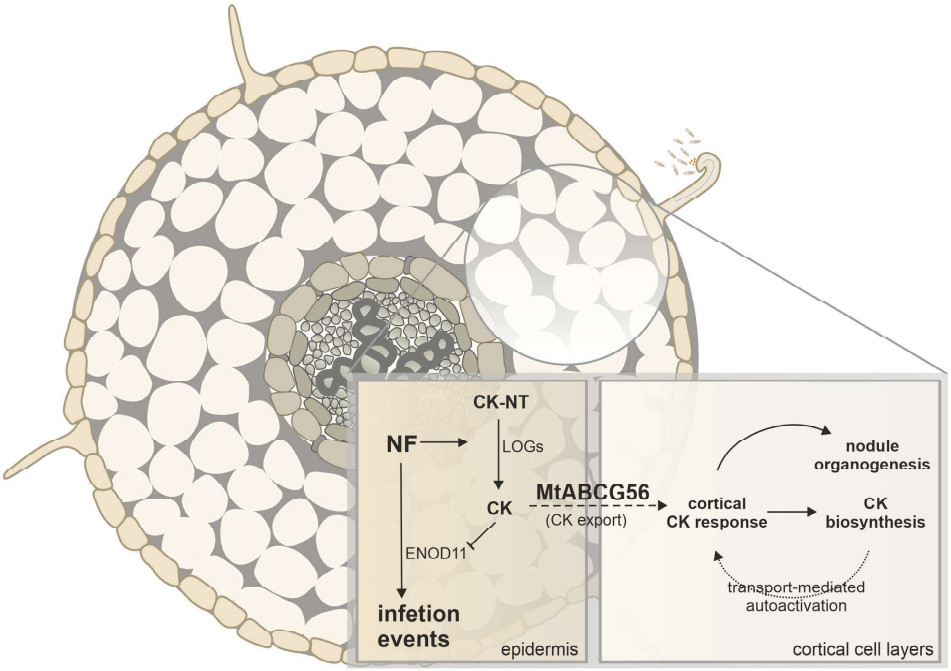

Figure 4. Proposed function of the cytokinin transporter MtABCG56 in the early stages of symbiotic interaction. MtABCG56 transports bioactive cytokinins (CK) from the epidermis to the root cortex, acting as a key component of a positive feedback loop that amplifies the cellular response to CK in the inner cortical layers. This process stimulates cell divisions in the cortex, leading to the formation of root nodule primordia. At the same time, MtABCG56 counteracts the negative effects of epidermal CK on the infection process. (NF, Nod factor; CK-NT, nucleotide precursors of CK; CK, bioactive forms of CK) (Based on publication **H4**).

3.5 The role of the abscisic acid transporter ABCG20 in the nodulation and seed germination of *M. truncatula* (H5)

My research on the role of hormone transport during LRS concerned not only CKs but also abscisic acid (ABA). ABA is one of the key hormones that adjust plant physiology to environmental conditions, particularly those related to water shortage. Interestingly, it has been shown that the energetically costly nodulation is inhibited under drought conditions, with ABA playing a crucial role in controlling this process at various stages (Ding et al., 2008). Considering this, we aimed to identify the ABA transporter influencing nodulation. This was

detailed in publication **H5**, in which I am the co-first author. Article H5 is the outcome of the HARMONIA grant titled "ABA transport in legumes - between response to drought stress and efficient fixation of atmospheric nitrogen", led by Prof. M. Jasiński. In this project, I was employed as a postdoc and completed an internship at the Department of Plant and Microbial Biology, University of Zurich.

Combining phylogenetic analysis with information about functionally characterized Arabidopsis ABA transporters enabled us to identify the half-size transporter MtABCG20 as a potential candidate involved in ABA translocation in *M. truncatula*. Expression of MtABCG20 was induced in roots both after exogenous application of ABA and under conditions mimicking osmotic stress (treatment with polyethylene glycol, PEG). Interestingly, the ABA-dependent accumulation of *MtABCG20* transcripts was not affected in Medicago hairy roots overexpressing the dominant-negative allele of *abi1-1* from Arabidopsis. The latter suppresses the ABA core signaling pathway in effector cells where this phytohormone triggers stress responses (Wu et al., 2003). These findings suggest that MtABCG20 functions at the site of ABA biosynthesis, further supported by promoter activity analyses showing its expression in the parenchyma cells of the root stele.

Transport experiments and multicolor Bimolecular Fluorescence Complementation (mcBiFC) assays, performed by Aleksandra Pawela as part of her PhD thesis, demonstrated that MtABCG20, as a homodimer, is capable of exporting ABA.

The next phase of the research focused on determining the biological role of MtABCG20. It is known that ABA affects root architecture differently depending on the plant species. In Arabidopsis, it inhibits lateral root formation, whereas in *M. truncatula*, it stimulates their development. It is assumed that the altered response to ABA in legumes is linked to their ability to form root nodules (Liang and Harris, 2005). Notably, ABA is considered a negative regulator of LRS. To investigate whether disruptions in the distribution of this phytohormone influence root morphogenetic processes, phenotypic analyses were conducted. These involved comparing the number of lateral roots and nodules formed under drought stress-mimicking conditions between *mtabcg20* insertion mutant lines and wild-type plants. The lack of the MtABCG20 transporter in both cases resulted in a slight attenuation of the ABA response, manifesting as a decrease in lateral root number and an increase in nodule formation. The obtained results suggest that MtABCG20 plays a role in exporting ABA from its biosynthesis site to response cells, leading to the stimulation or inhibition of cell division at the sites of lateral root and nodule formation, respectively (Fig. 5).

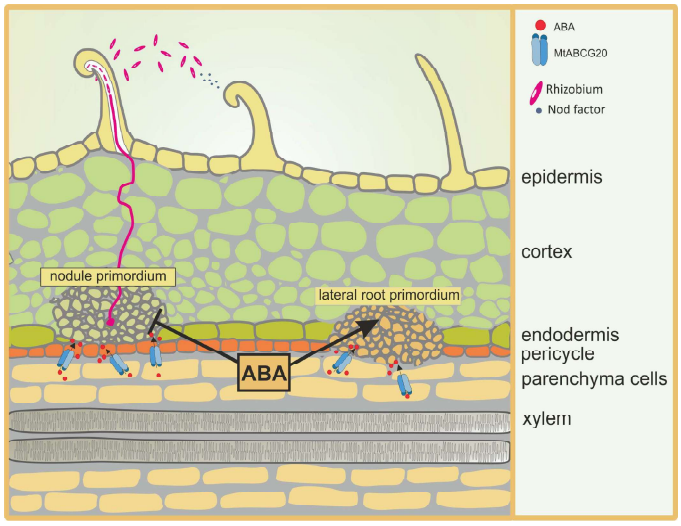

Figure 5. Proposed role of MtABCG20 in *Medicago truncatula* root. MtABCG20 functions as an exporter of abscisic acid (ABA) from its biosynthetic site in parenchyma cells to its response site. Consequently, MtABCG20 positively influences lateral root formation while negatively affecting root nodule development. (From publication **H5**).

Since ABA plays a crucial role in seed dormancy and germination, we decided to examine MtABCG20 in this context. In Arabidopsis, several ABCG transporters involved in ABA distribution in seeds have been characterized. Among them, AtABCG25 and AtABCG31 function as ABA exporters from the biosynthetic site (the endosperm), while AtABCG30 and AtABCG40 import ABA into the embryo. Their coordinated activity leads to suppressing seed germination under unfavorable conditions. Our analyses revealed that *MtABCG20* promoter activity in seeds is highly specific and restricted to the hypocotyl–radicle transition zone. However, the presence of an ABA exporter in the embryo of *M. truncatula* raises questions about its function, which appears to differ from that described for ABA transporters in Arabidopsis. Phenotypic analyses of *mtabcg20* mutants revealed that MtABCG20 promotes seed germination and that ABA accumulates to higher levels in the embryos of *mtabcg20* mutants compared with the wild type.

In summary, MtABCG20 likely participates in the removal of ABA from the specific growth zone of the embryonic root, thereby relieving this area from ABA's inhibitory effects and promoting germination (Fig. 6). This led to the proposal of a previously undescribed mechanism of germination control, which indirectly supports the idea of spatially separated decision-making centers in the embryo and the necessity of communication between them, possibly through hormone transport (Topham et al., 2017). However, whether this mechanism is unique to legumes remains an open question.

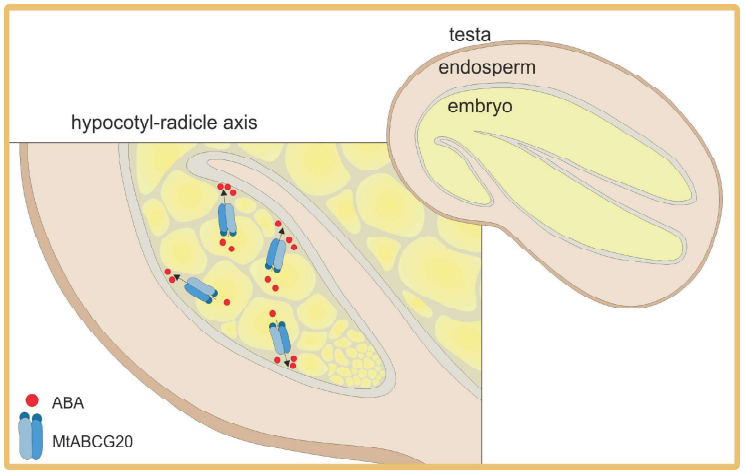

Figure 6. The proposed role of MtABCG20 in seed germination. MtABCG20 removes ABA from the embryonic axes cell, thus positively influencing germination. (From publication **H5**).

3.6 Determining the role of the MtABCG59 transporter in the secretion of stigolactones during the establishment of arbuscular mycorrhiza - in the context of the specific anatomical structure of legume roots (H6)

Developing my own research directions, I focused on determining the role of ABCG proteins from *M. truncatula* in another type of symbiotic interaction – arbuscular mycorrhiza (AM). AM is an ancient and widespread symbiotic interaction established between most terrestrial plants and fungi from the subphylum Glomeromycotina (Parniske, 2008). The main benefit of AM for the plant is improved acquisition of mineral nutrients, particularly phosphates. In return, the plant supplies its fungal partner with carbohydrates and lipids. The exchange of nutrients occurs through the membrane surrounding the highly branched fungal structures inside the plant cell, known as arbuscules. The origins of AM coincide with the emergence of land plants (Pirozynski and Malloch, 1975), and the early stages of the AM signaling pathway were adapted by legumes to establish a specific symbiosis with nitrogen-fixing bacteria (Oldroyd and Downie, 2006).

It is known that the establishment of AM is preceded by the release of signaling molecules into the rhizosphere. Among them, host-derived strigolactones (SL) play the most important role, stimulating the metabolism of arbuscular mycorrhizal fungi (AMF) and possibly guiding fungal hyphae toward host roots (Akiyama et al., 2005). In publication **H6**, of which I am the first and corresponding author, a functional analysis of the potential SL transporter in *M. truncatula* - MtABCG59 was described. Importantly, it was proposed that SL secretion, mediated by ABCG proteins, depends on specific anatomical features of the roots, including the presence or absence of the so-called exodermis. Article H6 is the result of the implementation of the SONATA grant entitled "Active transport systems as an element that controls the molecular cross-talk between symbionts during arbuscular mycorrhiza formation", of which I was principal investigator, and cooperation with foreign partners (Lorenzo Borghi and Enrico Martinoia). In addition, some of the results described in the publication were obtained during my internship at the Department of Plant and Microbial Biology, University of Zurich.

Previous studies conducted in Prof. Enrico Martinoia's laboratory demonstrated that SL secretion in petunia, a member of the Solanaceae family, is mediated by the full-size ABCG transporter PDR1, which is localized in the so-called hypodermal passage cells (HPCs) located within the exodermis. These cells also serve as entry points for AMF hyphae into the root (Kretzschmar et al., 2012). Importantly, most legume plants do not form a suberized cell layer called the exodermis, an apoplastic diffusion barrier located below the epidermis (Peterson and Perumalla, 1990). This is also true for *M. truncatula*, as confirmed by analyzing its roots stained with the fluorescent dye Fluorol Yellow 088, used to visualize suberin in plant tissues (Fig. 7A). This observation served as a starting point to examine how differences in root anatomical features affect SL release and the establishment of AMS, as well as to determine whether the SL transport mechanism based on full-size ABCG proteins functions in plants beyond the Solanaceae family.

To investigate this, we first performed phenotypic analyses on *M. truncatula* plants overexpressing PaPDR1 from *Petunia axillaris*. The transgenic plants displayed several traits associated with SL activity, including increased AMF colonization compared to the wild type. Demonstration of PaPDR1 functionality in Medicago motivated the identification of the endogenous ABCG protein responsible for SL translocation in this legume. Based on phylogenetic analyses and examination of ABCG gene expression in Medicago under conditions promoting AMS (phosphate deficiency) and following treatment with exogenous synthetic SL (GR24), MtABCG59 was selected as a candidate for further functional studies. *MtABCG59* was found to be root-specific, with promoter activity primarily localized to the outer cortical cells of the root, consistent with its proposed role in SL secretion into the rhizosphere. In collaboration with Lorenzo Borghi, phenotypic analyses of Tnt1 insertional *mtabcg59* mutants were conducted. Loss of MtABCG59 function resulted in reduced colonization of Medicago roots by *Rhizophagus irregularis*, particularly at early stages of the interaction, while the arbuscular fungal structures, which mediate nutrient exchange, remained intact. To investigate whether MtABCG59 is involved in SL secretion, *mtabcg59* root exudates were assessed for their ability to stimulate parasitic weed *Phelipanche ramosa* seed germination, a process entirely dependent on the presence of SL. Our experiments revealed that root exudates derived from *mtabcg59* possess lower germination-inducing activity than exudates from the WT. Interestingly, the effect observed concerned only seeds that were at some distance from the root, suggesting potential diffusion of SL, possible in the absence of an exodermis in *M. truncatula* roots.

Summarizing, the obtained results allowed us to propose a model comparing the first, SL-dependent stages of AMS formation between *P. axillaris* (Solanaeae) and *M. truncatula* (Fabaceae). In the case of *P. axillaris,* SLs are released into the rhizosphere by HPCs-localized PaPDR1. A steep concentration gradient of SLs, created by PaPDR1, guides AMF to access penetration sites – i.e., unsuberized HPCs. In the *M. truncatula*, due to a lack of exodermis, SLs could passively enter the rhizosphere because they do not encounter the apoplastic diffusion barrier. However, we suggest that to achieve the full extent of AMF colonization, the active

export of SLs, mediated by MtABCG59, is required. Its action ensures sufficiently high concentrations of SLs around the roots to attract AM fungi, which, together with the characteristic root anatomical traits in *M. truncatula*, enable the colonization of the whole root surface (Fig. 7B).

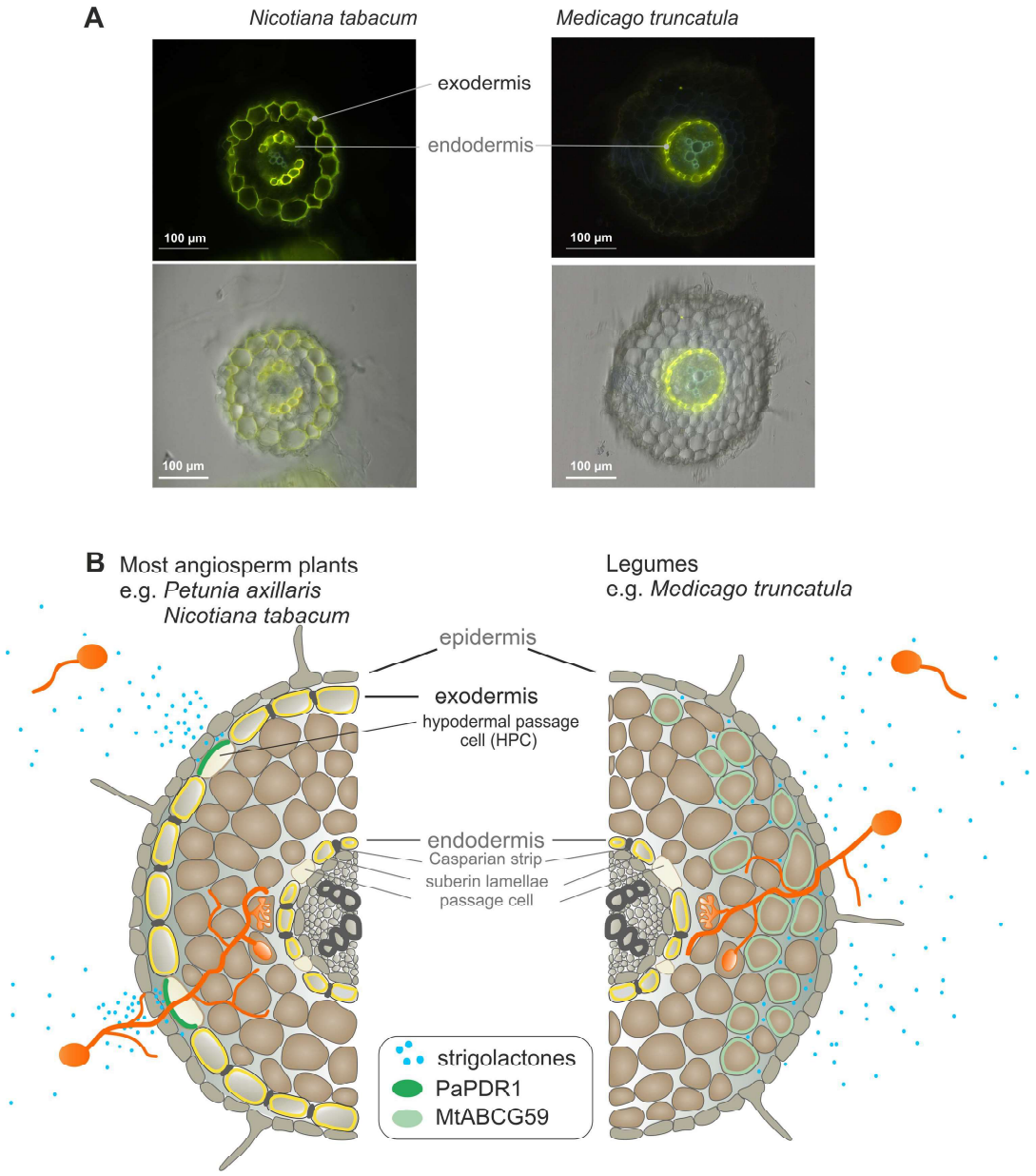

Figure 7. Influence of the root anatomical structure on the arbuscular mycorrhizal symbiosis (AMS) formation. Cross-section of *Nicotiana tabacum* (Solanaceae) and *Medicago truncatula* (Fabaceae) roots, stained with Fluorol Yellow 088, showing lack of the exodermis in *M. truncatula* roots **(A)**. Comparison of strigolactone (SL) exudation into the soil and SL-dependent guidance of arbuscular mycorrhizal fungi to the host root between *Petunia axillaris* and *Medicago truncatula*. In *P. axillaris*, SLs are secreted into the rhizosphere by PaPDR1, which is localized in the hypodermal passage cells (HPCs) within the exodermis. A steep concentration gradient of SLs, created by PaPDR1, guides arbuscular mycorrhizal fungi (AMF) to access penetration sites – i.e., unsuberized HPCs. In the *M. truncatula*, due to a lack of exodermis, SLs could passively enter the rhizosphere because they do not encounter the apoplastic diffusion barrier; however, to achieve the full extent of AMF colonization, the active export of SLs, mediated by MtABCG59, is required (B). (From publication **H6**)

In addition to AM, LRS may also be regulated by SLs during the early stages of the interaction, as suggested by studies conducted on various legume species (Foo and Davies, 2011; Liu et al., 2013). The absence of phenotypic differences in nodule number and morphology between wild-type plants and mutants indicates that MtABCG59 does not play a major role in nodulation. However, this function may be performed by its close homolog, *MtABCG43*, whose expression is induced by nitrogen deficiency and treatment with the NOD factor that initiates LRS.

3.7 ABCG gene expansion associated with adaptation to chemodiversity and new functions (H7, H8)

Review article **H7**, in which I am the first co-author, and review article **H8**, in which I am the first and corresponding co-author alongside Prof. M. Jasiński, summarize the achievements and results from the **H1-H6** publications and place them in a broader context of the current knowledge. Specifically, they address: i) the functioning of transport systems (including ABC proteins) at various stages of AM and LRS, and ii) plant proteins ABC, with an emphasis on their evolution, adaptation to new functions, and their potential importance in the development of sustainable agriculture.

Publication **H7** provides a comprehensive overview of the roles of various transport systems during the establishment of AM and LRS. The article summarizes current knowledge about the transport of signaling molecules at the presymbiotic stage, which determines partner recognition, as well as the potential roles of transporters during the invasion process. Additionally, it describes in detail the transporters located in the periarbuscular membrane and the symbiosome membrane, which are responsible for nutrient exchange between symbionts. The review highlights recent discoveries in the field, including the crucial role of lipid compounds belonging to the sn2-monoacylglycerols class as a major carbon source for mycorrhizal fungi and their translocation, as well as the identification of iron transporters essential for atmospheric nitrogen fixation in root nodules. Furthermore, the article points out significant gaps in knowledge, such as the still poorly understood mechanisms underlying the transport of plant hormones that modulate symbiotic interactions.

Publication **H8** focuses on ABC transporters, moving beyond the classic model organism Arabidopsis, which has been extensively reviewed in the past. First, transcriptomic data from the One Thousand Plant Transcriptomes (1KP) project related to plant *ABC* genes were summarized. This analysis allowed us to trace changes in the distribution of ABC transporters across various taxonomic groups within Archaeplastida (glaucophytes, red algae, green algae, and land plants). The article briefly discusses the evolutionary mechanisms driving changes in the number of ABC genes, as well as the role of ABC transporters in facilitating the colonization of land by plants.

The second part of the article focuses on full-size ABCG proteins in three economically important families of flowering plants: Fabaceae, Solanaceae, and Brassicaceae. Within these families, we observed a significant expansion of ABCG genes, which appears to be linked to

adaptations to specific biochemical backgrounds and the production of distinct groups of secondary metabolites, including flavonoids, terpenoids, and glucosinolates (Fig. 8).

It is worth noting that the ABCG proteins from *M. truncatula* presented in the habilitation achievement – MtABCG10/46 **(H2, H3)**, MtABCG56 **(H4)**, and MtABCG59 **(H6)** – belong to a expanded and the largest clade referred to as "G40", which includes only one representative from *A. thaliana*, AtABCG40 (Fig. 8). Both the results obtained in this work and those of other research groups suggest that the "G40" clade plays an important role in the broad interactions between plants and microorganisms. This clade comprises proteins responsible for both defense against pathogens (transport of secondary metabolites – NtPDR1, NpPDR1, NbABCG2, MtABCG46) and symbiotic interactions (LRS, AMS – MtABCG56, PhPDR1, MtABCG59). Its presence emphasizes the importance of conducting basic research on ABCG transporters beyond the canonical *A. thaliana* model, which is crucial for fully understanding their functions. The uniqueness of the "G40" clade is further highlighted by the fact that ABCG transporters forming another separate clade, "G32", which are responsible for the translocation of cutin monomers, are widespread and conserved among flowering plant species. This is consistent with their universal function (Fig. 8). Within the "G40" clade, a legume-specific subclade can be identified that includes the MtABCG56 **(H4)** protein, which is involved in promoting LRS. This suggests that the acquisition of the ability to establish specific symbiotic interactions may have been linked to the expansion and functional specialization of ABCG genes in legumes. Additionally, within the branch that groups strigolactone transporters (signaling compounds in AMS), including MtABCG59 **(H6)**, there are no representatives of ABCG transporters from Brassicaceae species, which do not form symbiotic relationships with arbuscular mycorrhizal fungi (Fig. 8) **(H8)**.

Considering the application potential of ABC transporters, in the final part of the publication **(H8)**, we were the first to gather examples of ABC proteins that positively influence agronomic traits, making them important targets for enhancing crop yield and quality.

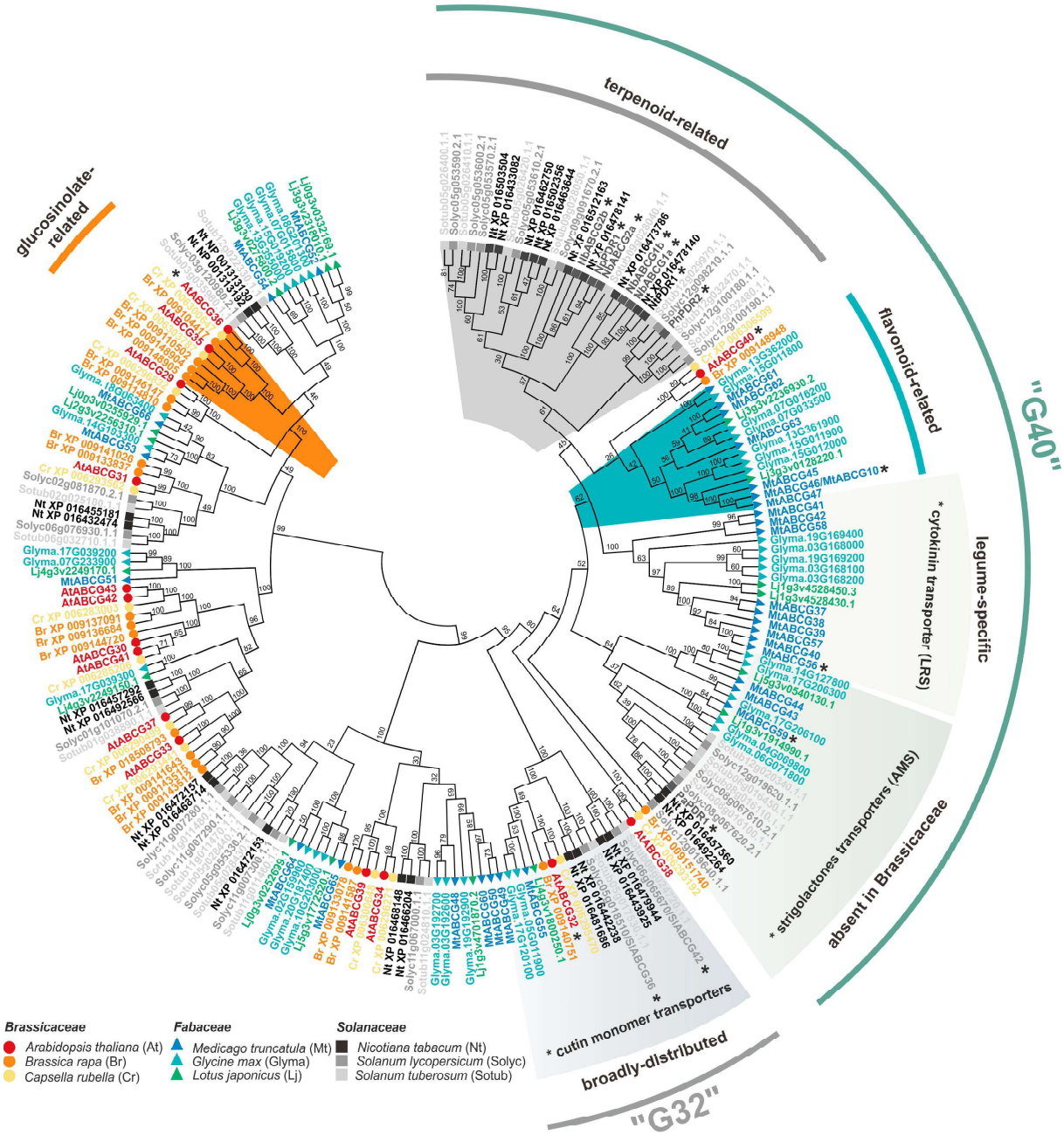

Figure 8. Phylogenetic tree of full-size ABCG proteins from plant species belonging to the families Brassicaceae, Fabaceae, and Solanaceae. The clades "G32" and "G40" are named after their homologs in *Arabidopsis thaliana*. The tree also includes additional subgroups of ABCG proteins, categorized based on phylogenetic relationships and potential functional specialization. An asterisk marks previously characterized ABCG transporters for which substrates are known or postulated. (From publication **H8**).

3.8 Summary of the most important achievements of the postdoctoral researcher in the presented series of publications

The research discussed above, which constitutes my scientific achievement entitled „Expansion and specialization of ABCG transporters in legumes", was conducted at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences (IBCH PAS) in Poznań and at the Department of Plant and Microbial Biology, University of Zurich (during two internships). My

scientific achievement comprises a collection of eight multi-authored publications. Five of these present the results of original research, one is a book chapter, and the remaining two are review articles summarizing the achievements/results of my research on ABC transporters, which were placed in the broader context of current knowledge. I am the first author of three of these publications, the co-first author in three additional ones, and the corresponding or co-corresponding author in two articles. These works have been published in prestigious journals, including Nature Plants, New Phytologist, Plant Physiology, Plant Journal, Journal of Experimental Botany, Frontiers in Plant Science, and Cellular and Molecular Life Sciences. Several of these publications have received awards, and the results have been presented at numerous international conferences in the form of scientific posters and oral presentations.

The findings presented in this series of publications have significantly advanced our understanding of the role of ABCG proteins in the transport of secondary metabolites and plant hormones, as well as their importance in developmental processes, responses to environmental stresses, and interactions with microorganisms. Importantly, these studies were unique and highlighted the crucial role of ABCG proteins in the biology of legume plants. It was proposed that the expansion of ABCG transporters and their functional specialization in legumes may be associated with adaptation to specialized metabolism and acquisition of new functions, such as establishing specific symbiotic interactions.

One of the key achievements was determining the role of the MtABCG10/46 protein in the biosynthesis of legume-specific 5-deoxy(iso)flavonoids and their derivatives. It was demonstrated that MtABCG10/46 is responsible for redirecting precursors (*p*-coumaric acid and liquiritigenin), under biotic stress conditions, towards the branch of the phenylpropanoid pathway responsible for medicarpin biosynthesis. This was the first time an ABCG transporter activity was linked to the distribution of early precursors and the modulation of secondary metabolite biosynthesis, rather than the transport of end products. Additionally, the transient substrate access pathway to the central cavity of the MtABCG10/46 transporter was characterized, and a key amino acid residue affecting its selectivity was identified.

Another significant achievement was the discovery of the long-sought CK transporter involved in the initiation of root nodule formation. It was shown that MtABCG56 is responsible for the translocation of CK from the rhizodermis to the root cortex at early stages of symbiosis. This influences the distal cortical response to CKs and the formation of the nodule primordium, and limits the negative effects of rhizodermal CKs on the infection process. This discovery provided new evidence supporting the hypothesis that CKs act as a mobile signal coordinating events between the rhizodermis and the root cortex during legume-rhizobium symbiosis establishment.

An important step was elucidating MtABCG20's role in nodulation and root architecture under drought-simulating conditions. The results suggest that MtABCG20 exports ABA from its synthesis site to modulate cell divisions during lateral root and nodule formation, helping

adjust root morphology to environmental conditions. It was also shown that MtABCG20 may participate in removing ABA from the embryonic root growth zone, thus releasing this area from the inhibitory effect of ABA and promoting germination. Consequently, a new mechanism of seed germination control by ABA transporters was proposed, which differs from the mechanism previously described for Arabidopsis.

Further studies identified MtABCG59 as a potential SL transporter, indicating a universal mechanism of SL transport across different plant taxonomic groups (Solanaceae, Fabaceae). At the same time, it was proposed that differences in SL secretion may reflect adaptations to root anatomy, particularly the presence (Solanaceae - Petunia) or absence (Fabaceae - Medicago) of the exodermis, influencing SL dispersal and fungal entry points.

Beyond its scientific importance, these findings could support the development of sustainable agriculture by promoting legume use, which can reduce chemical inputs. Based on my expertise, I was invited to join the management committee of the COST Action (European Cooperation in Science and Technology) CA22142 "Beneficial root-associated microorganisms for sustainable agriculture (ROOT-BENEFIT)" and elected as the vice leader of the working group focusing on the molecular mechanisms underlying plant–microorganism interactions. The goal of this initiative is to foster multidisciplinary collaboration, enhance research capacity, and facilitate knowledge transfer to socio-economic stakeholders regarding beneficial soil microorganisms in sustainable agriculture.

3.9 Future Directions

In the future, I plan to continue my research on the molecular mechanisms underlying the agronomic traits of legume plants. One such trait is the control of seed germination, which directly affects crop quality and yield. My interest in this topic stems from my experience studying the role of ABA transport in the germination process, which inspired me to further investigate the still poorly understood seed biology of *M. truncatula*. Germination, that is, the transition from the embryonic resistant state of mature seeds to the sensitive seedling, is a key stage of plant development that determines its survival. This process is tightly regulated, and understanding the mechanisms that underlie it has fundamental and practical importance. Accordingly, I am currently engaged as a PI in implementing my own OPUS research project entitled "New mechanisms of MFT protein action determining *Medicago truncatula* seed sensitivity to abscisic acid during germination."

The MFT (MOTHER OF FT AND TFL1) protein belongs to the phosphatidylethanolamine-binding protein (PEBP) family and is considered the ancestor of the well-studied FT (FLOWERING LOCUS T) and TFL1 (TERMINAL FLOWER1) proteins, which control flowering time (Karlgren et al., 2011). It is postulated that MFT modulates the

activity of transcription factors and thereby regulates the expression of target genes responsible for controlling seed dormancy and germination (Xi et al., 2010). Given the complexity of these processes and their species-specific requirements, I have undertaken a comprehensive functional analysis of the MFT protein from *M. truncatula*. This study aims to determine the potential role of MtMFT in establishing dormancy during seed development, as well as in inhibiting germination under unfavorable environmental conditions. At the same time, I intend to identify the signaling pathways and protein partners (transcription factors) involved in these processes and interacting with MtMFT, as well as potential genes whose expression is regulated by MtMFT. Moreover, in collaboration with crystallographers, we plan to determine the crystal structure of the MtMFT protein.

Using my experience in seed biology and ABCG transporter studies, I plan to further investigate the role of ABCG proteins in this context. My future research will focus on elucidating the contribution of ABCG transporters to both physiological and physical seed dormancy, as well as their function in preventing germination under unfavorable environmental conditions. This is particularly relevant for legumes, in which physical dormancy plays a key role in seed viability and uniform germination, with *M. truncatula* serving as an established model for studying this process at ecological and genetic levels (Renzi et al., 2020). There is increasing evidence that several ABCG transporters participate in the transport of suberin and cutin monomers, which are essential for the formation of lipid-based apoplastic diffusion barriers in the seed coat, a factor known to significantly impact seed quality. Understanding how these transporters mediate the deposition of these lipid compounds could shed light on the molecular basis of physical dormancy and seed longevity. In addition, my preliminary data suggest that the same group of ABCG proteins may be involved in establishing oxygen diffusion barriers in root nodules. This mechanism likely balances the high energy requirements for nitrogenase activity with the need to protect this oxygen-sensitive enzyme from inactivation, thereby supporting efficient biological nitrogen fixation. Identifying ABCG transporters that simultaneously contribute to key agronomic traits such as optimized germination and effective nitrogen fixation holds strong potential for fundamental discoveries and for developing novel selection markers in legume breeding. In the coming years, I aim to expand this research to deepen our understanding of the multifunctional roles of ABCG transporters in seeds and nodules, thereby exploring this still poorly understood area of plant science.

In the longer term, I also plan to expand the analyses of ABC proteins to include regulatory aspects, focusing on transcriptional control mechanisms. In recent years, the existence of specific operational units has been proposed, comprising transcription factors that coordinate the expression of genes involved in both the biosynthetic pathway and the transport of particular substrates. A well-documented example is the lipid export pathway regulated by the transcription factor RAM1 (Required for Arbuscular Mycorrhization 1), which includes arbuscular mycorrhiza-specific enzymes FatM and RAM2 that fine-tune lipid biosynthesis and ABCG transporters essential for the establishment of AM (Keymer et al., 2017). Identifying such functional units will significantly advance our knowledge; moreover, it may have practical

applications in systems biology and contribute to the development of new biotechnological tools for the production of natural products.

Additionally, my scientific interests include questions related to the evolution, expansion, and specialization of ABC proteins. Currently, in collaboration with Dr. Markus Geisler at the University of Fribourg, I am working on identifying full-size ABCB proteins, including potential auxin transporters, from diverse taxonomic groups within Archaeplastida, as well as Xanthophyta, Phaeophyta, and Bacillariophyta. These analyses are part of a broader project aiming to trace the evolutionary origins of auxin transport.

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

My scientific achievements have been obtained in three research institutes:

Poland:

1. Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan
2. Department of Biochemistry and Biotechnology, Poznań University of Life Sciences

Abroad:

3. Department of Plant and Microbial Biology, University of Zurich.

My scientific career has been primarily associated with the *Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań*, focusing on research into ABC transporters in the model legume *M. truncatula*. At IBCH PAS, I completed my doctoral studies and obtained my PhD degree in 2013. During my doctoral studies, I was awarded a promoter's grant as well as a scholarship under the program "Scholarship support for PhD students in fields considered strategic for the development of the Wielkopolska region." After earning my PhD, I worked as a postdoctoral researcher and project investigator on the HARMONIA and OPUS research grants, and subsequently, I led my own SONATA project. Currently, as the principal investigator of an OPUS grant, I am studying the role of the MFT protein in seed biology of *M. truncatula.*

Supported by the NCN HARMONIA and SONATA grants, I carried out two research internships at the Department of Plant and Microbial Biology, University of Zurich, in the group led by Professor Enrico Martinoia. Outcomes of this research were incorporated into publication H6 and presented in the form of posters and oral presentations at various international conferences.

In addition to my main line of research on ABC transporters in *M. truncatula*, I also participated in studies on the potential role of MATE transporters (Multidrug and Toxic Compound

Extrusion) in another model legume, *Lotus japonicus*, during an internship at the Poznań University of Life Sciences. This work focused particularly on their involvement in interactions with mycorrhizal fungi and their role in drought stress. The results were presented as scientific posters at international conferences, including the EMBO Workshop on Plant Membrane Biology (WPMB) in Helsingør, Denmark (2025).

VI. Description of other scientific and research achievements

List of publications not included in the scientific achievement *(the name of the candidate is in bold; *corresponding author; bibliographic data are given according to Web of Science Core Collection)*

Articles published after obtaining the PhD degree:

N1. Xia J, Siffert A, Torres O, Iacobini F, **Banasiak J**, Pakuła K, Ziegler J, Rosahl S, Ferro N, Jasiński M, Hegedűs T, Geisler MM*.

A key residue of the extracellular gate provides quality control contributing to ABCG substrate specificity. *Nature Communications*. (2025) 5;16(1):4177.

- doi: 10.1038/s41467-025-59518-3
- $IF_{(2024)} = 15.7$; $IF_{(5\ Year)} = 17.2$
- $MN_{(2024)} = 200$
- Citations: 0

Collaboration with Dr. Markus Geisler has resulted in a publication describing an important structural element that determines the substrate selectivity of ABCG proteins. Using one of the best-characterized ABCG transporters from Arabidopsis, AtABCG36/PEN3, the study highlighted the key role of a single amino acid (L704), which forms part of an extracellular hydrophobic gate that controls the exit of substrates from the central cavity and ensures substrate specificity. It was demonstrated that this region can be regulated allosterically, which in turn influences the protein's preference for its two structurally related substrates: the auxin precursor IBA and the defense compound camalexin. This precise discrimination and selection of substrates enables the plant to maintain a balance between growth processes and defense mechanisms.

N2. Banasiak J*

System odpornościowy roślin – model zygzakowy. The plant immune system – zig-zag model, *Postępy Biochemii* (2022)

- doi: doi.org/10.18388/pb.2021_427
- $MEiN_{(2021)} = 70$
- Citations: 0

N3. Stec N[#], **Banasiak J**[#], Jasiński M*. [#]These authors contributed equally

Abscisic acid - an overlooked player in plant-microbe symbioses formation? *Acta Biochimica Polonica*, (2016) 63(1) 53-58.

- doi: 10.18388/abp.2015_1210
- $IF_{(2015)}$ = 2.403; $IF_{(5\ Year)}$ = 1.534
- $MNiSW_{(2015)}$ = 15
- Citations: 26

Articles published during PhD studies

N4. **Banasiak J**, Biala W, Staszków A, Swarcewicz B, Kepczynska E, Figlerowicz M, Jasinski M*.

A Medicago truncatula ABC transporter belonging to subfamily G modulates the level of isoflavonoids. *Journal of Experimental Botany*, (2013) Feb;64(4):1005-15

- doi: 10.1093/jxb/ers380
- $IF_{(2012)}$ = 5.242; $IF_{(5\ Year)}$ = 5.542
- $MNiSW_{(2012)}$ = 45
- Citations: 74

N5. Staszków A, Swarcewicz B, **Banasiak J**, Muth D, Jasiński M, Stobiecki M*.

LC/MS profiling of flavonoid glycoconjugates isolated from hairy roots, suspension root cell cultures and seedling roots of *Medicago truncatula*. *Metabolomics*, (2011) Dec;7(4):604-613.

- doi: 10.1007/s11306-011-0287-2
- $IF_{(2010)}$ = 3.608; $IF_{(5\ Year)}$ = 3.637
- $MNiSW_{(2010)}$ = 30
- Citations: 39

N6. Jasiński M, **Banasiak J**, Radom M, Kalitkiewicz A, Figlerowicz M*.

Full-Size ABC Transporters from the ABCG Subfamily in *Medicago truncatula*. *Molecular Plant-Microbe Interactions*, (2009) 22(8): 921-931.

- doi: 10.1094/MPMI-22-8-0921
- $IF_{(2008)}$ = 4.136; $IF_{(5\ Year)}$ = 4.303
- $MNiSW_{(2008)}$ = 24
- Citations: 24

N7. Jasinski M, **Banasiak J**, Figlerowicz M*.

Transportery PDR – czyli historia jeszcze nienapisana. Od syntezy chemicznej do biologii syntetycznej, *Ośrodek Wydawnictw Naukowych,* (2009)147-160.

Articles published during master's degree studies

N8. Jasiński M, **Banasiak J**, Figlerowicz M*.

Roślinne transportery ABC. Na pograniczu chemii i biologii, Wydawnictwo Naukowe UAM, (2007) t. XVII: 257-274.

N9. Jasiński M, **Banasiak J**, Frankowska M, Figlerowicz M*.

Rośliny jako reaktory do produkcji biofarmaceutyków. Plants as the reactors for production of biopharmaceuticals. Biotechnologia, (2006) 3 (73): 53-66.

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

Teaching achievements:

I was the scientific supervisor/auxiliary supervisor of graduate and PhD students:

Auxiliary supervisor of PhD thesis:

- **Wanda Biała**
  Title: „The role of ABCG10 transporter in the biosynthetic pathway of medicarpin – *Medicago truncatula* phytoalexin"
  Poznań University of Life Science, defense 2017
- **Karolina Jarzyniak**
  Title: „Functional analysis of selected ABCG transporters from *Medicago truncatula* involved in symbiotic interaction"
  Poznań University of Life Science, defense 2018
- **Tomasz Jamruszka**
  Title: „Functional analysis of the ABCG transporter involved in the negative regulation of lateral root density and nodule number in Medicago truncatula"
  Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan, defense 2023
- **Maciej Nowak**
  Topic: „New mechanisms determining MFT-mediated sensitivity to abscisic acid during the germination of Medicago truncatula seeds"
  Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan, in progress

Supervisor of Master's thesis:

- **Joanna Matuszek**
  „Selected elements of functional analysis of MtPDR23 – a potential strigolactones transporter in *Medicago truncatula*"
  Poznań University of Life Science, 2018
- **Adwoa Ansomaa Tenkorang**
  „Subcellular Localisation Of Mother of FT and TFL1 in *Medicago truncatula*„
  Poznań University of Life Science, 2021

Supervisor of trainees:

- **Natalia Stec**
  Faculty of Biology, Adam Mickiewicz University, 2016
- **Maria Klimas**
  Faculty of Biology, Adam Mickiewicz University, 2018
- **Wiktoria Brożyńska**
  Faculty of Biology, Adam Mickiewicz University, 2020
- **Magdalena Brzózka**
  Faculty of Biology, Adam Mickiewicz University, 2022
- **Abinaya Manoharan Geetha Ponmalar**
  Poznań University of Life Science, 2025
- **Aleksandra Marszałek**
  Faculty of Biology, Adam Mickiewicz University, 2025

Reviewer of the Engineer's thesis:

- **Paulina Bierwagen**
  „Optimization of the purification method for BMV and RCNMV viruses isolated from plant tissues"
  Poznan University of Life Sciences, 2014

Organizational achievements:

Managing projects financed from external sources after obtaining PhD:

- National Science Centre (NCN) grant, SONATA9: „Active transport systems as an element that controls the molecular cross-talk between symbionts during arbuscular mycorrhiza formation". 2015/17/D/NZ3/03625, 2016-2020, IBCh PAS, **(principal investigator)**
- National Science Centre (NCN) grant, OPUS22: "New mechanisms determining MFT-mediated sensitivity to abscisic acid during the germination of *Medicago truncatula* seeds". 2021/43/B/NZ3/00672, 2022-2025, IBCh PAS, **(principal investigator)**

Conference organization:

- 11th PSEPB Conference, Poznan, September 19–22, 2023 (member of the organizing committee)

Achievements promoting science

- Popularization of science during the Night of Scientists and the Festival of Science and Art
- Popularization of science at School and Preschool Complex No. 11 in Poznań:
  "Krasnale" group (Bajkonutki Preschool) – 2020

Class II b and common room (Primary School No. 11 in Poznań) – 2022

- Popularization of science at Primary School No. 64, Class III a – 2025

VIII. In addition to the information provided above, the applicant may include any other details they consider important regarding their professional career

Awards:

1. Bronze Cross of Merit for achievements in scientific-research activities, granted by the President of the Republic of Poland (2023)
2. Director's Award of IBCH PAS for the best review paper in 2022 titled: "ATP-binding cassette transporters in nonmodel plants" (awarded in 2023)
3. Team award from the Division II – Biological and Agricultural Sciences for an outstanding research achievement entitled “Understanding the mechanism of cytokinin distribution in the process of biological nitrogen fixation in legumes”, awarded to Jasiński M., Jarzyniak K., **Banasiak J**., Jamruszka T., Pawela A. (awarded in 2022)
4. Distinction in the Professor Kazimierz Bessalik competition awarded by the Committee on Molecular Biology of the Cell, Polish Academy of Sciences, for the article "Early stages of legume–rhizobia symbiosis are controlled by ABCG-mediated transport of active cytokinins" published in the journal Nature Plants 2021; Committee on Molecular Biology of the Cell, Polish Academy of Sciences (awarded in 2022)
5. Director's Award of IBCH PAS for the best experimental works in 2021, titled: "Early stages of legume–rhizobia symbiosis are controlled by ABCG-mediated transport of active cytokinins" (awarded in 2022)
6. Director's Award of IBCH PAS for the best experimental works in 2019, titled: “MtABCG20 is an ABA exporter influencing root morphology and seed germination of *Medicago truncatula”* (awarded in 2020)
7. Director's Award of IBCH PAS for the best experimental works in 2017, titled: “*Medicago truncatula* ABCG10 is a transporter of 4-coumarate and liquiritigenin in the medicarpin biosynthetic pathway” (awarded in 2018)
8. Director's Award of IBCH PAS for the best experimental works in 2013, titled: “A *Medicago truncatula* ABC transporter belonging to subfamily G modulates the level of isoflavonoids” (awarded in 2014)
9. Director’s Award of IBCH PAS, for one of the best doctoral dissertations defended at the Institute in 2013 (awarded in 2014).
10. Stefan Barbacki National Scientific Award in Plant Genetics (awarded in 2012)
11. Scholarship for PhD students with specializations considered to be strategic for the development of Greater Poland; Voivodeship Employment Office (awarded in 2012)
12. Award for outstanding academic performance during studies at Adam Mickiewicz University in Poznań (awarded in 2007)

Oral presentations at scientific conferences:

1. **EMBO Workshop on Plant Membrane Biology** (WPMB). Helsingør, Denmark (2025) **(wykład na zaproszenie)**
2. Cytokinin Forefront Research. Olomouc, Czech Republic (2024)
3. **Gordon Research Conference**: Plant Communication: Understanding Signal Creation, Transmission, and Perception in Plant Systems. Holderness, New Hampshire, USA (2024)
4. Third International Legume Society Conference ILS3. Poznań, Poland (2019)
5. XI Parnas Conference - Young Scientists Forum, "Biochemistry and Molecular Biology for Innovative Medicine". Kyiv, Ukraine (2018)
6. 1st Congress of the Polish Biochemistry, Cell Biology, Biophysics and Bioinformatics BIO. Warsaw, Poland (2014)

Additional activities:

- Secretary of the Scientific Board of IBCh PAS 2015-2018; 2019-2022; 2023-2026.
- Member of the Management Committee and Vice-Leader of Working Group 2 (WG2) in the European COST Action CA22142 “Beneficial root-associated microorganisms for sustainable agriculture (ROOT-BENEFIT
- Editorial Board Member of journal “Postępy Biochemii”
- Member of working group of HR Excellence in research in IBCh PAS
- Reviewer of New Phytologist; Plant Molecular Biology; DNA Research; Plant, Cell & Environment; BioTechnologia; Acta Physiologiae Plantarum; Journal of Soil Science and Plant Nutrition

Literature:

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Joanna Banasiak