Łukasz Piotr Haliński, Ph.D.

Chemotaxonomic analysis of selected solanaceous plants and estimation of their domestication phase by using secondary metabolite profiles

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SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

Laboratory of Natural Product Analysis
Department of Environmental Analysis
Faculty of Chemistry, University of Gdańsk

Gdańsk 2019

1. Name and surname

Łukasz Piotr Haliński

2. Diplomas and academic degrees

28.06.2005	**MSc diploma in the field of Protection of the Environment**, Faculty of Chemistry, University of Gdańsk; MSc thesis: "Cuticular wax extraction from the potato (*Solanum tuberosum*)", was executed in the Department of Environmental Analysis under supervision of Beata Szafranek, Ph.D.
27.01.2010	**Ph.D. diploma in the field of Chemistry**, Faculty of Chemistry, University of Gdańsk; Ph.D. dissertation: "Chemical analysis of leaf cuticular waxes of the brinjal eggplant (*Solanum melongena* L.) and allied species, with their importance in plant biology and taxonomy", was executed in the Department of Environmental Analysis under supervision of Janusz Szafranek, Prof., Ph.D.

3. Information on previous and the current employment in scientific units

01.10.2009-30.09.2010	Senior technical consultant, Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk
from 01.10.2010	Assistant professor, Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk

4. Scientific achievement according to Article 16 Paragraph 2 of the Act of 14 March 2003 on Academic Degrees and Title and on Degrees and Title in Art. (Journal of Laws from 2017, item 1789):

A) Title of the scientific achievement

Chemotaxonomic analysis of selected solanaceous plants and estimation of their domestication phase by using secondary metabolite profiles

B) List of publications included in the scientific achievement

O1 **Ł.P. Haliński**, M. Paszkiewicz, M. Gołębiowski, P. Stepnowski, The chemical composition of cuticular waxes from leaves of the gboma eggplant (*Solanum macrocarpon* L.). *Journal of Food Composition and Analysis*, 2012, **25**, 74-78 (IF 2.088; MNiSW 40, where MNiSW is the Polish Ministry of Science and Higher Education)

O2 **Ł.P. Haliński**, P. Stepnowski, Short-term water deficit changes cuticular sterol profile in the eggplant (*Solanum melongena*). *Chemistry & Biodiversity*, 2016, **13**, 719-726 (IF 1.440; MNiSW 20)

O3 **Ł.P. Haliński**, J. Samuels, P. Stepnowski, Multivariate analysis as a key tool in chemotaxonomy of brinjal eggplant, African eggplants and wild related species. *Phytochemistry*, 2017, **144**, 87-97 (IF 3.186; MNiSW 35)

O4 **Ł.P. Haliński**, A. Puckowski, P. Stepnowski, Glycoalkaloid, phytosterol and fatty acid contents of raw and blanched leaves of the gboma eggplant (*Solanum macrocarpon* L.). *Journal of Food and Nutrition Research*, 2015, **54**, 9-20 (IF 1.676; MNiSW 20)

O5 **Ł.P. Haliński**, A. Topolewska, A. Rynkowska, A. Mika, M. Urasińska, M. Czerski, P. Stepnowski, Impact of plant domestication on selected nutrient and anti-nutrient compounds in Solanaceae with edible leaves (*Solanum* spp.). *Genetic Resources and Crop Evolution*, 2019, **66**, 89-103 (IF 1.130; MNiSW 30)

O6 **Ł.P. Haliński**, M. Kalkowska, M. Kalkowski, J. Piorunowska, A. Topolewska, P. Stepnowski, Cuticular wax variation in the tomato (*Solanum lycopersicum* L.), related wild species and their interspecific hybrids. *Biochemical Systematics and Ecology*, 2015, **60**, 215-224 (IF 0.988; MNiSW 15)

O7 **<u>Ł.P. Haliński</u>**, P. Stepnowski, Cuticular hydrocarbons and sucrose esters as chemotaxonomic markers of wild and cultivated tomato species (*Solanum* section *Lycopersicon*). *Phytochemistry*, 2016, **132**, 57-67 (IF 3.205; MNiSW 35)

The scientific achievement presented is composed of seven original articles published in journals included in Journal Citation Reports database. The total impact factor of the journals was **13.713**, and the total number of MNiSW (Polish Ministry of Science and Higher Education) points was **195** (according to the publication date). I was the first and corresponding author in all above-mentioned articles. Copies of articles are given as Appendix 4, and statements of author contribution in Appendix 5.

C) Description of scientific objectives of the above-mentioned works and the results obtained, with the discussion of their potential application

Introduction

Nightshades (Solanaceae) are a diverse and very large (9000-10000 species) plant family, including taxa of great economical importance (tomato, potato, eggplant, tobacco) (Knapp 2002). About 2000 species are classified within cosmopolitan genus *Solanum* L., which includes the majority of solanaceous plants used in agriculture. The whole genus was last treated taxonomically in the middle of the 19th century, while more modern studies focused mostly on relations within the genus. Despite applying both the conventional classification based on morphological traits and modern methods using molecular markers, taxonomical position of some species remains unclear. Moreover, the actual distinctiveness of some taxa, as well as correct identification of many plants already described and often available in germplasm banks, are still questionable.

Above-mentioned scientific achievement is focused on two groups of economically important solanaceous plants. First group includes the brinjal eggplant (*Solanum melongena* L.), several wild related species and African nightshades grown for edible leaves: gboma eggplant (*S. macrocarpon* L.) and the scarlet eggplant (*S. aethiopicum* L.) with their wild ancestors (*S. dasyphyllum* Schum. and Thonn. and *S. anguivi* Lam, respectively). The second group consists of the common tomato (*S. lycopersicum* L.) and several allied species

(*S. pimpinellifolium* L., *S. cheesmaniae* (L. Riley) Fosberg, *S. chilense* (Dunal) Reiche, *S. peruvianum* L. and *S. arcanum* Peralta), as well as the wild, genetically diverse and highly abiotic and biotic stress-resistant species *S. pennellii* Correl. Despite using both morphology-based approach and molecular markers in numerous scientific publications, in both groups there are still some taxa with unclear taxonomic position. In the same time, in both cases wild species are considered as a potential source of resistance or tolerance to abiotic stress (water deficit, soil salinity), as well as to insect feeding and pathogen infections (Collonier et al. 2001; Simmons and Gurr 2005). Therefore, they are species of interest in both basic and applied research.

In group of plants consisted of the brinjal eggplant and allied species, several major taxonomic uncertainties are still present, including: the exact status of wild/feral brinjal eggplant forms *S. melongena* subsp. *cumingii* (Dunal) J. Samuels (Mace et al. 1999; Samuels 2013a); time and place of the brinjal eggplant domestication, together with its true wild ancestors (Lester and Hasan 1991; Meyer et al. 2012; Samuels 2013b); and the taxonomic position of wild and domesticated African nightshades. For the long time, the gboma eggplant and its wild relative were classified together with the brinjal eggplant in section *Melongena*, while the scarlet eggplant and *S. anguivi* were placed in section *Oliganthes* (Lester and Daunay 2001). Currently, both complexes are usually treated as a part of a diverse group of poorly resolved taxa that are distinct from the brinjal eggplant (Vorontsova et al. 2013; Aubriot et al. 2016). Taxonomy of the *Solanum* subgenus *Leptostemonum*, which includes above-mentioned plant species, remains complicated because of the large diversification in terms of plant morphology in certain taxa. Some species were also described in the past using a number of synonymic names. For example, *S. aethiopicum* plants are now described as belonging to one of four groups of different morphology and diverse use in the agriculture. In the past, however, these plants were described as belonging to more than 20 species from several different genera (Lester 1986). Even now, taxonomic studies on this group of plants is often based strictly on plant morphology, which significantly affects the possibility of unambiguous identification of certain taxa (Knapp et al. 2013). Additionally, the domestication stage of African nightshades (*S. aethiopicum* and *S. macrocarpon*) also remains unclear. While they are both morphologically different from their wild ancestors, there are some reports suggesting that this is not followed by genetic distinctiveness (Bukenya and Carasco 1994; Stedje and

Bukenya-Ziraba 2003; Acquadro et al. 2017). The presence of significantly toxic steroidal glycoalkaloids in tissues of solanaceous plants is well documented (Mensinga et al. 2005). Reduction in toxic substances, including glycoalkaloids, is often suggested as one of the mechanisms in plant selection during domestication process and was already described also for Solanaceae (Johns and Alonso 1990; Moreira et al. 2018). Both gboma eggplant and the scarlet eggplant are grown for edible leaves and both are economically important in Africa. Therefore, it is of interest to find out, if the above-mentioned mechanism of selection was the part of domestication process of African eggplants, in order to determine their safety as green leafy vegetables.

Taxonomy of wild and grown tomato species has changed over time. Historically, tomatoes were classified as a distinct genus *Lycopersicon*, but sometimes were also placed in genus *Solanum* (Peralta and Spooner 2000). Later on, tomatoes were finally transferred to genus *Solanum* (Child 1990), several new species were described, and the modern taxonomy of this group of plants was established (Peralta et al. 2008). Possibly the most problematic issue in systematics of tomatoes is taxonomic position of biotic and abiotic stress-resistant species *S. pennellii*. Its high compatibility with all the species from *Lycopersicon* group was already described (Rick 1960; Bedinger et al. 2011). On the other hand, basing on several studies utilizing different molecular markers, the position of *S. pennellii* was difficult to establish (Palmer and Zamir 1992; Marshall et al. 2001; Spooner et al. 2005; Zuriaga et al. 2009; The 100 Tomato Genome Sequencing Consortium 2014). Some authors suggested its significant distinctiveness from all the other tomato species (Alvarez et al. 2001; Dodsworth et al. 2016). This species is of a special interest because of the presence of insecticidal glucose and sucrose esters on the surface of its aerial parts (Goffreda et al. 1990; Shapiro et al. 1994). Therefore, it is particularly important to clarify its taxonomic position, with the special attention paid to its affinity to the common tomato.

Cuticular (or surface) waxes consist of a complex mixture of organic compounds, that are present on the surface of all non-woody, aerial parts of higher plants and are responsible for the protection of plant from the environment (Kerstiens 1996; Müller and Riederer 2005). Because of their common occurrence, and because the majority of wax components is synthesized on quite well-documented metabolic pathways (Kunst and Samuels 2003), cuticular waxes could be considered as useful chemotaxonomic markers. As it was already mentioned, the use of morphological traits and molecular markers did not allow to fully clarify the taxonomy of described plant species. Therefore, it is of highest interest to obtain the additional set of data, that was generated basing on different markers. Cuticular wax components were in the past used as chemotaxonomic markers in classification of higher plants, including Solanaceae (Zygadlo et al. 1994; Haliński et al. 2011; Da Silva et al. 2012). Taking this into account, **the possibility of using cuticular wax components as chemotaxonomic markers in the classification of both above-mentioned groups of the plants was the main hypothesis of the whole scientific achievement**. Some other groups of metabolites were also used in determining the current domestication phase of several African nightshades with edible leaves.

Discussion of the scientific purpose and the results obtained

The main objectives of the research were:

a) **identification of cuticular wax components as potential chemotaxonomic markers** and evaluation of their composition stability, including the possible effects of environmental factors on this composition,

b) **classification of the plant species studied basing on their chemical characteristics**; plants used in the research included wild and domesticated solanaceous plants, namely: (1) the brinjal eggplant (*S. melongena*), complexes of scarlet eggplant (*S. aethiopicum*) and gboma eggplant (*S. macrocarpon*) with their wild relatives; and (2) the common tomato (*S. lycopersicum*) and allied species, with the special attention paid to the stress-resistant *S. pennellii*,

c) **estimation of the phase of domestication process** (understood as distinctiveness from the wild ancestor) for the number of emerging plants from *Solanum* L. genus grown in Africa, basing on the profiles of selected primary and secondary metabolites

Identification of chemotaxonomic markers and classification of the brinjal eggplant (*Solanum melongena*) and related species [O1-O3]

The use of cuticular wax components in chemotaxonomic approach to classification of so-called "spiny *Solanum*" plants was reported in the past several times (Zygadlo et al. 1994; Haliński et al. 2011; Da Silva et al. 2012). All these reports, however, did not primarily aim to clarify the unclear taxonomic position of the most problematic species, but more to determine usefulness of plant secondary metabolites as chemotaxonomic markers. Therefore, the objectives of publications **O1-O3** were: to identify components of cuticular waxes from the leaves of the gboma eggplant (*Solanum macrocarpon*) and to determine their stability between different plant accessions (**O1**); to estimate the effect of abiotic stress on leaf cuticular wax composition of the brinjal eggplant (*S. melongena*) as a model species in the group of taxa studied (**O2**); and finally to select markers of the highest stability and to use them in chemotaxonomic analysis of several species of unclear position within *Solanum* genus (**O3**). The latter was focused mainly on two species of African nightshades on suggested early phase of domestication: the gboma eggplant and the scarlet eggplant (*S. aethiopicum*), together with wild species usually considered as wild ancestors of these plants (*S. dasyphyllum* Schum. & Thonn. and *S. anguivi* Lam., respectively). Additionally, it was supposed that it is possible to use chemotaxonomic markers for the purpose of identification of wild/feral forms of the brinjal eggplant, that were in the past classified as several different taxa, usually basing only on their morphology.

Deep and complex chemical analysis of cuticular waxes from gboma eggplant leaves (**O1**) has revealed, that their chemical composition is much different than the one described for other domesticated *Solanum* species, including the potato (*S. tuberosum* L.) and closely related brinjal eggplant (Szafranek and Synak 2006; Haliński et al. 2009). First of all, while cuticular hydrocarbons were dominating fraction of cuticular waxes of both above-mentioned species with their contribution to the total wax coverage as high as 70-82%, in the gboma eggplant they were still the main fraction, but contributed only to 47-56% of the total wax. The overall wax coverage was also much lower and did not exceed 3 μg cm^{-2} of the leaf surface. Reported values were twice as high in case of the number of potato varieties (Szafranek and Synak 2006), and almost six-fold higher for the brinjal eggplant (Haliński et al. 2009). Other differences were mostly related to the presence of cyclic compounds: cuticular waxes of the gboma eggplant were in 19-32% composed of sterols. As far as I concerned, such a large contribution of these substances was never described before for cuticular waxes of any higher plant species. Cuticular hydrocarbons of *S. macrocarpon* were characterized by very high stability of their composition between plant accessions. This was consistent with previous report concerning cuticular lipids of the brinjal eggplant (Haliński et al. 2011). Similarly, in both *S. melongena* and *S. macrocarpon* waxes, other components, including fatty acids, triterpenes and sterols displayed much more variable chemical composition. Primary alcohols in the gboma eggplant waxes were, on the other hand, represented only by few dominating compounds. Low total wax coverage and smaller contribution of hydrocarbons when compared to other Solanaceae species were for the first time suggested as favoured in plant selection during the gboma eggplant domestication process, because – unlike the majority of domesticated solanaceous plants – *S. macrocarpon* is the species bearing edible leaves.

The brinjal eggplant was chosen as the model plant species for the group of plants studied in experiments aiming to determine the effects of abiotic stress on cuticular wax composition (**O2**). Brinjal eggplant is known to be domesticated quite early in the history (Meyer et al. 2012; Aubriot et al. 2018). It is diverse in terms of plant morphology (Kumar et al. 2008), but in the same time quite uniform genetically (Ali et al. 2011), and it was described as relatively tolerant to moderate water deficit (Behboudian 1977). Other plant species, that were planned to use in the chemotaxonomic analysis, are much more diverse genetically and better adapted to difficult environmental conditions (Lester and Daunay 2001). Because of this, expected observed effects of stress related to water deficit would be lower. In brinjal eggplant, water deficit affected the average leaf area, but did not significantly change the total wax coverage expressed in the mass unit per the leaf area unit. The differences in the total wax coverage were high between single plants, particularly when water-deprived plants were taken into account. Cuticular hydrocarbons, including *n*-alkanes, 2-methylalkanes and 3-methylalkanes, revealed very high stability of their chemical composition, which was expressed by small differences in the average chain length (ACL), calculated as follows:

$$ACL = \frac{\sum \%C_n \times n}{100}$$

where: n – carbon atom number in the chain, $\%C_n$ – relative contribution of the alkane with n carbon atoms to the total fraction. Fatty acids and aliphatic alcohols have displayed much higher variability. Among cyclic compounds, the composition of triterpene fraction was relatively stable, but these were minor wax components. The composition of the sterol fraction, on the other hand, was affected by the water deficit. In water-deprived plants, the amounts of cholesterol were significantly higher, while amounts of stigmasterol and β-sitosterol were lower when compared to the control group. This is one of the few reports, where cholesterol is described as the main plant cuticular sterol. The results obtained were consistent with the previous report, where hydrocarbons were the most stable fraction of the brinjal eggplant cuticular waxes both within a single accession grown in different seasons and between accessions (Haliński et al. 2011). Changes that were observed in the cuticular wax composition of plants grown under water deficit are different from the ones usually reported, which are: change in the total hydrocarbon content and increase in the hydrocarbon average chain length value (Shepherd and Griffiths 2006). The results obtained suggest that in case of the brinjal eggplant (and possibly also other related species) these effects were absent, which makes cuticular hydrocarbons potentially useful as chemotaxonomic markers.

Basing on the results reported in publication **O1-O2**, cuticular hydrocarbons were chosen as markers in chemotaxonomic classification of the brinjal eggplant and related species (**O3**). The relative composition of the fraction was determined, with *n*-alkanes, 2-methylalkanes and 3-methylalkanes as markers. Plants used in the study were all grown from seeds in comparable, semi-natural conditions. Hydrocarbons were extracted from the fresh plant material, identified basing on the results of gas chromatography – mass spectrometry (GC-MS) experiments, and their quantitative analysis was performed using gas chromatography with flame ionization detector (GC-FID). Plant seeds used in the study were obtained from commercial distributors (brinjal eggplant) and from two well-known genebanks (all species; World Vegetable Center, Tainan, Taiwan; Center for Genetic Resources, Wageningen, The Netherlands). Plant identification, that was declared by the germplasm suppliers, was verified basing on plant morphology by Dr John Samuels, who is a specialist in taxonomy of brinjal eggplant and related species. Plant species used included: the brinjal eggplant *S. melongena* and several wild, closely related species (*S. campylacanthum* Hochst. ex A. Rich., *S. capsicoides* All., *S. linnaeanum* Hepper & P.-M.L. Jaeger, *S. violaceum* Ortega), African eggplant complexes of the gboma eggplant (*S. macrocarpon – S. dasyphyllum*) and the scarlet eggplant (*S. aethiopicum – S. anguivi*), and the potato (*S. tuberosum*) as an outgroup. Quantitative results were processed using multivariate data analysis techniques, including hierarchical cluster analysis (HCA) with bootstrapping, two-way HCA and principal component analysis (PCA).

The results obtained in publication **O3** allowed to clearly separate 15 lines of the brinjal eggplant and accessions of two closely related wild species (*S. campylacanthum*, *S. capsicoides*) from all the other taxa. Advanced cultivars and landraces of the brinjal eggplant could not be distinguished basing on the results. Complexes of African eggplants were both separated from each other, and in the same quite closely related to *S. linnaeanum* and *S. violaceum*. What was the most important, the classification obtained allowed to address all the major issues in taxonomy of the plant species studied as described in the *Introduction* section. It has been shown that wild/feral forms of the brinjal eggplant (*S. melongena* subsp. c*umingii* (Dunal) J. Samuels.), which are distinct morphologically, could not be separated from cultivated forms of this plant species. Moreover, it was supported that both gboma eggplant and the scarlet eggplant are not different from their respective wild ancestors, which was already suggested by other authors basing on plant morphology and molecular markers (Bukenya and Carasco 1994; Stedje and Bukenya-Ziraba 2003; Acquadro et al. 2017). Finally, close relationship was confirmed between the gboma eggplant and the scarlet eggplant, as described in the most recent studied (Vorontsova et al. 2013; Aubriot et al. 2016). It could be then concluded that cuticular hydrocarbons are very useful markers in the classification of *S. melongena* and related species. Therefore, the results of chemotaxonomic analyses could contribute to a better understanding of the classification of this difficult group of plant species, and should be used together with the results obtained basing on plant morphology and molecular marker analysis, with the unit cost much lower than in case of methods based on molecular biology.

Estimation of the stage of domestication process of African nightshades with edible leaves basing on primary and secondary metabolite profiles [O4-O5]

The scarlet eggplant and gboma eggplant are important vegetable species grown in many African countries for edible fruits and leaves. The latter makes them atypical plants among economically important species from the Solanaceae family. As it was already mentioned, wild forms of both species are also described, and they are classified as separate taxa even if they are not genetically distinct from cultivated forms. The consumption of green leafy vegetables (including the gboma eggplant) in African countries was already described as the activity enriching the diet in vitamins A, C and selected micronutrients (Uusiku et al. 2010).

It was also suggested as a factor preventing anaemia and vitamin A deficiency in children (Egbi et al. 2018) and reducing the risk of neurodegenerative diseases (Ogunsuyi et al. 2018). Knowledge about the nutrition values of African eggplant leaves is still incomplete. There are some reports describing relatively high amounts of phenolic compounds (Oboh and Akindahunsi 2004) and total protein, fat, fibre, selected micronutrients, as well as anti-nutrient compounds, in the leaves of the gboma eggplant (Oboh et al. 2005). In both cases, the effect of some common food processing methods on above-mentioned values was also reported. It was also pointed out that consumption of unprocessed *S. macrocarpon* leaves can be associated with significant risk, without identifying chemical compounds responsible for this effect (Oboh 2005). More detailed results concerning the chemical composition of fatty acids, amino acids and selected micronutrients were also reported (Glew et al. 2010). To the best of my knowledge, there are no similar reliable reports on the chemical composition of the scarlet eggplant leaves. In the same time, the data on the amounts of potentially toxic steroidal glycoalkaloids in leaves of both species are still missing, while it is already known that such substances are present in scarlet eggplant and gboma eggplant fruits in considerable amounts, particularly in *S. macrocarpon* fruits (Sánchez-Mata et al. 2010). Therefore, the aim of publications **O4-O5** was to identify primary and secondary metabolites from selected classes in leaves of the gboma eggplant, which is more widely used as a green leafy vegetable, and to evaluate, if domestication of both species resulted in higher amounts of nutrients and reduction in toxic substances.

The chemical analysis of free and bound fatty acids and sterols, as well as determination of glycoalkaloids in leaves of the gboma eggplant, were reported in publication O4. The effect of thermal processing of the leaves on the chemical composition was also described. The total fatty acid content was much higher than reported before (Glew et al. 2010) and reached values up to 32 mg g^{-1} of the dry weight (DW) of the plant material after the processing, which makes *S. macrocarpon* potentially reach source of these compounds. Polyunsaturated fatty acids 18:2 and 18:3 were the most abundant compounds, with their contribution to the total fatty acid content in range of 64-69%, which is consistent with previous report. The amount of sterols was also high, reaching values of 3-4 mg g^{-1} DW. In comparison, respective values for other vegetables were in range of 0.6-4.1 mg g^{-1} DW, with the highest sterol content reported in cauliflower and broccoli (Piironen et al. 2003). The source cited described β-sitosterol as the main phytosterol in the majority of vegetables, and it was also the most abundant compound in gboma eggplant leaves (35-38% of the total sterols). Glycoalkaloids present in *S. macrocarpon* leaves were not fully identified, but their total amount exceeded 200 mg kg^{-1} of the fresh weight (FW), which is considered as safety level in food as described for potato glycoalkaloids (Mensinga et al. 2005). Short thermal processing of the leaves decreased glycoalkaloid levels in a statistically significant way to the values in range of 91-157 mg kg^{-1} FW. The results have shown that the amount of selected nutrients in *S. macrocarpon* leaves is high and comparable with other vegetables with the highest contents of fatty acids and sterols. This is somehow surprising, as it is suggested that the gboma eggplant is still in initial phase of its domestication. However, levels of potentially toxic defensive compounds were high and therefore caution is required when *S. macrocarpon* leaves are to be consumed without thermal processing. Probably, this could be the explanation of previously reported negative effects of the diet containing fresh *S. macrocarpon* leaves on rodents (Oboh 2005).

Taking into account previous reports and the results presented in publication **O4**, the attempt was made to estimate the phase of domestication process of African eggplants. Four lines of *S. macrocarpon* and five accessions of *S. aethiopicum* (Aculeatum, Kumba and Gilo groups) were used in the study. Profiles of selected primary and secondary metabolites produced by plants were compared to those obtained for their wild relatives *S. dasyphyllum* and *S. anguivi*. In case of *S. macrocarpon*, one line classified as var. *sapinii* was analysed. While its taxonomic position is unclear, it was already suggested as a hybrid between cultivated *S. macrocarpon* and wild *S. dasyphyllum* (Bukenya and Carasco 1994). Additionally, two lines of *S. scabrum* Mill. were also used as a model of a taxonomically more distant nightshade that is grown for edible leaves (Lester and Daunay 2001). Cultivated and wild forms of *S. scabrum* are genetically and morphologically uniform (Manoko et al. 2008), which suggests very initial phase of its domestication even when compared to African eggplants, as their cultivated and wild forms are morphologically distinct. In both cases, however, it is impossible to indicate when first domestication attempts were made. All the plants described in publication **O5** were obtained from the collection of Center for Genetic Resources (Wageningen, The Netherlands) and were grown from seeds in a greenhouse. Metabolites analysed included fatty acids, sterols, phenolic compounds (together with their antioxidant properties), steroidal glycoalkaloids and saponins.

Mechanisms of plant selection during domestication process could be described as conscious (aiming to select some desired plant traits) and unconscious, that result from the transfer of a wild plant to the human-driven environment and include consequent loss of traits that are not essential in new growing conditions (Heiser 1988; Zohary 2004). Hypothetical conscious mechanisms of selection, that could appear in domestication of the plant species studied, include: (1) achieving higher nutritional value and lower amounts of bitter and toxic compounds, and (2) reduction of mechanical means of protection (trichomes and spines). Both mechanisms are supposed to be more important in plant accessions that are grown for edible leaves. The results obtained indicate, however, that the content of nutritionally beneficial chemical compounds (fatty acids, sterols, phenolics) is similar in leaves of wild and cultivated forms, as well as in accessions bearing edible and inedible leaves. Moreover, in scarlet eggplant complex higher amounts of unsaturated fatty acids were found in leaves of wild plants. When scarlet eggplant and gboma eggplant complexes were compared, it was clear that the former was a better source of phenolic compounds. In the same time, however, it contained higher amounts of potentially harmful substances. In leaves of the gboma eggplant and its postulated wild ancestor only moderate amounts of glycoalkaloids based on the solasodine were found. Their amounts in wild and cultivated forms did not differ significantly, and only suggested hybrid form *S. macrocarpon* var. *sapinii* contained glycoalkaloids in potentially dangerous dose. Leaves of *S. aethiopicum* and *S. anguivi,* on the other hand, contained high, but quite variable, amounts of saponins based on diosgenin. Reduction in defensive compounds was already postulated as a trait characteristic for domesticated plants (Moreira et al. 2018), and it was confirmed in Solanaceae for the potato and wild related species (Johns and Alonso 1990). Similar effect was not observed in the species studied. Reduction in mechanical means of protection (mainly spines) seems the only conscious mechanism of selection in case of African eggplants, which was already described for other Solanaceae plants (Heiser 1988). This confirms the initial stage of domestication of both African eggplant species. Very low content of nutrients, especially fatty acids, and very high amounts of saponins based on tigogenin, were observed in *S. scabrum* leaves, which is consistent with the recent report (Yuan et al. 2018). Possibly, it confirms the very initial phase of domestication of this plant species (Manoko et al. 2008). Publication **O5** is one of the first attempts to estimate the stage of domestication of plants with edible leaves. The results suggest that the approach based on metabolite profiling may be useful in identification of plant species, in which conscious mechanism of selection are not yet dominating.

Chemotaxonomic classification of the tomato (*S. lycopersicum*) and related species [O6-O7]

Publications **O6-O7** aimed to evaluate the usefulness of cuticular wax components as markers in chemotaxonomic classification of the tomato and wild related species, just like it was described above for the brinjal eggplant and allied species. Special attention was paid to abiotic stress resistant *S. pennellii* (Corell) D'Arcy. Fractions of cuticular waxes were analysed in terms of their contribution to the total wax and variability of the chemical composition, both between plants within a single line and between different accessions within a species (publication **O6**). Hydrocarbons were the most abundant wax fraction and were characterized by the most stable chemical composition, like in case of the brinjal eggplant and the gboma eggplant. The chemical composition of the fraction was similar to the one reported before (Smith et al. 1996), but much higher number of compounds was detected in the current study. Diversity in chemical composition, which was reflected in differences in average chain length values (ACL), was very low within six lines of the common tomato and moderately low within five accessions of *S. pennellii*. The latter seems to confirm already suggeted significant genetic diversity of *S. pennelli* when compared to other tomato species (Rick and Tanksley 1981). All the other wax fractions displayed much higher variability in their chemical composition, therefore suggesting particularly high usefulness of cuticular hydrocarbons as taxonomic markers. Additionally, *S. pennellii* accessions were characterized by higher total wax load when compared to other species studied, which was related to higher amounts of hydrocarbons and – in most cases – also sterols and fatty acids. Consequently, the total leaf cuticular wax load of *S. pennellii* was in a range of 2.3-4.0 μg g^{-1} DW, while in case of the common tomato respective values were 1.0-2.1 μg g^{-1} DW. Interspecific hybrid *S. lycopersicum x S. pennellii* had the intermediate amount of the total waxes (2.7 μg g^{-1} DW) and their main components. The chemical composition of the triterpene fraction was of interest in terms of searching for taxonomic markers: the main triterpene in *S. lycopersicum* waxes was β-amyrin, which was absent in *S. pennellii* waxes, where lupeol or α-amyrin was the main fraction component. Interestingly, triterpenes were not reported in cuticular waxes of *S. pennellii* fruits, and the chemical composition of *S. lycopersicum* fruit waxes was much different, with dominating δ-amyrin, which in the present study was detected in leaf waxes only in trace amounts (Yeats et al. 2012). However, due to very low concentrations of triterpenes in leaf waxes, they were not chosen as chemotaxonomic markers.

Basing on the results obtained in publication **O6**, cuticular hydrocarbon profile was applied to classify 27 lines of tomatoes belonging to seven different species (**O7**). The chemical composition of the fraction was determined in 11 accessions from *Lycopersicon* group (seven lines of *S. lycopersicum*, three lines of *S. pimpinellifolium* and one line of *S. cheesmaniae*), 4 accessions from *Eriopersicon* and *Arcanum* groups (one line of *S. chilense*, two lines of *S. peruvianum* and one line of *S. arcanum*), 10 accessions from *Neolycopersicon* group, which consists of *S. pennellii* exclusively, and two interspecific hybrids (*S. lycopersicum x S. pennellii* and *S. lycopersicum x S. pimpinellifolium*). The main objective of the study was to clarify taxonomic position of *S. pennellii*, and therefore sucrose esters with short-chain fatty acids from the leaf surface of this species were used as additional markers. *Solanum pennellii* accessions used in the study originated from the whole area of its natural distribution, i.e. from the south-western Peru (Rick and Tanksley 1981). Plants were grown in greenhouse conditions, from seeds obtained from Tomato Genetics Resource Center (TGRC, Davis, USA) and Center for Genetic Resources (Wageningen, The Netherlands) collections. Hydrocarbons were analysed as described before (publication **O3**), while sucrose ester profiles were obtained using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The results of quantitative analyses were then processed using multivariate data analysis techniques, including hierarchical cluster analysis with bootstrapping and principal component analysis.

The results have shown limited usefulness of cuticular hydrocarbons in classification of the species with red (*Lycopersicon* group) and green fruits (*Eriopersicon* and *Arcanum* groups). Species from all above-mentioned groups were clustered together, basing on relatively high contribution of *n*-alkanes with 30-32 carbon atoms in chain, and *S. lycopersicum* accessions were only slightly separated from wild species from *Eriopersicon* and *Arcanum* groups. On the other hand, classification of *S. pennellii* accessions was much more interesting. Basing on the results, three different chemotypes of this species could be separated: the first was characterized by very high contribution of 2-methylalkanes to total hydrocarbons (ca. 41%), second revealed higher than in other plants amounts of 3-methylalkanes (ca. 18%), while the third one displayed hycrocarbon profile similar to the one determined for species from *Lycopersicon* group and accessions from this chemotype were clustered together with *S. lycopersicum* plants. Moreover, third chemotype included mainly lines described as *S. pennellii* var. *puberulum*, which is characterized by reduced number of glandular trichomes on the surface of green parts and by minimal production of glucose and sucrose esters (Shapiro et al. 1994). Interspecific hybrid *S. lycopersicum x S. pennellii* was also clustered together with these plants. Each chemotype was characterized by a distinct sucrose ester profile, which was quite variable in the third chemotype, particularly in case of interspecific hybrid, where the profile was somehow disturbed and strongly dominated by several compounds of a certain molecular weight. Application of sucrose esters as additional chemotaxonomic markers resulted in more clear separation of *S. pennellii* accessions from other tomato species, in the same time not affecting the whole classification. Basing on the results, it was suggested that *S. pennellii* var. *puberulum* lines, which were similar to the interspecific hybrid, could be an affect of a past hybridization event between *S. pennellii* and one of the species from *Lycopersicon* group. It cannot be confirmed basing on the result obtained so far, but in the same time it opens some new possibilities to better understand diversity observed within *S. pennellii* and – in the future – to help in developing hybrid lines of economical importance, that would be more resistant to biotic and abiotic stress. Hybridization between *S. lycopersicum* and *S. pimpinellifolium* was already suggested as a possible source of selected lines of the wild form of the common tomato, classified as *S. lycopersicum* var. *cerasiforme* (Ranc et al. 2008).

Summary

Despite the intensive development of molecular biology techniques, which are now frequently used in taxonomy and, in much extent, have replaced classical approach, based on plant morphology, exact taxonomic position of many, particularly wild and diverse species, remains unclear. Publications described in previous paragraphs have documented the aim to fill the gap in a knowledge of classification of selected species of Solanaceae by using chemotaxonomic approach with components of cuticular waxes as markers. Basing on the results, it has been shown that in case of both the brinjal eggplant and allied species and the common tomato and its wild relatives, cuticular hydrocarbons were wax components of the most stable composition. Hydrocarbon fraction displayed no significant changes in its composition between plants from the same accession, as well as between different lines of a single species, and its profile was not affected by environmental factors, at least in case of *S. melongena*. Therefore, cuticular hydrocarbon profiles were used to classify both groups of the plants studied, with the special attention paid to species of unclear taxonomic status. The main conclusions of these studies are as follows:

- Chemotaxonomic approach confirmed that there are no significant differences between advanced cultivars, local landraces and wild/feral forms of the brinjal eggplant (*S. melongena*)
- Complexes of African eggplants with edible leaves, namely the scarlet eggplant (*S. aethiopicum*) and gboma eggplant (*S. macrocarpon*) are taxonomically distinct from the brinjal eggplant; taxa described as wild relatives of cultivated species are in fact their wild forms, that differ only morphologically; these conclusions are consistent with the most recent studies on taxonomy of the brinjal eggplant and relatives (Vorontsova et al. 2013; Aubriot et al. 2016)
- Cuticular hydrocarbon profiles did not allow to differentiate cultivated and wild forms of the common tomato (*S. lycopersicum*), as well as the most closely related wild species; they were, however, useful in separation of three significantly different chemotypes of the wild, genetically diversified species *S. pennellii*
- One of the *S. pennellii* chemotypes was for the first time suggested as the result of the past hybridization event with unidentified tomato species from the *Lycopersicon* group; however, confirmation of this hypothesis requires additional studies

Additionally, determination of selected nutrients (fatty acids, sterols), compounds that are desired in the diet (phenolic compounds) and potentially toxic anti-nutrients (glycoalkaloids, saponins) was performed in the leaves of African nightshades. Levels of above-mentioned substances were compared in wild and cultivated plants, as well as in plants with edible and inedible leaves. Basing on the results, it was concluded, that mechanisms of selection in this group of species are still mostly unconscious, and the only conscious mechanism of selection concerned some morphological traits, i.e. the number of spines and trichomes on the surface of leaves and stems. It confirms that plant species studied are still in initial phase of domestication and in the same time emphasizes the risk associated with the presence of significant amounts of toxic compounds in green parts of these cultivated plants.

Perspectives of the future studies

The results obtained during realization of experiments, described in publications O1-O7, have clearly shown the direction of the future studies concerning both groups of the plant species. Undoubtedly, special attention should be paid to the possibility of differentiation of different chemotypes of the stress-resistant species *S. pennellii* basing on changes in profiles of selected metabolites. Recently obtained and still unpublished results suggest that *S. pennellii* is able to synthesize a mixture of steroidal glycoalkaloids with a much different composition than all the other tomato species, and differences in their profiles between chemotypes are in line with those described for cuticular hydrocarbons and sucrose esters. It seems to support hypothesis about the hybrid origin of some of *S. pennellii* lines. It is planned to examine this phenomenon on a broader scale, including experiments with a sister species of similar genetic diversity, namely *S. habrochaites* S. Knapp & D. M Spooner. Determination of biosynthetic pathways, which are involved in synthesis of glycoalkaloid aglycones, is also planned if possible. In case of African eggplants with edible leaves, the most interesting question is if mechanisms of conscious selection were more widespread than suggested by the results obtained so far. It is planned to find out, if some traits other than reduction in mechanical means of protection were also preferred. This subject will be continued in close cooperation with Dr John Samuels and experiments will aim to define more precisely the range of conscious mechanisms of selection that are possible to implement in extensive agriculture systems, that were dominating in suggested time of domestication of the species concerned in the area of their natural growth and cultivation.

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5. Discussion of other scientific achievements

In addition to publications that were discussed as the part of scientific achievement, my academic achievement consist of 13 publications in journals indexed in Journal Citation Reports database, with a total IF = **25.322** (MNiSW = 317), 6 publications in journals not indexed in above-mentioned database and two peer-reviewed book chapters.

5.1. Scientific achievements before obtaining a Ph.D. degree

My academic achievement before obtaining a Ph.D. degree consists of three JCR-indexed publications and one manuscript published in journal not included in this database (**I1**):

I1. **Ł.P. Haliński,** B.M. Szafranek, Cuticular waxes from potato leaves – evaluation of extractions methods. *Herba Polonica*, 2006, **52**, 65-70

I2. M. Gołębiowski, B. Ostrowski, M. Paszkiewicz, M. Czerwicka, J. Kumirska, **Ł. Haliński**, E. Maliński, P. Stepnowski, Chemical composition of commercially available essential oils from blackcurrant, ginger, and peppermint. *Chemistry of Natural Compounds*, 2008, **44**, 794-796 (IF 0.468; MNiSW 10)

I3. M. Gołębiowski, E. Maliński, M. Paszkiewicz, **Ł. Haliński**, P. Stepnowski, Chemical composition of commercially available essential oils from eucalyptus, pine, ylang and juniper. *Chemistry of Natural Compounds*, 2009, **45**, 278-279 (IF 0.572; MNiSW 10)

I4. **Ł.P. Haliński**, J. Szafranek, B.M. Szafranek, M. Gołębiowski, P. Stepnowski, Chromatographic fractionation and analysis of main components from eggplant (*Solanum melongena* L.) leaf cuticular waxes. *Acta Chromatographica*, 2009, **21**, 127-137 (IF 0.676; MNiSW 15)

My early scientific interests included mainly the method development for the chemical analysis of natural compounds. Works that were published at this stage of my career were focused on the method development for the extraction of cuticular waxes from leaves of higher plants (**I1**) and on the identification of plant essential oil components (**I2-I3**). Additionally, the procedure of the brinjal eggplant leaf cuticular waxes extraction, their fractionation using flash chromatography, and qualitative and quantitative analysis, was also developed (**I4**). It was the first report on the chemical composition of cuticular waxes from leaves of this species, and the method developed was successfully used in later works, including those from the scientific achievement.

5.2. Scientific achievements after obtaining a Ph.D. degree

My academic achievement after obtaining a Ph.D. degree consists of ten JCR-indexed publications:

I5. **Ł.P. Haliński**, J. Szafranek, P. Stepnowski, Leaf cuticular *n*-alkanes as markers in the chemotaxonomy of the eggplant (*Solanum melongena* L.) and related species. *Plant Biology*, 2011, **13**, 932-939 (IF 2.395; MNiSW 32)

I6. Ł.P. Haliński, P. Stepnowski, GC-MS and MALDI-TOF MS profiling of sucrose esters from *Nicotiana tabacum* and *N. rustica*. *Zeitschrift für Naturforschung C, A Journal of Biosciences*, 2013, **68**, 210-222 (IF 0.569; MNiSW 15)

I7. Ł.P. Haliński, D. Śmigiel, M. Czerwicka, M. Paszkiewicz, J. Kumirska, P. Stepnowski, The derivatization and analysis of anticancer pharmaceuticals in the presence of tricyclic antidepressants by gas chromatography. *Acta Chromatographica*, 2014, **26**, 473-484 (IF 0.577; MNiSW 15)

I8. Ł.P. Haliński, P. Stepnowski, Fractionation of cuticular waxes from the leaves of Solanaceae plant species using microextraction by packed sorbent. *Acta Chromatographica*, 2015, **27**, 729-741 (IF 1.187; MNiSW 15)

I9. A. Topolewska, K. Czarnowska, **Ł.P. Haliński**, P. Stepnowski, Evaluation of four derivatization methods for the analysis of fatty acids from green leafy vegetables by gas chromatography. *Journal of Chromatography B*, 2015, **990**, 150-157 (IF 2.689; MNiSW 30)

I10. M. Borecka, G. Siedlewicz, **Ł.P. Haliński**, K. Sikora, K. Pazdro, P. Stepnowski, A. Białk-Bielińska, Contamination of the southern Baltic Sea waters by the residues of selected pharmaceuticals: method development and field studies. *Marine Pollution Bulletin*, 2015, **94**, 62-71 (IF 3.099; MNiSW 40)

I11. M. Borecka, A. Białk-Bielińska, **Ł.P. Haliński**, K. Pazdro, P. Stepnowski, S. Stolte, The influence of salinity on the toxicity of selected sulfonamides and trimethoprim towards the green algae *Chlorella vulgaris*. *Journal of Hazardous Materials*, 2016, **308**, 179-186 (IF 6.065; MNiSW 45)

I12. P. Śramska, A. Maciejka, A. Topolewska, P. Stepnowski, **Ł.P. Haliński**, Isolation of atropine and scopolamine from plant material using liquid-liquid extraction and EXtrelut® columns. *Journal of Chromatography B*, 2017, **1043**, 202-208 (IF 2.603; MNiSW 30)

I13. I. Fischer, **Ł.P. Haliński**, W. Meissner, P. Stepnowski, M. Knitter, Seasonal changes in the preen wax composition of the Herring gull *Larus argentatus*. *Chemoecology*, 2017, **27**, 127-139 (IF 1.298; MNiSW 25)

I14. A.M. Naczk, A.K. Kowalkowska, N. Wiśniewska, **Ł.P. Haliński**, M. Kapusta, M. Czerwicka, Floral anatomy, ultrastructure and chemical analysis in *Dactylorhiza incarnata/maculata* complex (Orchidaceae). *Botanical Journal of the Linnean Society*, 2018, **187**, 512-536 (IF 3.124; MNiSW 35)

The area of my scientific interests included three main topics: (1) method development for determination of several groups of primary and secondary metabolites in plant material, and application of these methods to chemotaxonomic analyses **(I5, I6, I8, I9, I12)**; (2) the chemical analysis of pharmaceuticals in the environment and estimation of their toxicological potential **(I7, I10, I11)**; (3) the use of instrumental analytical chemistry in studying phenomenons of biological nature, performed in cooperation with Ph.D. students and academics from the Faculty of Biology, University of Gdańsk **(I13, I14)**.

First subject concerned mainly secondary metabolites of solanaceous plants, and methods developed were used also in experiments, which were described above as a part of the scientific achievement **(I5, I6, I9)**. Publication **I5** describes the first approach to the use of cuticular hydrocarbon profiles in chemotaxonomic analysis of the brinjal eggplant and related species. The most important results included the estimation of the variability of the chemical composition of each cuticular wax fraction between different growing seasons and the possibility of applying the profiles of selected metabolites to classify plant species in line with the current knowledge of their taxonomy. The method used applied a simplified approach, that utilized *n*-alkanes only as chemotaxonomic markers, and the results were presented earlier as a part of my Ph.D. dissertation. Method development for determination of sucrose esters, that are secreted as defensive compounds on the surface of leaves and stems of some solanaceous plant species, was the main objective of publication **I6**. Cultivated tobacco (*Nicotiana tabacum* L.) and Aztec tobacco (*N. rustica* L.) were used as model species. The main issue in the chemical analysis of plant sucrose esters is the presence of a large number of isomeric compounds, which severely affects the performance of their chromatographic determination, usually using gas chromatography coupled to mass spectrometry, and makes the interpretation of the results quite challenging. The method developed included direct analysis of sucrose esters in plant extracts by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Such an approach results in loosing the information about the presence of certain isomers, but in the same time allows to determine profiles of sucrose esters basing on their molecular weight. The results obtained have clearly shown that using this method for determination of the relative composition of the fraction for chemotaxonomic purposes may give the results of the precision similar to the one, obtained using more standard methods based on gas chromatography. Method was then successfully applied to the analysis of sucrose esters produced by the wild tomato species (publication **O7** in the scientific achievement). Publication **I8** documents the aim to use miniaturized microextraction to packed solvent technique in fractionation of cuticular wax extracts from leaves of solanaceous plants. As it was initially supposed, low resolution of the technique did not allow to fully fractionate wax components, but it was sufficient to obtain pure cuticular hydrocarbon fraction for the purpose of chemotaxonomic analyses with the minimal labour input and organic solvent use, which makes it an attractive alternative for usually used classical liquid chromatography techniques. Publication **I9** describes a method development for an optimal derivatization procedure used in gas chromatographic analysis of fatty acids. Four different procedures were evaluated, each in different conditions of the reaction. The specificity of extracts from the green parts of the plants was included, and the main objective was to develop a method useful in the chemical analysis of fatty acids extracted from edible parts of the plant. The method developed was successfully used in publication O5 from the scientific achievement described above. Publication **I12** aimed to compare several isolation methods for the analysis of tropane alkaloids (represented by atropine and scopolamine) in the plant material. These compounds are produced by some of solanaceous plants and in certain conditions they may be potentially toxic when present in food. They may also be useful chemotaxonomic markers. Application of the method for the food analysis and taxonomic studies is planned in the future.

Publications **(I7, I10, I11)** concerned the presence of pharmaceuticals used in veterinary and human medicine as new, emerging pollutants in the aqueous environment. Description of the analytical method allowing simultaneous determination of tricyclic antidepressants and selected anti-cancer drugs using gas chromatography was given in publication **I7**. Extensive study on the presence of selected pharmaceuticals in Baltic Sea and an effort to identify areas that are exposed to their highest concentrations were described in publication **I10**. Moreover, there was an attempt to find the correlation between physiochemical water characteristic and effectiveness of the extraction procedures utilized. On this stage of experimentation, it was concluded that concentrations of compounds studied are still to low to result in serious toxic effects in the environment. Ecotoxicity of selected antibacterial drugs, namely several sulphonamides and trimethoprim, was studied using green algae *Chlorella vulgaris* Beijer. and the results were presented in publication **I11**. Additionally, the effect of water salinity on observed toxic effects was determined. Toxicity of some of the compounds studied was relatively high, suggesting possible risk associated with their presence in the environment in the future. It was also found out that a growing water salinity resulted in lowering toxicity of pharmaceuticals, and mechanism of this phenomenon including lower bioavailability of chemicals due to the higher concentration of ions close to the algal cell surface was suggested.

In last few years, I have also started cooperation with academics from the Faculty of Biology, University of Gdańsk, in order to broadly apply possibilities of instrumental analytical chemistry to interpretation of biological processes by using the results of chemical analyses. So far, two works have been published as the result of this cooperation (**I13, I14**). Publication **I13** describes the seasonal changes in the chemical composition of the preen gland waxes of the herring gull (*Larus argentatus* Pontoppidan). The most important conclusion is that there is a significant difference in the composition of waxes between birds captured in winter and during the breeding season. Moreover, differences were larger in case of males, which incubate at night more frequently than breeding females. Therefore, we suggested defensive mechanism that makes localization of the incubating birds by predators more difficult by an olfaction at night. In the same time, it has been shown that in the herring gull the seasonal change in the chemical composition of preen gland waxes did not result in the shift from more volatile monoesters produced in winter to diesters of higher molecular weight synthesized during the breeding season, which was described for several other bird species. The differences found were much more subtle and included only changes in the structure of monoesters that are produced during the whole year. Publication **I14** describes differences in anatomy and ultrastructure of flowers, as well as in the chemical composition of compounds secreted by flowers of four orchid taxa from the *Dactylorhiza incarnata*/*maculata* complex. Two of the taxa studied were diploid parental lineages, while the remaining two – their tetraploid derivatives. Differences were found in flower anatomy, ultrastructure and the chemical composition of the volatile fragrance compounds. This is quite a novel report aiming to characterize changes in physical form and functioning of flowers of higher plants that could be effects of polyploidization. Cooperation in both areas will be continued and other joint publications are expected in the future.

In addition to reports published in journals indexed by Journal Citation Reports database, my academic achievements include 2 peer-reviewed book chapters and 5 short reviews written in Polish:

I15. M. Gołębiowski, M. Paszkiewicz, **Ł. Haliński**, P. Stepnowski, HPLC of plant lipids. In: *High performance liquid chromatography in phytochemical analysis* (Ed. Monika Waksmudzka-Hajnos, Joseph Sherma), s. 425-452. Boca Raton: CRC Press Taylor & Francis Group, 2011

I16. M. Caban, **Ł.P. Haliński**, J. Kumirska, P. Stepnowski, Gas chromatography applied to the analysis of drug and veterinary drug residues in food, environmental, and biological samples. In: *Determination of target xenobiotics and unknown compound residues in food, environmental, and biological samples* (Ed. Tomasz Tuzimski, Joseph Sherma), s. 133-168. Boca Raton: CRC Press Taylor & Francis Group, 2018

I17. M. Caban, **Ł. Haliński**, A. Białk-Bielińska, J. Kumirska, P. Stepnowski, Efekty matrycowe w chromatografii gazowej (*Matrix effects in gas chromatography*). *Analityka*, 2015, R. 16, nr 4, 4-7

I18. M. Gołębiowski, **Ł. Haliński**, P. Stepnowski, Analiza chemiczna lipidów kutykularnych roślin wyższych i owadów, cz. 1 (*The chemical analysis of higher plant and insect cuticular waxes, part 1*). *Laboratorium: przegląd ogólnopolski*, 2017, nr 11-12, 62-65

I19. M. Gołębiowski, **Ł. Haliński**, P. Stepnowski, Analiza chemiczna lipidów kutykularnych roślin wyższych i owadów, cz. 2 (*The chemical analysis of higher plant and insect cuticular waxes, part 2*). *Laboratorium: przegląd ogólnopolski*, 2018, nr 1/2018, 16-22

I20. **Ł. Haliński**, Taksonomia roślin wyższych – rola analityki chemicznej (*The role of chemical analysis in the taxonomy of higher plants*), *Laboratorium: przegląd ogólnopolski*, 2018, nr 4/2018, 26-29

I21. **Ł. Haliński**, Metabolity wtórne roślin jako alternatywne pestycydy (*Plant secondary metabolites as alternative pesticides*), *Laboratorium: przegląd ogólnopolski*, 2019, nr 1/2019, 62-67

Publications **I15-I16** are both reviews in form of peer-reviewed chapters in English-written books, published by CRC Press Taylor & Francis Group. Chapter **I15** describes the use of high-performance liquid chromatography (HPLC) in the chemical analysis of plant lipids and its subject is partially associated with publications **O1-O7** described earlier as the scientific achievement. Chapter **I16** reviews the most recent literature on the chemical analysis of veterinary drugs in food, biological and environmental samples using gas chromatography. Publications **I17-I21** are short popular reviews written in Polish and concern the occurrence of so-called matrix effects in gas chromatography (**I17**), the chemical analysis of cuticular waxes from plant and insect surface (**I18, I19**), the use of plant metabolites as chemotaxonomic markers (**I20**) and the usefulness of plant secondary metabolites as potential alternative pesticides of low toxicity (**I21**).

5.3. Other aspects of scientific activity

The results of my studies were presented at national and international conferences in the form of my presentation (2), presentations of other co-authors (5) or as poster presentations (33). I participated in one project financed by the European Union as an expert, in three projects financed by Polish Ministry of Science and Higher Education and later by National Science Centre (in one as a supervisor, in two other as participant) and in six projects financed by University of Gdańsk as a supervisor. I received three Team Awards of the Rector of University of Gdańsk for series of scientific publications. Between 2013 and 2017 I carried out three short-term scientific fellowships in research centres in Germany and Portugal.

A detailed list of my scientific, didactic and popularizing achievements is given as **Appendix 3**.

5.4. Overview of the scientific achievements and bibliometric data

Data from 27.03.2019	Before Ph.D. (A)	After Ph.D. (B)	In scientific achievement	Total (A + B)
H-index (Web of Science)	7			
Number of publications in JCR-indexed journals	3	17	7	20
Number of other publications	1	5	0	6
Total IF	1.716	37.319	13.713	39.035
Number of citations • excluding self-citations • with self-citations	 16 23	 75 90	 10 17	 91 113
Author position in publications included in scientific achievement • first author • corresponding author • other			 7 7 0	
Number of book chapters	0	2	0	2
Number of oral presentations at national and international scientific conferences • as presenting author • as co-author	 0 0	 2 5		 2 5
Number of poster presentations	10	23		33
Number of scientific projects realized • as principal investigator • as participant	 0 0	 7 3		 7 3

Łukasz Haliński