

Theses of doctoral (Ph.D.) dissertation



**RESISTANCE OF APRICOT AGAINST BLOSSOM
BLIGHT CAUSED BY
(*MONILINIA LAXA* ADERH. ET RUHL.)**

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INTRODUCTION

Resistance against pests and diseases became the second most important point of fruit breeding, because growing of most fruit species can happen economically only by using pesticide treatment. (Gardiner et al., 2007).

Blossom blight caused by *Monilinia laxa* is one of the most severe diseases of sour cherry and apricot. In the case of rainy or wet weather during the blooming period plant protection of apricot plantations is determined by chemical treatments against *Monilinia* infection. (Holb, 2003).

Gyula Magyar's apricot selection experiments can be considered as the first apricot breeding program in Hungary. Winter hardiness and *M. laxa* resistance were the primary goals of his project (Pedryc, 2003).

One can't study the resistance of a plant without knowing the evolution and procession of infection. Species of *Monilinia* genus are typical necrotroph fungi. After penetrating the host organization with the help of extracellular enzymes they cause disintegration of their tissues. Their fitotoxins directly destroys the infected tissues (Honey, 1936).

Destroyed tissues shrivel due to dehydration. Dried and rotten fruits form mummies, remaining on trees until the next vegetation period. The survival of disease is provided by the fruit mummies and infected shoots. It is widely accepted that conidia can enter pistils across stigmata, similarly to pollen tubes (Glits, 2000). According to Byrde and Willetts (1977) all parts of the flower can serve as a first site of infection. Start points of infection can be petal, stamen or stigma. Pathogen fully colonizes the flower in 48 hours and penetrates the phloems of fruit sets through the sepal and stem (Weaver, 1950).

Stigma is rich in nutrients therefore it is a target of phitopathogen fungi and bacteria. In avoiding stigma's infection with fungi and bacteria the role of RN-ase enzyme was assumed by Schrauwen and Linskens (1972). Nectar secreted in flowers contains sugars, lipids and proteins in great quantities. It was Hilwig et al. (2010) who identified in plants, namely from petunia's nectar first time 2 RN-ase proteins very similar to S-RN-ase, which play role in nectar's protection from pathogens.

Hugot et al. (2002) proved that in tobacco 'S-like RNase' enzyme *in vitro* prevents growtf of *Fusarium oxysporum* and *Phytophthora parasitica*. In apricot the infection of flowers

in connection with pollination was examined by Tzoneva és Tzonev (1999). In their experiments they found increased resistance of pollinated pistils against unpollinated ones. Their hypothesis explaining this observation was that the pollinated pistils produce protective substances, which does not allow the infection and also stops the pathogen in other flower parts.

Sensitivity and resistance of apricot varieties to *M. laxa* was studied by Benedek et al. (1990) in Hungary, Komar-Tyomnaya and Richter (2000) in Ukraine, Tzonev and Yamaguchi (1999) in Japan, Balan et al. (1999) in Romania, Crossa-Raynaud (1969) in Tunisia, Nicotra et al. (2006) in Italy. In the Mediterranean region ‘Hamidi’ was described as *M. laxa* resistant variety by Crossa-Raynaud (1969), high-rate resistance of ‘Precoce de Italia’ is reported both by Nicotra et al. (2006) and Balan et al. (1999). Komar-Tyomnaya and Richter (2000), researchers of the Nikita Botanical Garden, ranked all the examined apricot varieties into susceptible group. Resistance against *M. laxa* flower infection in close relatives of apricot was studied by several researchers. Tzonev and Yamaguchi (1999) report about immunity of *P. mume* and its hybrids with apricot as well as two varieties of *P. salicina*. Komar-Tyomnaya and Richter (2000) describe resistance of interspecific hybrids *P. cerasifera* × *P. armeniaca*: ‘Sintez’, ‘Uryuko-alyca’, *P. domestica* × *P. armeniaca*: ‘Kleimen x ‘Krasny partizan’.

Resistance to pathogens plays a key role in the breeding program of *Prunus* species. In resistance breeding of apricot against *Plum pox potyvirus* (PPV) NBS-LRR (nucleotide binding site – leucine-rich repeats) RGAs (Resistance Gene Analogue) were developed by several research groups. All the amino acid sequences from apricots belonged to TIR-NBS-LRR group. (Vilanova et al., 2003, Dondini et al., 2004, Soriano et al., 2005). In the case of apricot unequal distribution of TIR-NBS-LRR and CC-NBS-LRR RGAs was found. This is a unique phenomenon among dicot species studied until now. It could not be decided whether it can be explained by the small number of CC-NBS-LRR type sequences or by the unsuitability of the PCR techniques applied for isolation (Soriano et al., 2005).

Enzymes responsible for the degradation of cell-wall are secreted by fungi in defined order. (Albersheim et al., 1969). The first enzyme is endo-polygalacturonase (PG) (Jones et al., 1971). Only polygalacturonase enzymes are able to initiate degradation of pectins in the cell-wall, without them polisacharid-degrading enzymes secreted later are not able to break down the pectins which means that infection can not be realized. (Karr and Albersheim, 1970).

The inhibiting of PG enzymes was observed by Fielding (1981) during fruit infection with *M. laxa* and *Botrytis cinerea* of plum, peach, apple, pear and grape. PG activity could not be detected from fruit extracts infected with *M. laxa* and *Botrytis cinerea*. Mixing experiments confirmed the presence of an inhibitor of polygalacturonase which was separable from the enzyme by isoelectric focusing and the PG activity appeared again at the isoelectric point pI 9-10. Endo-polygalacturonase inhibiting protein (PGIP) was first time isolated from (Cervone et al., 1987). It was established that evolving PG-PGIP complex caused complete inhibition of PG activity.

The first successful transformation experiment with PGIP gene by Powell et al. (2000) The PGIP gene of pear was transformed to tomato. Compared to untransformed control plant damaged caused by *Botrytis cinerea* in tomato decreased by 15% on fruits, 25 % on leaves. Phytopathogen PG inhibition from plant derived PGIP was confirmed in case of *Arabidopsis* (Ferrari et al., 2003) grape (Aguero et al., 2005), tobacco (Joubert et al., 2007) in transformation experiments.

AIM OF OUR STUDY

1. Ranking of apricot varieties and hibryds according to blossom blight caused *M. laxa* susceptibility.
2. Exact description of infection of fruitsets, exploration the relationship between pollination and stigma infection.
3. Description the background of resistance and characterisation of response to infection.
4. Elaboration of routines suitable for identifying *M. laxa* resistant genotypes.
5. Identification of RGAs linked to *M. laxa* resistance based on homology.
6. Identification from phloem differently expressed genes which produce determined responses to *M. laxa* infection in susceptible and resistant varieties.

MATERIALS AND METHODS

Plant material

Varieties and hybrids (table 1) came from the Szigetcsép germplasm collection of Corvinus University of Budapest, Department of Genetics and Plant Breeding. The artificial infection of shoots was executed in the Soroksár plantation of our Department.

The infection was carried out on 'Korai Zamos' and field resistant 'Zard' varieties and their 48 progenies crossed in 2003.

Table 1. Varieties and hybrids of apricot, cherryplum and Japanese plum under examination

Name of variety	Origin	Pedigree
Aurora	USA	RR17-62 × NJA-13 / Rutbhart
Baneasa 4/11	ROU	Unknown
Ceglédi arany	HUN	Rózsabarack C. 1668 × Ceglédi óriás; 1994
Ceglédi bíborkajszi	HUN	Random seedling; 1953
Ceglédi óriás	HUN	Landscape selection, Izsák; 1953
Ceglédi Piroska	HUN	Ceglédi óriás × Magyarkajszi C. 1789; 1995
CR-2-63	USA	Unknown
Effekt	UKR	Open pollinated seedling of Krupnoplodnij
Goldrich	USA	Sunglo × Perfection
Gönci magyarkajszi	HUN	Selected clone of Magyarkajszi, 1960
Harcot	CAN	(Geneva × Narmata) × Morden 604 × NJA1 (Perfection × Phelps); 1977
Harmat	HUN	Open pollinated seedling of Salah; 2002
Karola	SVK	Kloboucka × Velkopavlovicka
Kecs-pszar	UZB	Local variety in Central-Asia; Kosztina, 1930
Konkurencia	UKR	Effect × Priuszadebnij rannij
Konzervnij pozdnij	UKR	Random seedling
Korai zamos	HUN	Open pollinated seedling of Jubilar; 2002
Magyarkajszi C.235	HUN	Selected clone of Magyarkajszi
Mamaia	ROU	(Ananas × Ananas) × (Tarzii de Bukuresti × Ananas); 1985
Mandulakajszi	HUN	Unknown; 1954
Marculesti 18/6	HUN	Unknown
Mari de Canad	ROU	Local variety of unknown origin
Nikitszkij	UKR	Selected clone of Krasznoscsokij

Observation of Natural Infection

Observation of natural infection in open was carried out in germplasm in Szigetcsép through 3 vegetation periods. Rate of infection was surveyed every year in 5 weeks after petal falls during the intensive growing period of shoots. Categories of disease level were formed according to percentage of fruitset damage. (table 2.)

We studied the natural twig blight symptoms on *P. mume* and *P. sibirica* in Vácrátót Botanical Garden of the Institute of Botany of the Hungarian Academy of Sciences from 2009 till 2011.

Table 2 Categories of *M. laxa* infection

Category of infection	Fruitset damage (%)
0	0
0,5	1-5
1	6-10
1,5	11-15
2	16-20
2,5	21-25
3	>26

Molecular research

DNA isolation

Genomic DNA was extracted from fully expanded young leaves using DNeasy Plant Mini Kit (Qiagen, Germany).

PCR techniques for identification of NBS sequences

Twenty-six NBS-LRR resistance genes (8 from the TIR-NBS-LRR and 18 from the non-TIR-NBS-LRR class) were retrieved from the NCBI GenBank. The sequences represented 9 species (*Arabidopsis thaliana* 11, *Lycopersicon esculentum* 4, *Oryza sativa* 3, *Solanum tuberosum* 2, *Linum usitatissimum* 2, *Cucumis melo* 1, *Nicotiana tabacum* 1, *Capsicum chacoense* 1, *Helianthus annuus* 1). The nucleotide and amino acid sequences were

aligned with ClustalW program (Thompson et al., 1994). Degenerated primers were designed manually. The applied primers' sequences are presented in table 3.

Table 3. Sequences and permuted indexes of degenerated primers designed for NBS-LRR type resistance genes

Primer	Sequence 5'–3'	Permutation index	Source
CUB P-loop F	5'-RTT GNA TGG GNG GNN TDGG-3'	1536	Own design
CUB Kin2 F	5'-TND TNH TNG ATG AYR TNT GG-3'	9216	Own design
CUB HD R	5'-GCY ARW GGY AVH CCW YYA CA-3'	1152	Own design
P-loopGent F	5'-GAA TGG GKG GAG TYG GYA ARA C-3'	16	Gentzmittel et al. (1998)
LM637 R	5'-ARI GCT ARI GGI ARI CC-3'	2048	Kanazin et al. (1996)

M=A+C, S=C+G, Y=C+T, R=A+G, W=A+T, N=A,+T+G+C,

RNA-based experiment

Sample collection for gene expression survey

Sampling was performed in Szigetcsép germplasm collection of Corvinus University of Budapest, Department of Genetics and Plant Breeding from variety 'Zard' two times: 2 and 4 weeks after blooming respectively. Sample collection of intact and infected shoots from 'Orange Red' was realised 2 weeks after blooming. The intact and infected shoots were cut and placed in liquid nitrogen after packaging and labelling. In this form they were transported to our Department's lab, where they were stored at the temperature of -80 °C until processing.

RNA isolation and synthesis of single-stranded cDNA

The intact phloem under the infected part was peeled with sterile scalpel and ground in liquid nitrogen. Sampling from the intact shoots happened in a similar way. The RNA isolation was performed with hot borate method according to Wan és Wilkins (1994). cDNS

synthesis was realized by First Strand cDNA Synthesis Kit (Fermentas Life Sciences, Biocenter Ltd, Szeged) using biotin labelled oligo dT primers according to the instructions of the manufacturer.

cDNA AFLP

cDNA AFLP techniques were applied according to Vuylsteke et al. (2007) with just minor modifications.

Primers designed for PGIP sequences

We harvested *Rosaceae* sequences from NCBI database with BLASTn and BLASTp (Altschul et al., 1990) which showed high similarities (E -value $<10^{-50}$) with apple and peach PGIP genes.

After having aligned the sequences with ClustalW (Thompson et al., 1994) four primers (Table 4) were designed on the conserved regions.

Table 4. Primers based on alignment of PGIP genes from *Rosaceae* species

Primer	Sequence 5'–3'	length (bp)
PGIP CONS1 F	CATCCTAAACC(C/A)AGC(T/G)CTCTC	20
PGIP CONS2 F	AGC(T/G)CTCTC(C/A/T)GAGCTCTGC	19
PGIPZ R	GATCCAAGGAAGTCAAGCTCTTC	23
PGIP R	AACTGTTGTAGCTCACGTTTCAGGA	24

RESULTS

Apricot varieties and closely related diploid *Prunus* species sensitivity to twig blight caused by *M. laxa*

Based on the extent of fruitset decay of the *Prunus* species and apricot varieties we can conclude that the susceptibility level showed a continuous distribution. (Figure 1.). We measured an average fruitset decay of the apricot varieties from 5 to 30 percent. Based on that resistance of the apricot varieties can be characterised as a quantitative trait – except 'Zard' variety that shows full resistance (Figure 2).

In their studies Balan et al. (1999) from 124 apricot varieties found only one hybrid completely resistant while 4 of them were resistant or moderately resistant. The 'Goldrich' variety they categorize as susceptible, which is in harmony with the 20 % fruitset decay detected by us.

In the Nikita *Prunus* germplasm Komar-Tyomnaya and Richter (2000) ranked all the apricot varieties among the susceptible group. Apricot and cherryplum hybrids showed complete immunity to infection. From the *P. dasycarpa* varieties 'Grossa tardiva', 'Manaresi' 'Tlor Ciran', 'Picole precocte', from the plumcot species 'Zamshevy' and 'Krasny' varieties were ranged among resistant category. Individuals of the species *P. sibirica* were put among susceptible group, which is in accordance with our result in the Vácrátót Botanical Garden where we detected serious *M. laxa* infection on *Prunus sibirica* specimens. Tzonev and Yamaguchi (1999) identified 'Red June' and 'Sordum' varieties of *P. salicina* as completely resistant. In our research we also placed two Japanese plum varieties – namely 'Methley' and 'Burbank' – to the completely immune group. Resistance of apricot and plumcherry hybrid 1/33 is also consonant with results of Komar-Tyomnaya and Richter (2000). It is an important finding of Surányi (2003) that *P. dasycarpa* is a constant hybrid of *P. armeniaca* and *P. cerasifer*, as a matter of fact its pedigree is identical with our 1/33 hybrid.

In the sensitivity rank we found two types of resistance. In the first one the fruitset decay is less than 2-3 %. *P. salicina*, *P. cerasifera*, *P. dasycarpa*, 1/33 apricot- cherryplum hybrid and the *P. armeniaca* 'Zard' varieties represent that level of resistance. On those genotypes following flower infection a few mm decay of shoot phloem takes place, which was closed round by a thickened tissue. On those genotypes in the course of our 3 year research we have never experienced decay of long fruitsets and the total fruitset decay has never reached a 5 % level. (figure 2).

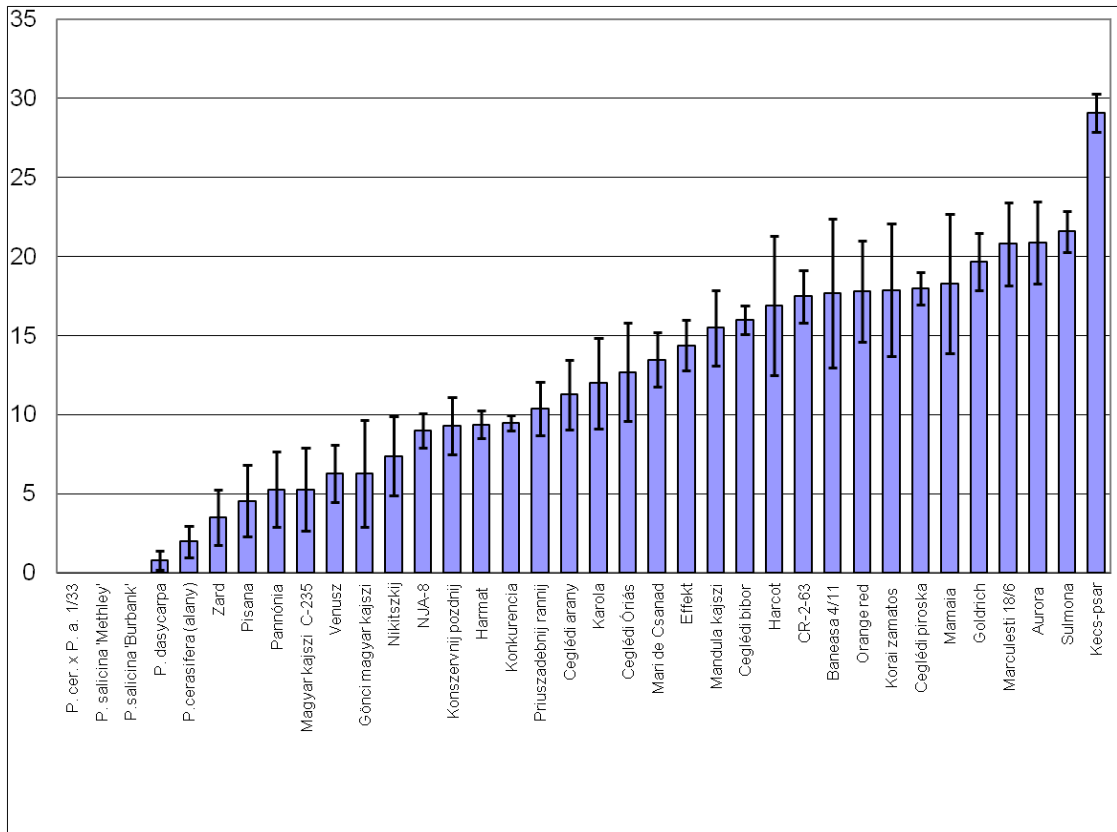


Figure 1 Susceptibility of apricot and (*P. cerasifera*, *P. salicina*) genotypes to *M. laxa* infection. Diagram shows the percentage of the infected fruitsets.



2. ábra. *Monilinia laxa* damage on two apricot varieties. On the left shoot decay of the 'Orange red', higher than 30 %. On the right 'Zard' with a twig decay less than 2 %. Photo from the abandoned Szigetcsép germplasm, taken in 2010.

Relationship between pollination and flower infection

It is widely accepted that *M. laxa* conidia can enter pistils across stigmata. Based on this hypothesis Tzoneva and Tzonev (1999) investigated if pollination has any effect on the frequency of infection. This question emerged because the apricot breeders often experienced an increased resistance on the crossed branches. They have observed no infection on emasculated and pollinated branches while the branches without treatment were infected.

We clarified the effect of pollination on infection by installing an experiment on two susceptible varieties. In case of ‘Goldrich’ we have experienced infection on the 111 emasculated and pollinated flowers just one time, while in case of 118 emasculated and non-pollinated flowers we could not find any flower infection. In case of ‘Goldrich’ variety lack of infection was caused not by the pollination process, but the mechanical removal of susceptible parts of flowers functioning as gate of infection. The pollination did not have any effect on the frequency of flower infection, therefore in case of apricot *M. laxa* infection could not happen.

While growing fungi on solid media we have experienced an inhibition effect around stigmata. From the stigmata placed on media extracellular ribonuclease was secreted as it was demonstrated also by Halász (2007), which has been detected by staining the RNA of *Torula* yeast. The growing of mycelia was inhibited around stigmata, the size of it was similar to the zone demonstrated by RNA staining.

Intercellular liquid soaked from stigmata inhibited the germination of conidia or if they have still germinated the growing of mycelia was completely halted. ‘S-like’ ribonuclease from tobacco similarly inhibited the germination of two phytopathogen fungi. Hugot et al. (2002). Therefore we suppose that S-RNase expressed in stigmata play role in the stigmata’s protection from pathogens, beside enabling self-incompatibility.

Results of artificial infection of fruitsets

Crossa-Raynaud (1969) published an artificial infection technique to survey the *M. laxa* resistance of apricot and almond varieties. They have made cuts on excised two-year old branches and infected them with *M. laxa* inoculum. They have found an unambiguous connection between the susceptibility of the varieties in the field and the length of decayed tissues on artificially infected branches. This technique was applied in several apricot breeding programs (Gulcan et al., 1999, Nicotra et al., 2006) as a method to excelerate

fenotyping. Contrarily Szügyi et al. (2012) artificially infected sour cherry shoots and found a same length fruitset decay on variety ‘Csengődi’ considered to be resistant and ‘Érdi bőtermő’ which has been ranked as highly sensitive.

We applied the above mentioned infection method on ‘Zard’, identified as resistant in the field and ‘Kecs-psár’ considered to be susceptible. Decay of the phloem was identical on both varieties and in 30 days the necrosis has extended on the full length of the excised branches. Gulcan et al. (1999) reported the same result after artificially infecting apricot shoots. We expected an exaggerated effect from an artificial infection executed in summertime therefore we chose the autumn period to infect the varieties ‘Zard, ‘Korai zamatos’ and their 48 hybrids. We infected shoots instead of branches since the natural *M. laxa* infection also appears on them.

We have got a surprising result. Average length of infected tissues of ‘Zard’ variety was higher than that of susceptible ‘Korai zamatos’, however the difference was not significant because of the major deviation. This result is in complete contradiction with Crossa-Raynaud’s (1969) findings.

The above mentioned result shows that the length of the infected phloem is not feasible to determine the resistance of individuals, however we have achieved a relevant result, namely that on the border surface of infected and intact tissues there was a significant difference between the resistant ‘Zard’ and the sensitive ‘Korai zamatos’ varieties. On the resistant variety the phloem of the shoot was separated with a sharp edge from the brown coloured ruined tissues, while the intact tissue has been thickened. On the susceptible variety we found a vague border zone between the intact and infected tissues. All the 48 progenies were evaluated based on that principle. Inheritance of *M. laxa* resistance was formerly described as a dominant trait by Crossa-Raynaud (1969), Gulcan et al. (1999) and Nicotra et al. (2006)., because the utmost majority of the hybrids were resistant similarly to the parent, applied as source of resistance. Of course the number of individuals is not enough to draw unambiguous conclusions about the process of inheritance, but assuming that the variety ‘Zard’ contains the resistance in a heterozygous form the ratio of susceptible and resistant progenies approaches the theoretical 3:1 ratio of dominant process of inheritance described by Mendel.

The linkage of NBS-type RGAs and the *M. laxa* resistance

In case of grape and apple while applying RGA markers a linkage with plant resistance could have been demonstrated. In grape Di Gaspero and Cipriani (2002) identified linkage with downy mildew while Donald et al. (2002) with powdery-mildew related NBS-type RGA markers. Calenge et al (2005) published NBS-type RGA sequences linked to *Venturia*-resistance. In our research for the study of polymorphism of NBS-type RGA sequences we have chosen SSCP technique. In favour of isolating the more NBS genes we elaborated a new PCR strategy.

During SSCP analysis the self-designed primers enabled us to detect both NBS LRR gene families. With this technique we could harvest fragments in a threefold quantity in both groups compared to number of sequences amplified by formerly designed primer combination of Soriano et al. (2005).

In case of *M. laxa* resistant ‘Zard’ apricot variety we could not detect a fragment unique to this variety by SSCP analysis of PCR products which doesn’t exclude the role of NBS-LLR type genes in setting up resistance.

Differences in gene expression response to *M. laxa* infection of susceptible ‘Orange red’ and resistant ‘Zard’ varieties

In the process of PAGE applying 4 AFLP primer combinations we have identified 29 fragments which have appeared as a response to infection. From the total 29 we have sequenced 18 fragments. In his doctoral thesis Zubini (2008) detected the transcriptomic changes to *M. laxa* infection of peach fruit with cDNS- AFLP technique. She has identified 50-70 fragments for every primer combination in all samples. As an effect of the infection the appearance of the fragments has been changed in 20-35 %. In our research the estimated number of the fragments gained from 4 primer combinations has been changed from 50 to 200. In the samples from infected shoots the percentage of the new fragments was 10 %.

In one case our TDF (transcript derived fragment) was identical to the gene of *Pseudomonas* saprophyte bacteria, in spite of the fact that RNA has been isolated from an uninfected part. Zubini (2008) found that 50 % of the total 339 TDF sequences was identical to plant genes, while 40 originated in *Pseudomonas* species and 10 % derived from fungi. This result can be explained by the phenomenon that the infected part of the fruit can not be

separated from the intact part by a sharp border, therefore the half of the fragments having appeared in the infected samples derived from bacteria and fungi.

Out of the 18 sequences we have found only 11 in the peach genome that can be explained by directsequencing of the short fragments around 100bp.

One of the identified fragments (15th TDF from the first set of infected ‘Zard’ samples) was homolog with the sequence of Polygalacturonase Inhibiting Protein (PGIP) which has been identified from many plant species. Based on research of fruit infections Fielding (1981) reported of a polygalacturonase inhibiting plant protein which was expressed in fruits (apple, plum and peach) under infection. It was able to inhibit largely the polygalacturonase activity of the *Monilinia* species.

The PGIP enzyme in the plant is able to inhibit polygalacturonase activity of different fungi species in a specialized way (Albersheim and Anderson, 1971). We could establish about the expression of PGIP genes that they could not have been found neither in the resistant nor in the susceptible varieties of the intact samples. As an effect of the infection on the shoots of both resistant and sensitive varieties several PGIP alleles could be detected however we could not conclude on the level of the expression of the alleles. It means that the differences experienced in cDNA AFLP that is the 15th TDF appeared only in the first set of infected ‘Zard’ samples were caused by differences in sequence of PGIP alleles and not by the differences in expression. In the AFLP we could find only the *Tru* I restriction site on the PGIP sequence because the sequenced clones haven’t contained 3’ end of 15th TDF where the *Eco* RI cleavage happened. The *Eco* RI cleavage site haven’t been found on any of the PGIP sequences of the NCBI database therefore it is likely that on this special sequence part of 15th TDF there is a difference in the sequence of ‘Orange red’ and the other *Prunus* taxa.

In the genom of apple and peach the PGIP genes are present in 2 copies therefore we assumed that the maximum number of alleles in our samples of cDNA and genomical DNA is four. Contrary to our expectation from ‘Orange red’ we identified 6 various alleles while from ‘Zard’ the number of the identified alleles amounted to 5 in the cDNAs of infected samples. The above mentioned contradiction could be solved by identification of PGIP sequences from genomic DNA. This way the mismatches in the reverse transcription could be avoided.

When infecting plant tissues with fungi the expression of the PGIP genes was multiplied as published about *Arabidopsis* by Ferrari et al., (2003), apple by Yao et al., (1999), bean by Oliveira et al. (2010). According to Leckie et al. (1999) a single aminoacid

substitution in the PGIP gene of bean was responsible for the formation of an inhibition to a further pathogen.

NEW SCIENTIFIC RESULTS

1. Based on ranking 31 apricot varieties according to *M. laxa* shoot infection we have found a high level resistance of 'Zard' caused by the response of phloem tissue in the fruitset.
2. It was proved that in contradiction to former studies in case of apricot the infection never penetrates through stigmata. The pollination doesn't effect flower infection.
3. We established that extracellular secretum with ribonuclease activity originating in stigma inhibits the germination of conidia and development of mycelia in *M. laxa*.
4. We infected artificially varieties 'Zard', 'Korai zamatos' and also their hybrids. On some hybrids we observed phloem tissue responses special to 'Zard', which proves the inheritability of resistance.
5. We have elaborated a selection system which was based not on the usual method of shoot infection but on the artificial infection of petals and leaves. This technique allows fast fenotyping of varieties.
6. In the case of apricot we have isolated CC-NBS-LRR RGA sequences firstly. We could not find linkage between NBS RGA sequences and *M. laxa* resistance.
7. In the phloem transcriptome we have identified a sequence showing high similarity to PGIP gene, which has appeared as a response to *M. laxa* infection specially in the resistant varieties.

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PUBLICATIONS CONNECTED TO THE DISSERTATION

Article in impact factored journals:

Gutermuth, Á., György, Z., Hegedűs, A., Pedryc, A. (2011) Confirming the existence of nonTIR-NBS-LRR resistance gene analogs in apricot (*Prunus armeniaca* L.). *Acta Biologica Hungarica* 62 (2), 171-181. IF 0,551

Other journal articles:

Gutermuth, Á., Lendvay, B., Pedryc, A. (2010) Different responses of sensitive and resistant apricot genotypes to artificial *Monilia laxa* (Aderh. & Ruhl.) infection. *Acta Agronomica Hungarica*, 58(3), 289–294.

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Gutermuth, Á., Pedryc, A. (2009) The possible source of resistance against blossom blight (*Monilia laxa* Ehr.) in apricot. *Hungarian Agricultural Research*

Conference papers (Abstracts):

Gutermuth, Á., Pedryc, A. (2009) Kajszi genotípusok virágzáskori moniliával (*Monilia laxa* Ehr.) szembeni ellenállósága és a lehetséges rezisztencia források. Lippay János – Ormos Imre – Vas Károly Nemzetközi Tudományos Ülésszak, Gyümölcstudomány Szekció, okt. 28–30. Előadás és poszter összefoglaló

Gutermuth, Á., Pedryc, A. (2009) CC-NBS-LRR rezisztenciagén-analógok izolálása kajsziból XV. Növénynevelési Tudományos Napok, 2009. március 17., Összefoglalók (Szerk.: Veisz, O.), MTA, Budapest, ISBN 978-963-8351-34-0, p.156

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Conference papers (full paper): Gutermuth, Á., Pedryc, A. (2008) Targeted isolation of nonTIR-NBS-LRR resistance gene analogs in Apricot. *Acta Horticulturae* 839(2):531-536.