Ph.D. thesis

An assessment of walnut fruit morphology and walnut blight resistance in Hungarian cultivars and Transylvanian selections, including an examination of the antibacterial role of phenolic compounds

Attila Bandi

Supervisor: Magdolna Tóth DSc

Budapest, 2015

PhD School

Name:	Doctoral School of Horticultural Sciences
Field:	Crop Sciences and Horticulture
Head of Ph.D.School:	Prof. Dr. Magdolna Tóth Doctor of the Hungarian Academy of Sciences Head of Department of Fruit Sciences Corvinus University of Budapest, Faculty of Horticultural Sciences
Supervisor:	Prof. Dr. Magdolna Tóth Doctor of the Hungarian Academy of Sciences Head of Department of Fruit Sciences Corvinus University of Budapest, Faculty of Horticultural Sciences

The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

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Head of Ph.D. School	Supervisor

1. INTRODUCTION

Persian walnut production can greatly benefit from breeding research that targets the development of cultivars and hybrids that meet both production and market expectations. In Hungary, the selection research started by Dr. Péter Szentiványi and the hybridization and breeding research that followed from the beginning of the 1970s resulted in cultivars with excellent characteristics by international standards.

Nowadays it is still highly relevant the discovery and preservation of new genotypes for the further development of walnut production. Across Europe it has been realised an important selection work with the screening of local walnut population. The aim is to find such genotypes or to breed such hybrid cultivars that posses good market value, are disease tolerant and produce reliably while being adapted to the pedoclimatic conditions of a given region.

In Transylvania 90% of the walnut population is from seed. Consequently there is great genetic diversity that manifests in the variability of quantitative and qualitative attributes such as fruit characteristics and disease resistance. Those selections with appropriate fruit quality and good tolerance to the most important diseases might became valuable starting points for future breeding work.

In the past few years the bacterial blight disease (*Xanthomonas arboricola* pv. *juglandis* (Pierce) Vauterin *et al.* (*Xaj*) caused greater and greater difficulties for walnut production operations. Different cultivars have different levels of susceptibility to this pathogen. For this reason in a given year even complete crop failure is possible depending on the cultivar.

There is a wide range of scientific literature available on the susceptibility of different cultivars, however, this information can only be relied upon with caution because in Hungary, as in many walnut production countries, local cultivars are used. Consequently it is of great significance to broaden the knowledge about the susceptibility of different cultivars and to examine and assess the genotypes from a given walnut population.

2. THE AIMS OF RESEARCH WORK

The following research objectives were set for this PhD project:

1. To map the walnut populations in various regions of Transylvania:
    - ❖ to evaluate, based on field measurements, the variability of walnut populations in the study regions from fruit morphology and disease resistance perspectives.
    - ❖ to identify those individuals that are most valuable from a market perspective.
    - ❖ to assess the walnut populations' susceptibility to bacterial blight disease (*Xanthomonas arboricola* pv. *juglandis* (Pierce) Vauterin *et al.* in order to identify *Xaj* tolerant genotypes.

2. To compare from fruit morphology and quality perspectives our best selections with the best Hungarian registered cultivars.

3. To assess the more valuable selections' susceptibility to bacterial blight disease and to compare these with Hungarian cultivars.

4. To examine the antibacterial effect of phenolic compounds.

5. To identify the phenolic profile of *Xaj* tolerant selections and cultivars.

6. To examine the physiological background of *Xaj* infection as regards changes in phenolic compounds.

3. MATERIALS AND METHODS

3.1. The assessment of walnut populations' diversity in Transylvania

Our fieldwork targeted the mapping of walnut populations in Transylvania made up of walnut trees growing wild and around village houses that have not been studied previously. The methodical assessment of the Carpathian walnut's (or the Carpathian strain of the Persian walnut) Transylvanian populations resulted in the identification and selection of valuable fruiting trees from the various study regions. Between 2007 and 2011 we examined 648 fruiting trees from 57 settlements in Felső-Háromszék, Maros-tere, Kis-Küküllő mente and Nyárád mente regions.

In the study years we collected 40 fruit samples from each tree during September and October. The samples were kept in raschel bags, in dark rooms, at room temperature (20 °C) until processing.

The lengths, diameter and thickness of the fruit and that of the shell (endocarpium) were measured with the Mitutoyo CD-15APX digital sliding caliper with an 0.01 mm error margin. The weight of the fruits and kernels was measured with digital laboratory scale. The data was used to determine the kernel to shell ratio. The kernel fractions that resulted from breaking the nuts were categorized into half, quarter, small fraction and damaged categories.

The walnut populations' susceptibity to *Xanthomonas arboricola* pv. *Juglandis* was assessed in the field in July, after the critical period for the infection, using Hunter and Roberts' (1978) method. The assessment was carried out in 2009 and 2010 in the Felső-Háromszék region and in 2010 and 2011 in the Maros-, Nyárád- and Kis-Küküllő regions. For the assessment, 20 leaf and 20 fruit samples were collected randomly from each cardinal side of the tree. The level of infection was determined on a scale from 0 to 5 based on the proportion of infected leaf and fruit surface relative to the whole.

3.2. *Xaj* susceptibility assessment based on artificial inoculation

Between 2010 and 2013 we evaluated the *Xaj* susceptibility of registered Hungarian cultivars and that of Transylvanian selections. As controls we used the moderate resistant (mR) 'Pedro' and highly susceptible (hS) 'Milotai intenzív' cultivars. We collected the samples in June, before shell hardening, from the Trial Station of the National Food Chain Safety Office at Pölöske (HU) and from farmer orchards in Transylvania (RO). We collected the isolates used for inoculation from various walnut producing regions of Hungary and Transylvania.

The identification of the pathogenic agents was based on morphological, biochemical and physiological attributes. The pathogenicity tests were carried out in the Erwinia laboratory of the Department of Pomology, Corvinus University of Budapest. We examined 61 *Xaj* isolates. These were chosen based on the colony growth characteristics such as yellow pigment production, smooth, mucoid, convex colony type, and oxidative breakdown of glucose. After identification the isolates were placed in the NATIONAL COLLECTION OF AGRICULTURAL AND INDUSTRIAL MICROORGANISMS (http://ncaim.uni-corvinus.hu) at the Faculty of Food Sciences, Corvinus University of Budapest.

3.3. Biochemical assessment of Hungarian and Transylvanian isolates selected for artificial inoculation

Following the virulence assessment of the collected isolates, two were selected for detailed biochemical assessment. The B.02490 (HU) isolate from Hungary and B.02489 (RO) isolate from Transylvania originated from the green husk of a walnut and showed similar levels of virulence. The NCPPB 411 (NZ) strain from New Zealand was used as control.

As part of the biochemical assessment we examined the isolates' use of different substrates as carbon sources. We used the API 20 NE and API 50 CH rapid diagnostic kits by bioMérieux, France, and followed closely the manufacturer's instructions. The API 20 NE kit consists of 20 micro tubes which contain dehydrated substrates. The API 50 CH kit is based on colour changes. If a given bacteria uses a

given carbohydrate making acid out of sugar, then the originally red solution turns yellow. During positive gelatin (protein) break-down test, the gelatin becomes fluid and black.

The isolates' copper resistance was assessed with the agar diffusion method pipetting 0.15%, 0.25% and 0.35% copper hydroxide solutions in the 10 mm diameter wholes.

3.4. The creation of a bacterial cell suspension and subsequent inoculation

We carried out the artificial inoculation with a mixture of the Hungarian B.02490 (HU) and Transylvanian B.02489 (RO) isolates. For the bacterial suspension we used clean 24 hour cultures. For the bacterial suspension we used clean 24 hour cultures. We conducted the susceptibility tests based on the method of Özaktan *et al.*. (2008). For each cultivar and selection we collected 30 immature fruits, 20 pieces for the artificial inoculation and 10 pieces for the control treatment. Each of the 20 fruits was injected in 5, 0.5 square centimeter, area with a 20 µl bacterial suspension. The level of susceptibility was assessed based on the assessment of the 100 inoculated area per cultivar or selection (20 immature fruits * 5 inoculation points per fruit).

The control fruits were inoculated with sterile distilled water. The inoculated fruits were then placed in closed transparent plastic boxes in order to assure an adequate humidity (85%) and temperature (26-28 ˚C).

3.5. Assessment of fruit susceptibility to *Xaj*

When assessing the level of *Xaj* susceptibility, we took into account both the developed and missing symptoms. We used the dimensions of the necrotic area around the inoculation point to calculate the susceptibility indices using the five-stage infection scale developed by Özaktan *et al.*. (2008). Data from the infection scale was then used to construct indexes using formulas from relevant scientific literature. We determined the level of infection using the following disease rating

index developed by Bertrand and Gottwald (1986). This method allowed for the assessment of cultivar/selection susceptibility and resistance to *Xaj*.

$$Fm = \sum(ai \times fi)/n$$

Fm – level of infection on the fruit

a_i - a given value registered on the infection scale (infection intensity)

f_i- the count of the given infection scale value (infection frequency)

n – number of examined fruit per cultivar or selection

3.6. Physiological background of walnut blight infection

We collected the fruit samples in the Gf+45 phenological stage from the Trial Station of the National Food Chain Safety Office at Pölöske (HU) and from farmer orchards in Transylvania (RO). Based on the susceptibility results of our previous research we selected for inoculation the moderatly susceptible 'Hartley', the highly susceptible (ALB-22) and the moderate resistant 'Milotai kései' and 'Pedro' cultivars. The artificial inoculation was carried out in less than 24 hours after sample collection.

Sample preparation for the HPLC determination of phenolic compunds

To follow the inoculation induced biotic stress response, we collected husk tissue (*exocarpium* and *mezocarpium*) samples (10 mm ø) with a plug drill from the area surrounding the inoculation points. The samples were taken at 0, 24, 96 and 216 hours after inoculation. For each sample collection time we had three repeats per cultivar. For both the inoculated and control fruits, for each repeats we collected 6 disk samples.

We bought the HPLC standards for the examined phenolic compunds such as cinammic acid [140-10-3], gallic acid [149-91-7], pirocatechin [120-80-9], protocatechin [99-50-3], (+)-catechin [154-23-4], chlorogenic acid [327-97-9], vanillic acid [121-34-6], (-)-epicatechin [490-46-0], syringic acid [530-57-4], rutin [153-18-4], quercetin 3-glucoside [482-35-9], quercitrin [522-12-3], juglone [481-39-0] and quercetin [117-39-5] from Sigma Aldrich Chemical Co. (St. Louis, MO,

USA). We used HPLC methanol CAS [67-56-1] and Milli-Q water for the eluent. We introduced the standards in the HPLC as 0.5 g/50 ml stock solutions diluted 100 times and ran the analyses. We used the HPLC laboratory of the BCE Department of Pomology under Dr. György Végvári's supervision.

3.7. The *in vitro* method of assessing the antibacterial effect of phenolic compounds

We examined the following phenolic compounds: juglone [481-39-0], gallic acid [149-91-7], protocatechin [490-79-9], vanillic acid [121-34-6], syringic acid [530-57-4], pirocatechin [120-80-9], chlorogenic acid [327-97-9], (+)-catechin [154-23-4], (-)-epicatechin [490-46-0], rutin [153-18-4], cinammic acid [140-10-3]. For each of these phenolic compounds, we dissolved 1 mg in 200 µl DMSO and added 3 ml of sterile distilled water. Then we added 700 µl of bacterial cell suspension. We set the concentration of the bacterial cell suspension to OD_{570nm}=0.6 (optical density), which corresponds to approximately $3x10^8$ $cell \cdot mL^{-1}$ cell count. We carried out the colouring after an hour. We used an a.r. purity MTT (3-(4.5-Dimethylthiazol-2-yl)-2.5-Diphenyltetrazolium Bromide) dye reagent (Sigma) to make visible the antibacterial effect of the phenolic compounds.

We measured the colour coordinates immediately after colouring, and again after 16 hours. We determined the level of colour change (L*, a*, b* coordinates) with the Konica Minolta CR-400 tristimulus colorimeter. The measurement data was downloaded on laptop through the RS232 serial port.

We followed the antibacterial impact of the phenolic compound with the reverse isolation of the bacterial cells as well. Following inoculation, for each phenolic compound after 0 hour, 10 minutes, 1 hour, 4 hours and 24 hours we took ten fold serial dilutions and we dropped 100 µl samples on King-B agar and spread them uniformly with a glass stir stick. We incubated the samples at 27±1 °C temperature for 48 hours, then we counted the colonies that developed.

3.8. The method of assessment of the antibacterial effectiveness of expressed walnut husk liquid

We tested the antibacterial effectiveness of expressed walnut husk liquid that was obtained from cultivars and selections with different levels of *Xaj* susceptibility (‘Hartley’, ‘Pedro’, ‘Alsószentiváni 118’ ‘Milotai kései’, M-10-25, OZSD-37, SZEN-10, ALB-22, SOM-101). Using a plug drill, 10 mm diameter disks were cut from infected green husks and pressed. The tissue fluid used to fill the wholes was obtained from healthy and infected husk disks that were pressed 10 days after infection. In this experiment we used the previously isolated B.02490 (HU) és a B.02489 (RO) *Xaj* isolates, the same isolates that we used for our artificial inoculation experiment. We used the NCPPB 411 (NZ) strain for control. We tested the antibacterial effectiveness of the expressed walnut husk liquid with the agar diffusion method.

Determination of total polyphenol content

The total polyphenol content was determined in term of gallic acid equivalent with the spectrophotometric method of Singleton and Rossi (1965). The analysis was carried out in the Fruit Analytical Laboratory of the Department of Pomology under the guidance of Dr. Gitta Ficzek. The total phenolic content was calculated from the measured absorbtion using the calibration curve’s equation *($A = a \times c_{sample} + b$)*..

$$\boldsymbol{Polyphenol\ (mg\ GS/l)} = \frac{\boldsymbol{A-b}}{\boldsymbol{a}}$$

A= absorbtion value measured at 765 nm wavelenghts

a, b = parameters of the calibration curve

4. RESULTS AND DISCUSSION

4.1. Transylvanian walnut populations's diversity from fruit morphology and *Xaj* susceptibility perspectives

There are valuable walnut populations in the Felső-Háromszék region and on the rolling hills between the Maros, Nyárád and Kis-Küküllő rivers. Based on field observations and laboratory measurement data we conclude that our research area is rich in research specimens that show high diversity for a range of attributes. This rich diversity allowed us to identify a number of valuable specimens during our selection work. Based on our detailed analysis to date six genotypes proved to be the most valuable (promising) coded SZEN-10, FFA-11, ALB-22, OZSD-37, SOM-101, SOM-120 for future breeding work.

From a market value perspective, the weight of the Transilvanian walnut selections' inshell fruit is between 11.20 and 16.69 g. This interval overlaps with that of Hungarian registered cultivars (10.8–13.7 g). Bujdosó (2006) considers that a dried inshell fruit weight above 12-13 g is desirable. Measurement data from Szentiványi (2006) shows that the fruits of the 'Milotai kései', 'Milotai intenzív', 'Bonifác' and 'Tiszacsécsi 83' cultivars are relatively lighter, below 12 g. The inshell fruit of 'Alsószentiváni 117','Milotai 10', 'Alsószentiváni kései' and 'Milotai bőtermő' ranges between 12 and 14 g. Excluding SOM-120 the Transilvanian selection contains walnut inshell fruits above 12 g. From a dried inshell fruit weight perspective the SZEN-10 selection stands out with an average weight of 16.70 g.

The diameter of a great quality inshell walnut must be above 32 mm (G. Tóth, 2004), this value being the lower bound of the first dimension class (Bujdosó, 2006). The Transylvanian selections satisfy this criterium. The average diameter of the SZEN-10, FFA-11, OZSD-37 selections is significantly greater than that of other selections. From this point of view the cultivars bred by Dr. Péter Szentiványi at the Fruitculture Research Institute, Research Station of Érd can also be considered

outstanding. Their inshell fruit diameter is consistently between 32-34 mm based on long time series data (Bujdosó *et al.*, 2014).

The fruit morphology data from the Transylvanian samples showed that the rounded shape is the most frequently occurred. Exception from this is the SZEN-10 selection, which fruit shape is elliptical. The Hungarian registered cultivars have a shape index between 1.00 and 1.15, and consequently have a rounded shape.

The appearance, size and kernel content of walnuts is cultivar specific. From an economic point of view, the rounded or almost rounded shape is the most desirable due to advantages associated with transportation, handling and processing as well as aesthetics (Szentiványi, 1976).

The fruit shape showed great variability in the studied population. We registered 4 shapes: rounded (*forma rotunda*), elliptical (*forma elliptica*), egg-shaped (or oval) (*forma ovata*) and heart-shaped (*forma obovata*). There was correlation between fruit shape and shell thickness. The rounded fruit shape is associated with the thinnest endocarpium, followed by the egg-shaped and heart-shapped ones. The thickest endocarpium is associated with the elliptical shape. We found the fruit shape to influence the kernel ratio, and based on Bujdosó (2006) this influences breakability as well.

The inshell walnut's most important attribute as a market product is its kernel content which is expressed as the kernel's proportion in the total walnut weight. Based on Szentiványi (1976), the kernel content of good quality cultivars' inshell fruit should be at least 44%. Bujdosó considers a 46 to 50% kernel ratio as optimal in his market value research of various walnut cultivars.

In Hungary, based on a large timeseries dataset, the registered cultivars have a kernel content between 40.4 g and 52.4 g. In this interval the following cultivars have a kernel ratio greater than 50%: 'Milotai intenzív', 'Alsószentiváni 117' and 'Tiszacsécsi 83' (Bujdosó *et al..*, 2014). Similarly, among the Transylvanian selections, FFA-11 and SZEN-10 have a kernel ratio greater than 50%. The lowest kernel ratio (47.53%) was registered for the OZSD-37 selection. However, the

average ratio of this selection does not differ from the average ratio of ALB-22, SOM-120 and SOM-101 selections that register 1 to 2% greater ratios.

The selected genotypes from Felső-Háromszék and Alsó-Nyárádmente had very different breakability indexes. For the SZEN-10, FFA-11 and SOM-120 selections the proportion of the half size kernels was around 60%, in line with the Hungarian cultivars that have 50 to 80% of the kernel in the half size kernel category (Bujdosó, 2006). For the ALB-22 and SOM-101 selections this proportion is 20% less. The quarter size kernels are preponderant for the OZSD-37 genotype.

4.2. The assessment of the isolates' biochemical and physiological reactions

We identified categories of carbohydrates based on pathogen use; we differentiated between carbohydrates that were utilized fast and completely; carbohydrates that were utilized slow and completely; carbohydrates that were utilized to a low degree and poorly; and finally carbohydrates that were not utilized at all. We suppose that those carbohydrates that are used up fast and completely, and slowly and completely respectively, influence to a great extent the level of infection.

For certain carbohydrates the two isolates - B02490 (HU) and B02489 (RO) - were different only with regards the speed of breakdown. The NCPPB 411 (NZ) strain utilized four fewer carbohydrates (see carbohydrates 22, 31, 35, 37) than the isolates from Carpathian Basin.

We conclude that, due to the identical usage of carbohydrates by the B02490 (HU) strain from Hungary and B02489 (RO) strain from Transylvania, the cultivars originating from these regions could be grown mutually. The differing usage of carbohydrates by the NCPPB 411 (NZ) strain from New Zealand indicates that a cautionary approach is warranted with the naturalization of distant cultivars. Prior to such attempts the examination of their resistance to local strains is required.

4.3. Walnut cultivar and selection susceptibility to *Xaj*

The majority of the examined cultivars and selections show moderate susceptibility and susceptibility. Among the Hungarian cultivars, the 'Milotai kései',

‘Alsószentiváni 118’, ‘M-10-25’, and ‘Milotai bőtermő’ have low susceptibility. From the Transylvanian selections SZEN-10 and SOM-101 stood out with moderate resistance and moderate susceptibility. Based on the results of the three study years the susceptible ‘Milotai intenzív’ cultivar was the third most susceptible. Similarly susceptible were the `ALB-22`, `OZSD-37`, `SOM-274`, and `SAR-33` selections. For certain cultivars such as ‘Alsószentiváni 118’ and ‘Milotai kései’ the yearly results showed big variations and therefore the assessment of their susceptibility to the disease will require further research.

The results of these assessments could contribute to the efforts to minimize the damages caused by *Xaj* by helping characterize the susceptibility of different cultivars and selections.

4.4. The role of phenolic compounds in the defense mechanism of Persian walnut against *Xaj*

The examined walnut cultivars and selections showed great diversity regarding the various phenolic compounds’ natural level of activity. Quercetin was present in the greatest amount and proportion in the green husks that were collected in the Gf+45 phenophase. There were also significant amounts of juglone. The quercetin 3-glucoside had the third highest concentration, followed by cinammic acid, (+)-catechin, rutin, chlorogenic acid, quercitrin, pirocatechin, gallic acid, (-)-epicatechin, syringic acid and vanillic acid. Protocatechin was present in the lowest amount.

We observed that following infection there were differences in the activity of the different phenolic compounds based on the susceptibility level of cultivars. Among the fours cultivars and selections, ‘Pedro’ showed the biggest difference between the infected and control treatments.

In the ‘Pedro’ infected fruits, the total activity of the 14 examined phenolic compounds was 38.93% higher than in the control fruits. At the same time, the concentration of the phenolic compounds in the infected fruits was 23.06% higher than the naturally occurring concentration.

In the infected fruits of the highly susceptible ALB-22 selection the activity of the phenolic compounds was 2.45% lower than in the control fruits. At the same time, the concentration of the phenolic compounds was 2.29% lower than the naturally occurring concentration.

Solar *et al.* (2007) stated that there is no connection between the phenolic compounds' concentration and their antibacterial effectiveness. Based on our results, the group of flavanols (pirocatechin, (+)-catechin, (-)-epicatechin) showed significant increase in their synthesis relative to both the naturally occurring levels and the levels measured in the control fruits. However, their direct antibacterial effect could not be confirmed based on the results of the in vitro experiment.

In a latter experiment Solar *et al.* (2009) found that due to infection, the synthesis of (+)-catechin, chlorogenic acid and rutin increased. Based on our analyses, we reached a similar conclusion with the observation that an increased activity of these phenolic compounds could only be observed for the less susceptible cultivars and selection. The results of the *in vitro* experiment confirmed the antibacterial effect of chlorogenic acid, as opposed to the (+)-catechin and rutin. Out of the 14 studied phenolic compounds a further six compounds, namely vanillic acid, syringic acid, protocatechin, gallic acid, cinammic acid and juglone showed antibacterial effects similar to that of chlorogenic acid.

In summary, we conclude that certain phenolic compounds play a significant role in the defense mechanism of walnut cultivars and selections against *Xaj*.

Based on the results from the examination of the husk expressed husk liquid's antibacterial impact, we concluded that the expressed husk liquid from cultivars with different level of susceptability does not result in significant differences of inhibition zone. The antibacterial effect of the expressed husk liquid from infected and healthy husks does not differ significantly. At the same time we identified a correlation between polyphenol content and the dimensions of inhibition zone. Based on these findings we conclude that the fruit of walnut cultivars with higher polyphenol content are more resistant to bacterial blight infection.

5. NEW SCIENTIFIC RESULTS OF DOCTORAL RESEARCH

The PhD research had the following novel scientific findings:

1. The first mapping of walnut populations in the various regions of Central- and Eastern-Transylvania revealed high variability from fruit morphology and susceptibility to bacterial blight disease perspectives.

2. Two selections, SZEN-10 and SOM-101 were identified as valuable based on fruit morphology attributes and susceptibility to *Xaj*, and propagated to allow further comparative studies with other cultivars.

3. The need for using *in vitro* inoculation technique for the assessment of walnut cultivars' *Xaj* susceptibility was proved for the first time in a Hungarian context. Hungarian cultivars and Transylvanian selections have been classified based on their *Xaj* susceptibility and resistance.

4. The positive correlation between the polyphenol content of the green husk and resistance to bacterial blight disease was proven.

5. The biochemical identification of three *Xanthomonas arboricola* pv. *juglandis* bacterial isolates with differing origins was carried out based on their use of carbohydrates to identify the strains role in resistance breeding work.

6. A complex method was devised for the assessment of those phenolic compounds that have antibacterial effect in walnuts.

7. It has been confirmed that the physiological process of the bacterial blight disease can be traced through the analysis of the phenolic compounds. Those phenolic compunds that play a role in the defense mechanism of walnut cultivars and selections have been identified.

LITERATURE CITED

1. BERTRAND P.F., GOTTWALD T.R. (1986): Evaluating fungicides for pecan disease control, 179-181. p. In: HICKEY K.D. (Ed.). Methods for evaluating pesticides for control of plant pathogens. APS Press, St-Paul, Minnesota, 319. p.
2. BUJDOSÓ G. (2006): Hazai diófajták áruértéke. 54-58. p. In: SZENTIVÁNYI P., KÁLLAY T.-NÉ (Szerk.): *Dió.* Budapest, Mezőgazda Kiadó, 204 p.
3. BUJDOSÓ G., VÉGVÁRI GY., HAJNAL V., FICZEK G., TÓTH M. (2014): Phenolic Profile of the Kernel of Selected Persian Walnut (*Juglans regia* L.) Cultivars. *Not Bot Horti Agrobo*, 42(1), 24-29. p.
4. HUNTER R.E., ROBERTS D.D. (1978): A disease grading system for pecan scab. *Pecan Quarterly* 12(3), 3-6. p.
5. ÖZAKTAN H., ERDAL M., AKKOPRU A., ASLAN E. (2008): Evaluation of susceptibility of some walnut cultivars to *Xanthomonas arboricola* pv. *juglandis* by immature nut test. Cost 873, WG and Management Committee Meeting, Athens, Greece. http://www.cost873.ch/_uploads/_files/Ozaktan_WalnutSusceptibility.pdf
6. SINGLETON V. L., ROSSI J. A. (1965): Colometry of total phenolics with phosphomolybdic phosphotungstic acid "reagents". *Am. J. Enol. Vitic.*, 16, 144–158. p.
7. SOLAR A., DREO T., MICULIC-POTROVSEJ M., LIZOCAR A., SUSTARSIC M., VEBERIC R., MATICIC L., RAVNICAR M., STAMPAR F. (2009): Phenolic compounds as potential markers for walnut blight resistance. Annual Cost 873 Meeting, Cetara, Italy. http://www.cost873.ch//_uploads/_files/ASolar_Walnut-Phenolics_Italy.pdf
8. SOLAR A., JAKOPIC J., VEBERIC R., STAMPAR F. (2007): Phenolic compounds as a potential marker of walnut resistance against *Xanthomonas arboricola* pv. j*uglandis.* Cost 873, WG3/WG4 Joint Meeting, Murcia, Spain. http://www.cost873.ch/_up_loads/_files/m_Solar_murcia.pdf
9. SZENTIVÁNYI P. (1976): Dió. 9 – 83. p. In: SZENTIVÁNYI P., PEJOVICS

B., HORN E. (Szerk.): *Dió, Mandula, Mogyoró, Gesztenye*. Mezőgazdasági Kiadó, Budapest, 342. p.

10. SZENTIVÁNYI P. (2006): Az Országos Mezőgazdasági Minősítő Intézet által elfogadott, államilag elismert árúfajták. 62 – 65. p. In: SZENTIVÁNYI P., KÁLLAY T- NÉ: *Dió.* Mezőgazda Kiadó, Budapest, 204. p.

Publications of the author in the framework of the PhD thesis

Papers in journals with impact factors:

1. Thiesz R., **Bandi A.**, Tóth M., Balog A. (2009): Evaluation of an isolated Persian walnut (*Juglans regia* L.) population from Eastern Transylvania, Romania. Journal of Food, Agriculture and Environment 6 (3-4). pp. 132-136. IF: 0,517

Papers in journals without impact factors:

1. **Bandi A.**, Hevesi M., Szani Zs., Tóth M. (2015): Assessment of bacterial blight tolerance in Persian walnut based on immature nut test. Notulae Scientiae Biologicae 7(2). pp. 253-257. (ISI Thomson Master Journal List, BIOSIS Previews, CAB Abstracts, index Copernicus)
2. **Bandi A.**, Tóth M., Hevesi M. (2014): Comparison of *Xanthomonas arboricola* pv. *juglandis* isolates from walnut trees grown in Romania and Hungary. International Journal of Horticultural Science 20 (1-2). pp. 65-69. (BIOSIS Previews, CAB Abstracts)
3. Thiesz R., **Bandi A.**, Tóth M., Balog A. **(**2007)**:** Epidemiological survey of *Xanthomonas arboricola* pv. *juglandis* and *Gnomonia leptostyla* on natural population of walnut (*Juglans regia*) in eastern Transylvania. International Journal of Horticultural Science 13 (4). pp. 7-11. (BIOSIS Previews, CAB Abstracts).

Publications in conference rewievs

Hungarian conferences, full paper:

1. **Bandi A.**, Thiesz R., Daczó, L.(2007): Felső - Háromszék diópopulációjából tájfajta- szelekcióval kiválogatott genotípusok növénytani és gazdasági értékelése. Erdélyi Múzeum Egyesület Múzeumi Füzetek, Acta Scientiarum Transylvanica Agronomia, 15/2, pp. 55-67, Published by the Transylvanian Museum Society, Kolozsvár/Cluj

Hungarian conferences, abstract:

1. **Bandi A.**, Hevesi M., Thiesz R., Tóth M. (2012): Erdélyből származó diószelekciók fogékonyságának megállapítása a *Xanthomonas arboricola* pv.

juglandis baktériummal szemben. Növényvédelmi Tudományos Napok, Magyar Tudományos Akadémia, Budapest, Növénykórtani szekció.

2. **Bandi A.**, Tóth M., Thiesz R., Hevesi M. (2012): Magyarországi diófajták és erdélyi szelekciók baktériumos foltosság betegséggel szembeni ellenállóságának vizsgálata. Kihívások és megoldások a XXI. század élelmiszertudományában TÁMOP- 4.2.1/B-09/1/KMR-2010-0005 záró konferencia. 2012. január 18- 19.

International conferences, full paper:

1. **Bandi A.**, Tóth M., Hevesi M., Thiesz, R. (2010): Walnut selections susceptibility to *Xanthomonas arboricola* pv. *juglandis*. Preliminary results. University of Agronomical Sciences and Veterinary Medicine, Scientifical Papers, 54: 22-30, pp. 366-371. ISSN 1222-5312, index BDI-CABI
2. Thiesz R., Balog A., Kentelky E., **Bandi A.** (2007): Studies of physical characteristics on natural population of walnut (*Juglans regia* L.) fruits in Eastern Transylvania. In: "Agricultura durabilă – Agricultura viitorului" Ediţia a III-a, pp. 362-369. ISSN 1841-8317

International conferences, abstract:

1. Molnár R. L., Thiesz R., **Bandi** A. (2015): Evaluation of promising walnut genotypes (*Juglans regia* L.) from the region of Eastern and Middle Transylvania. In: 3. Transylvanian Horticulture and Landscape Studies Conference, Tîrgu Mureş (Marosvásárhely). p. 18.
2. **Bandi** A., Hevesi M., Thiesz R., Tóth M. (2010): Susceptibility to *X. arboricola* pv. *juglandis* of walnut elites selected from the natural population in Transylvania. In: 1. Transylvanian Horticulture and Landscape Studies Conference, Tîrgu Mureş (Marosvásárhely). p. 12.
3. **Bandi A**, Szani Zs., Thiesz R., Bujdosó G., Tóth M. (2010): Variability of Persian walnut (*Juglans regia* L.) fruit phenotype of the Carpathian Basin. In: 1. Transylvanian Horticulture and Landscape Studies Conference, Tîrgu Mureş (Marosvásárhely). p. 13.