Phytophthora Infestans Induced Gene Expressional Changes in Different Potato Cultivars

Phytophthora infestans által kiváltott génexpressziós változások különböző burgonya fajtákban

Quyen, Tran Dinh[1], János Taller[1]*, Erzsébet Nagy[1], Melinda Mészáros[2], Zsolt Polgár[2], István Wolf[2] and Krisztián Frank[2]

[1]*Hungarian University of Agriculture and Life Sciences, Institute of Genetics and Biotechnology; trandinhquyen26061997@gmail.com, nagy.erzsebet@uni-mate.hu*

[2]*MKSzN Non-profit LLC.; mmeszaros382@gmail.com, polgar.zsolt@uni-mate.hu, wolfistvan01@gmail.com, frank.krisztian@uni-mate.hu*

**Correspondence: taller.janos@uni-mate.hu*

Abstract: The oomycete fungus *Phytophthora infestans* is the most damaging pathogen of potatoes. Sources of resistance are identified in different wild potato species, and some of these genes have already been isolated. During the six decade long resistance breeding program in Keszthely, race-specific *P. infestans* resistance genes were used in breeding. One of these cultivars, White Lady (WL), was used in this study together with the susceptible Sárvári borostyán (Sb) and Kastia (K) for transcriptomic analysis. Purpose of the present experiment was to analyse the *Phytophthora* inoculation induced stress response in the three varieties. Transcriptomes were reconstructed from samples collected 18, 24, 48 and 72 hours after inoculation. The results clearly revealed, that in the resistant WL significantly more genes, in some cases three-five times more genes are upregulated, than in the two susceptible cultivars. Similarly, significantly more genes are downregulated in the WL, than in Sb or K. It is concluded that the response to *P. infestans* inoculation is more comprehensive in the resistant cultivar, and possibly this reorganisation of tens of thousands of functioning genes leads to a successful resistance response. This process is suggested to be triggered by the race-specific late blight resistance genes in White Lady.

Keywords: *Phytophthora infestans; late blight; potatoes; resistance; transcriptomes*

Összefoglalás: A burgonyavészt előidéző Phytophthora infestans a burgonya legveszélyesebb kórokozója. Ez idáig számos rezisztenciagént azonosítottak különféle vad burgonyafajokban, és néhányat ezek közül izoláltak is. A több, mint hat évtizedre visszatekintő keszthelyi rezisztencianemesítési program során rassz-specifikus P. infestans rezisztenciagéneket építettek be számos fajtába. Ezek egyikét, a White Lady-t (WL) használtuk a jelen tanulmány transzkriptomikai vizsgálataiban két másik fajtával a fogékony Sárvári borostyánnal (Sb) és a Kastiával (K) együtt. A jelen vizsgálatok célja a három fajtában a Phytophthora fertőzésre adott stressz válasz molekuláris genetikai jellemzése volt. A transzkriptomokat a fertőzés után 18, 24, 48 és 72 órával gyűjtött levélmintákból készítettük. Az eredmények világosan mutatják, hogy a rezisztens WL-ben szignifikánsan több gén, egyes esetekben 3-5-ször annyi gén expressziója növekedett, mint a fogékony fajtákban. Hasonlóképpen, szignifikánsan több gén expressziós szintje csökkent a WL-ben mint a fogékony fajtákban. Ezek az eredmények arra utalnak, hogy a rezisztens fajtában az egész genetikai apparátusra kiterjedő változások mennek végbe,

melyben gének tízezreinek működése módosul. Feltételezhetően ezen átfogó génexpressziós változásokat a White Lady-ben jelenlévő rassz-specifikus rezisztenciagének indukálják, és egyben ez a feltétele a sikeres rezisztencia válasznak.

Kulcsszavak: *Phytophthora infestans; burgonyavész; burgonya; rezisztencia; transzkriptom*

1. Introduction

The late blight disease caused by the oomycete fungus Phytophthora infestans is still considered the most damaging disease in potato (Solanum tuberosum) production (Gao et al., 2013). The yield loss caused by P. infestans is estimated to be about 16% globally (Sanju et al., 2015) and the annual cost of crop damage and chemical control is typically around 5.6 billion euros globally (Haverkort et al. 2009). Hence, genetics-based plant protection would be desirable for crop safety, as well as for lowering production costs, and not at least for the mitigation of agrochemical pressure on the environment. In 32 Solanum species 70 P. infestans resistance genes have been identified so far, and some of them, like the R1, R2, R3a, R3b, and R8, which derived from the hexaploid Solanum demissum (Ballvora et al. 2002; Huang et al. 2005; Li et al. 2011; Lokossou et al. 2009; Vossen et al. 2016), and the Rpi-blb1, Rpi-blb2, Rpi-blb3, Rpi-abpt, and Rpi-bt1, which originate from the diploid S. bulbocastanum (Ea et al. 2005; Vossen et al. 2003; Lokossou et al. 2009; Oosumi et al. 2009) have already been cloned. The R genes convey race-specific resistance to P. infestans, while the Rpi genes are considered broad spectrum resistance genes against late blight. The pyramiding of different resistance genes could be the solution to procreate durable late blight resistance in new potato varieties. Marker assisted selection based on isolated P. infestans resistance genes would facilitate such a breeding effort. The complex resistant potato cultivar White Lady (WL), that was bred at the Potato Research Centre (now MKSzN) at Keszthely, Hungary, contains most of the R1-R11 genes, and by molecular methods previously the R2, R3a and R3b genes have already been revealed from this cultivar.

To explore the genetic background of late blight resistance, as well as to understand the difference between a resistance and susceptibility response to *P. infestans* inoculation, in the present study we analysed the gene expression changes induced by *P. infestans* in three different potato cultivars. The cultivar Kastia (K) was previously found by Gergely (2004) to have outstanding good horizontal resistance to *P. infestans*, although the origin of its resistance was unclear. A susceptible cultivar, Sárvári borostyán (Sb) was used also in the experiments, as well as the cultivar WL for the identification of not yet isolated R-genes. In the present publication we focus on the *P. infestans* induced gene expressional changes, by identifying the up and down regulated transcripts in different time points after inoculation. The expressional differences are compared between the cultivars, and annotation of the significantly upregulated transcripts of the resistant cultivar WL was performed.

2. Materials and Methods

2.1. Plant material

Plants were grown from minitubers in 3 L pots filled with peat. The growing conditions in the phytotron were: 50% relative humidity, 16:8 hours day:night period, with the start of the lighting period the temperature increased gradually in two hours from 20ºC to 25ºC, and at the

end it decreased also in two hours from 25ºC to 20ºC. From each cultivar five plants were grown for inoculation and one for control.

2.2 *P. infestans* inoculations and samplings

The Polish *Phytophthora* isolate, MP-1548 was used. The culture was grown on tuber slices of the cultivar Hópehely. From the harvested mycelium sporangia were released in distilled water, and its concentration was adjusted to 15 000 sporangium/mL. One drop of this solution was applied to the abaxial surface of a leaflet on vigorously growing plants before flowering. The control plants obtained just a drop of clean distilled water.

For molecular analysis the neighbouring leaflet of the inoculated one on the same leaf was collected, and was put immediately on dry ice, and until RNA extraction the leaf samples were stored at -80ºC. Samples were collected at 18, 24, 48 and 72 hours after inoculation from the inoculated and control plants.

2.3. RNA-sequencing and transcriptome construction

RNA was extracted by Direct-zol RNA Miniprep kit (Zymo Research, USA). For poly-A enrichment the Poly(A) RNA Selection kit (Lexogen, Austria) was used. The sequencing libraries were prepared using the NEXTFLEX Rapid Directional RNA_Seq kit 2.0 (Perkin Elmer, USA), and transcriptome sequencing was done on a NextSeq 500 (Illumina, USA) platform, using a High Output 150 sequencing kit.

Using the SOAPdenovo-Trans (Xie et al. 2014), a *de novo* transcriptome assembly was done from White Lady RNA-seq data, and this *de novo* transcriptome was used as the mapping index. Quantification of transcripts in White Lady, Sárvári borostyán and Kastia samples was done with the "index" and "quant" commands of the Salmon program (Patro et al. 2017). Statistically significant differentially expressed genes (DEGs) were identified with the DESeq2 (Love et al. 2014) Bioconductor package in the R environment.

3. Results

The inoculation proved the expected resistance of WL, as well as the expected susceptibility of Sb to *P. infestans.* Surprisingly, the cultivar Kastia showed symptoms of infection, and similarly to the cultivar Sárvári borostyán, the late blight disease developed on the five tested Kastia plants, while the control plants remained healthy.

Transcriptomes of the control plants and of the four test time points were constructed for each cultivar. In the resistant cultivar WL with 8% ($\pm$0.3%) more transcripts were identified, than in the two susceptible cultivars. The total number of transcripts was the following: White Lady - 111 228 (100.0%), Sárvári borostyán - 102 654 (92.3%), Kastia - 101 919 (91.7%).

Besides the number of expressed genes, strong differences in the number of genes with inoculation induced expressional changes in the different time points was observed. As revealed in Figure 1., three to five times more genes were upregulated in WL than in the sensitive cultivars. In WL the number of upregulated transcripts continuously increased during the sampling period, while in both sensitive cultivars decrease in the number of upregulated genes could be observed at 24 hpi and 48 hpi, and an increase at 72 hpi. For the sensitive cultivars exactly the same tendency could be observed also for the downregulated genes. Further, the number of downregulated genes was in all cases characteristically higher in the sensitive cultivars, but in the resistant WL the tendency was the opposite, i.e.: there were in all time point much more up than downregulated genes. The number of downregulated genes was always higher, in WL than in SB and K, and in some cases this was 2-3 times more.

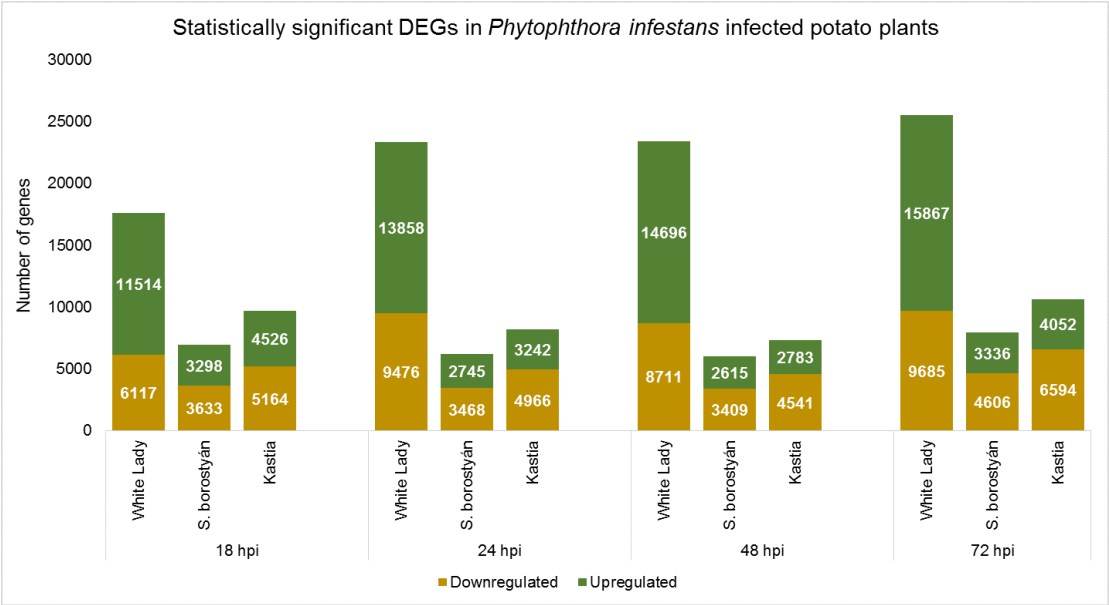

Figure 1. *The number of statistically significant differentially expressed genes (DEGs) of the three analysed cultivars in the four tested time points*

However, the transcripts revealed in Figure 1., are those which showed a two-times up- or down regulation, in each sampling time point a characteristically higher number of transcripts were found to be significantly up- or downregulated, but with a magnitude smaller than twice. The number of these transcripts are listed in Table 1.

Table 1. The number of differentially expressed transcripts in the three potato cultivars in different post inoculation time points

	Cultivars	LFC >1 and padj <0.05	LFC >0 and padj <0.05	LFC <0 and padj <0.05	LFC <-1 and padj <0.05
All time points	WL	6 735	8 315	4 272	2 014
	Sb	976	1 640	2 222	1 248
	Kastia	897	1 902	2 546	1 059
18 hpi	WL	11 514	14 368	9 191	6 117
	Sb	3 298	4 388	4 878	3 633
	Kastia	4 526	7 232	7 732	5 164
24 hpi	WL	13 858	16 614	12 429	9 476
	Sb	2 745	3 871	4 600	3 468
	Kastia	3 242	6 095	7 403	4 966
48 hpi	WL	14 696	17 462	11 866	8 711
	Sb	2 615	3 728	4 593	3 409
	Kastia	2 783	5 219	6 941	4 541
72 hpi	WL	15 867	18 519	12 839	9 685
	Sb	3 336	4 417	5 683	4 606
	Kastia	4 052	7 241	9 039	6 594

Notes: LFC: log to fold change; padj: adjusted P-value. LFC>1 and LFC<-1 are the more than two-times up- or downregulated transcripts, respectively. LFC>0 and LFC<0 are the less than two-times up- or downregulated transcripts, respectively. padj<0.05 indicates the 5% probability. hpi – hours post inoculation. WL – White Lady, Sb – Sárvári borostyán

4. Discussion

Instead of the expected high horizontal resistance of Kastia, that was previously described in unheated foil tent provocation experiments (Gergely, 2004), under the adjusted conditions of the phytotron this cultivar was found to be sensitive to *P. infestans*. Besides the differing test conditions of the two experiments, the *Phytophthora* isolate was also different, that may give an explanation for the observed alteration in stress response.

During the testing period more and more genes have been activated in WL and a high number of genes were downregulated, obviously a phenomenon closely linked to the successful resistance response. In contrast much less genes were affected by the *P. infestans* inoculation in the sensitive Sárvári borostyán and Kastia cultivars. This difference indicates that the late blight resistance genes of WL, similarly to other *P. infestans* resistance genes with proved function, are possibly transcription initiation factors, which after recognition of the pathogen trigger a cascade of genes expression changes to avoid infection.

For the identification of those genes and gene families which are mostly affected by the *P. infestans* inoculation we are using now a GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) annotation approach. Further, with the DNA motif-based identification of resistance gene like sequences we are identifying the possible late blight resistance gene candidates in the cultivar White Lady.

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