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# ROLE OF SOME PHENOLIC COMPOUNDS IN A RESISTANT GENE PYRAMIDED POTATO GENOTYPE TO LATE BLIGHT

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## Abstract

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In this study, we aimed to identify some phenolic compounds that determine resistance in a gene pyramided potato resistant genotype to late blight agent *Phytophthora infestans* and to compare it reaction with other resistance forms. Three resistant genotypes have been used; two genotypes from *Solanum tuberosum*: R4 (simple), MR (*R2, R3, and R4*) and the wild relative *Solanum demissum*. Inoculation of R4 genotype decreases the synthesis of Gallic acid and increase the amount of protocatecuic acid. In the case of *S. demissum*, an increase of the synthesis of protocatecuic acid, caffeic acid and ferulic acid. In the case of MR, inoculation induced a decrease of the synthesis of chlorogenic acid, caffeic acid, o-coumaric acid, quercetin and Kaempherol.

*Key words*: *Solanum* spp., *Phytophthora infestans*, resistance, phenolic compounds, gene pyramiding

## Introduction

*Phytophthora infestans*, the agent of late blight, is the most important pathogen on potato crop; also, it attacks tomato and other *Solanceae* species. It was the responsible for the Irish famine in the 1840. Until now, *P. infestans* still causes damages and loses on different *Solanaceae* crops, especially potato and tomato. It is responsible for multibillion dollars annually loses and treatment costs.

It is a necessity to develop potato and tomato crops that possess durable resistance against *P. infestans.* This necessity is increasing as more virulent, crop-specialized and pesticide resistant strains of the pathogen are rapidly emerging (van der Vossen Edwin et al., 2005). First, potato resistance breeding was based on gene introgression from the wild species *Solanum demissum*. 11 *R* genes from *S. demissum* were used (*R1*, *R2*,…*R11*). The pathogen was able to overcome this resistance. Actually, many other wild potato relatives are used in potato resistance breeding programs, such as *S. bulbocastanum*, *S. phureja*. Genes from *S. bulbocastanum* offers a broad spectrum against *P. infestans* (Song et al., 2003).

In this study, we tried to understand the difference between resistance of potato genotype with a simple one *R* gene, genotypes with multiple resistance genes (gene-pyramided genotypes) and the wild relative *S.*

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*demissum*. Gene pyramiding is advanced as a solution to *P. infestans* problems for both durability and level of resistance. It means the accumulation of resistance genes into a single genotype. It can be realized by the introgression of major *Rpi* genes, defeated *Rpi* genes, by different alleles of the same gene or the same allele (Tan et al., 2011).

Plants produce a large range of different compounds, which are toxic to pathogens, mostly considered secondary metabolites. The implication of phenolic compounds in plant disease is known for many years as the role of secondary metabolites in plant defense against pathogens and herbivores. Phenylpropanoid compounds implication in plant disease defense range from preformed or inducible physical and chemical barriers against pathogen infection to signal molecules involved in local and systemic signaling for defense gene induction [4]. The role played by phenols in plant resistance to pathogens, herbivores, and in allelopathy has been well discussed in many reviews (Field et al., 2006; Dixon et al., 2002; Bennet and Wallsgrove, 1994).

## Materials and Methods

In the study we used resistant potato plant to *P. infestans*, with the purpose to discover where the differences between the resistance form, are R4 (with the *R4* resistance gene), MR (with 3 resistance genes *R2*, *R3*, and *R4*) and the wild species *S. demissum* (Sd). Potato plants were grown *in vitro* in MS medium, and then acclimatized in greenhouse.

The *P. infestans* isolate A2.2 was chosen for infection. The isolate A2.2 (NL08009) was kindly sent to us by W. G. Flier, G. B. M. van den Bosch and G. J. T. Kessel from Plant Research International BV. The pathogen was grown on rye medium.

The infection procedure was performed according to CIP manual (Cip, 1997) for detached leaf test.

Total polyphenols extraction: The leaves have been ground into a fine powder in a mortar and pestle under liquid nitrogen, and then transferred to an eppendorf 2 ml tube. 1 ml of 70 % methanol was added, and mixed by sonification for 15 min, then centrifuged. 50 µl of supernatant was used for total polyphenol determination. For a microplate with 24 wells were used following quantities of reagents: 50 µl sample; 150 µl Folin Ciocalteu Reagent; 450 µl $Na_2CO_3$ (7.5%), 2350 µl bidistilled water. For data reading, we used the multidetection spectrophotometer BIOTEK Synergy HT

Standard curve was done using different concentrations of galic acid (mg/ml) (Figure 1). Absorption at 765 nm was measured. Total phenol contents were expressed in gallic acid equivalents (mg galic acid/g DW).

Preparation of HPLC samples: 0.2-0.5 g infested and healthy leaves have been used for extraction. The leaves (0.2-0.5 g) were ground into a fine powder in a mortar and pestle under liquid nitrogen, and then transferred to a 2 ml eppendorf tube, where 1 ml of 70% ethanol was added, followed by mixing by sonication for 15 minutes. The extract was filtered and 20µl were injected in HPLC system Agilent 1200 with UV-Vis detector.

HPLC protocol of phenolic compounds separation: 5µm Supelcosil LC18 column (250 x 4,6 mm) using a gradient A (methanol: acetic acid: water 10:2:88) and B (methanol: acetic acid: water 90:3:7) for 55 min, registered at λ =280 nm, at 25°C. Standard curve was done using different concentrations of chlorogenic acid (Figure 2).

The statistical analysis was performed with the software Statgraphics.

## Results and Discussions

*P. infestans* isolate was not able to succeed the infection of potato genotypes tested. However, the resistance

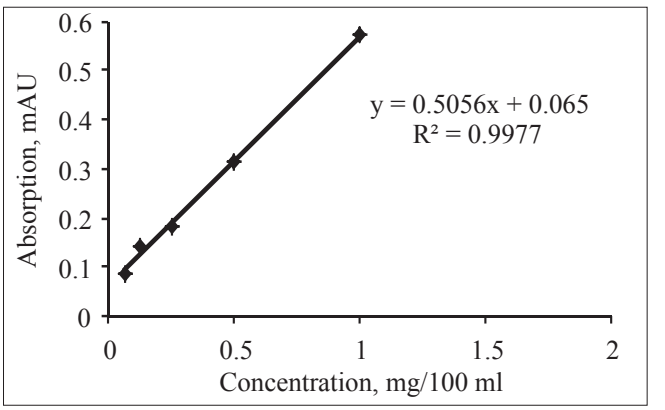


**Fig. 1. Calibration curve of gallic acid for total polyphenol extraction**

type differs. While R4 and *S. demissum* were immune, the genotype MR has expressed resistance via a hypersensitive reaction (HR). Figure 3 shows the reaction of different potato genotypes to inoculation by *P. infestans*.

At the metabolomics level, the content of polyphenols differed from a genotype to another (Figure 4). While the inoculation induced a decrease in total polyphenol content in *S. tuberosum*, it caused an augmenta-

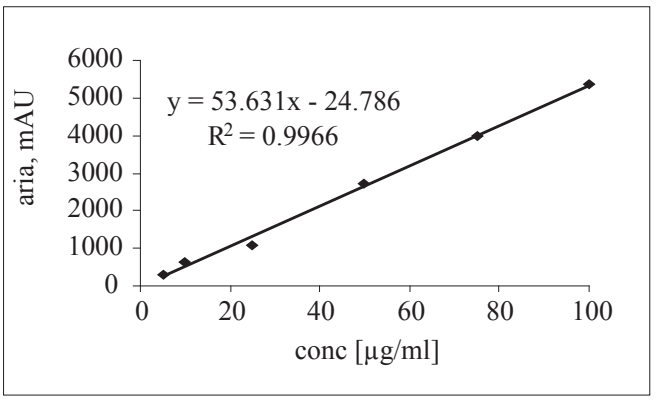


**Fig. 2. Calibration curve of chlorogenic acid for HPLC test**

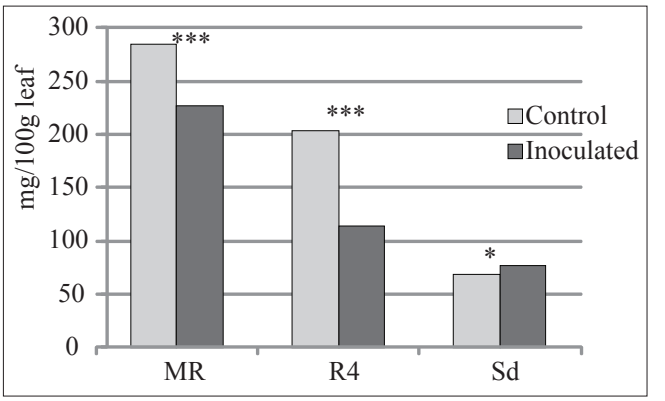


**Fig. 4. Content of total polyphenols for the inoculated and non-inoculated potato genotypes. *: P<0.05; ***: P<0.001**

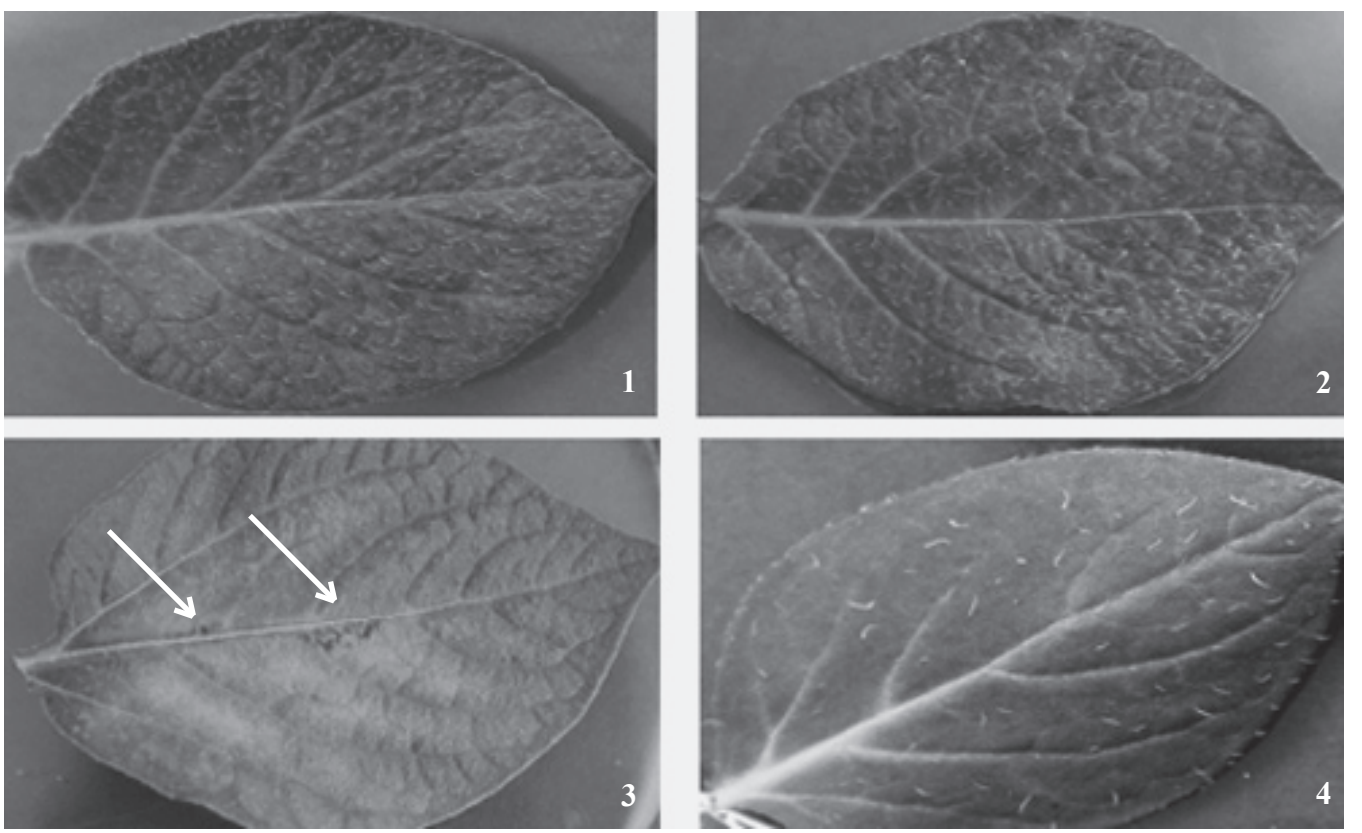


**Fig. 3. Reaction of potato to inocultation with A2.2 isolate of *P. infestans*. 1: control non-inoculated leaf; 2: R4; 3: HR in the case of the genotype MR. 4: *Solanum demissum***

tion in the case of *S. demissum*. The decreases of content in both *S. tuberosum* genotypes was high significant (P<0.001) when in the case of *S. demissum* the increase was significant (P<0.05).

From 13 detected, 10 phenolic compounds were identified: gallic acid, Protocatecuic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid, sinapic acid, o-coumaric acid, quercitin, kaempherol, and 3 not identified (NiA, NiB, NiC) (Figure 5).

In the case of R4, the synthesis of two acids was decreased after infection, and only for one, the synthesis has enregistered an increase. The most important effect was on gallic acid, which decreased significantly (P<0.01) after inoculation, protocatecuic acid, which is increased after infection (P<0.05), and chlorogenic acid with a significant decrease (P<0.05). Caffeic acid, ferulic acid, and quercetin have not been detected in the case of R4. Only one non-identified phenolic compound has been detected in this case. The inoculation induced an increase of catechin, kaempherol and a non-identified compound, but the increase was not significant (Figure 6).

The effect of inoculation is more visible on *S. demissum* and the genotype MR. In the case of *S. demissum* the most important phenolic compounds were Protocatecuic acid, ferulic acid, and all the 3 unknown phenolic compounds were highly significant induced (P<0.001). The unknown Nia was not detected in the control. It was induced totally by *P. infestans*. In the case of MR, for all the compounds detected, the inoculation has induced a decrease of phenolic compounds, with the exception of gallic acid. We did not detect the ferulic acid and catechin. Kaempherol has been suppressed after in-

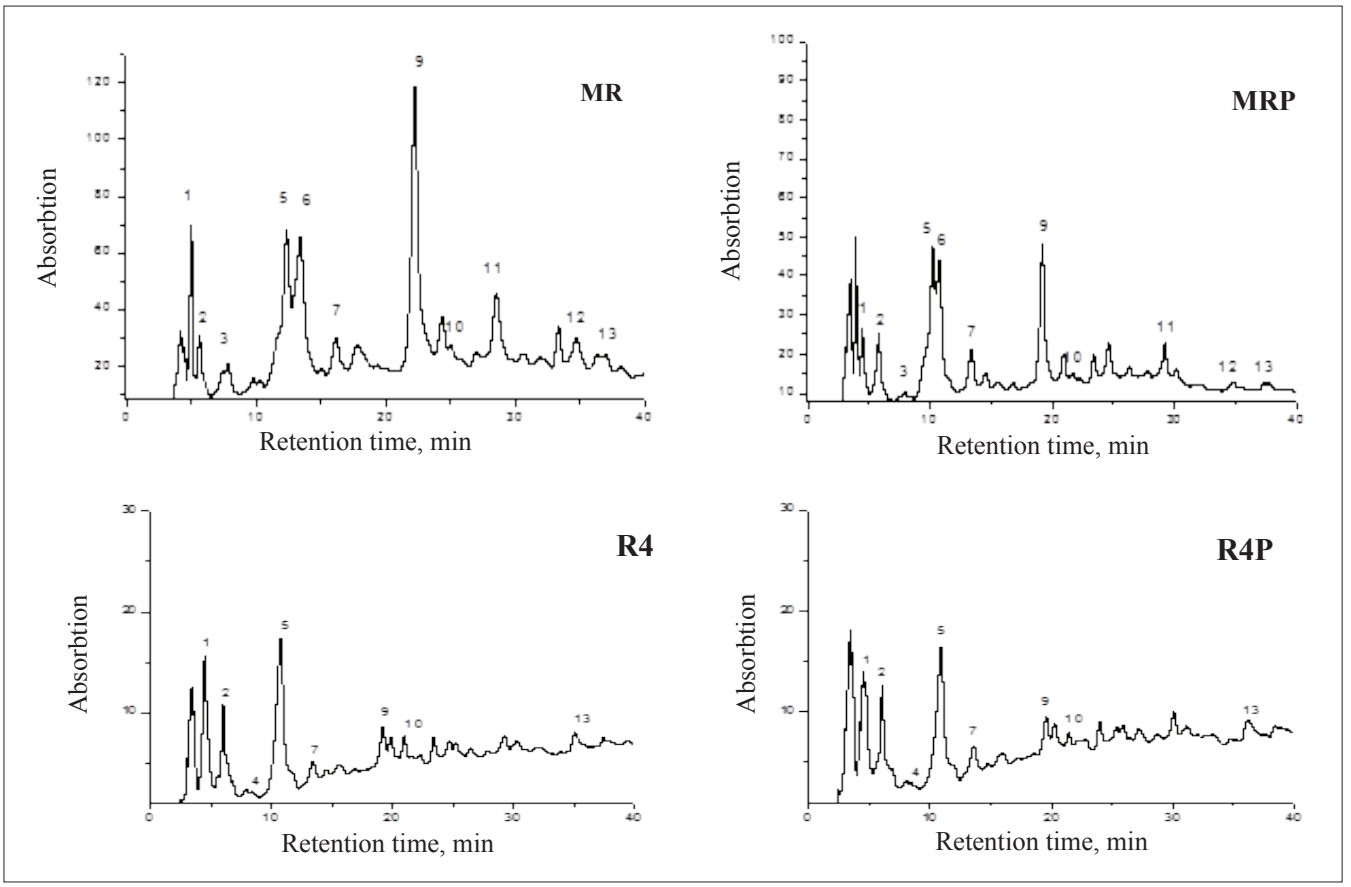


**Fig. 5. HPLC chromatograms for control and inoculated R4 and MR. 1: gallic acid, 2: Protocatecuic acid, 3: catechin, 4: chlorogenic acid., 5: caffeic acid, 6: p-coumaric acid, 7: ferulic acid, 8: sinapic acid, 9: o-coumaric acid, 10: quercetin, 11: Kaempherol**

oculation. Inoculation with *P. infestans* induced a very important decrease of biosynthesis of different phenolic compounds: chlorogenic and coffeic acid and Nib with a P<0.01, and sinapic, o-coumaric acids, quercetin, kaempherol and Nic with a P<0.001 (Figure 6).

The order of phenolic compounds in MR genotype is for hundreds, while in the case of R4 and *S. demissum* is under the amount of 30 μg/ml.

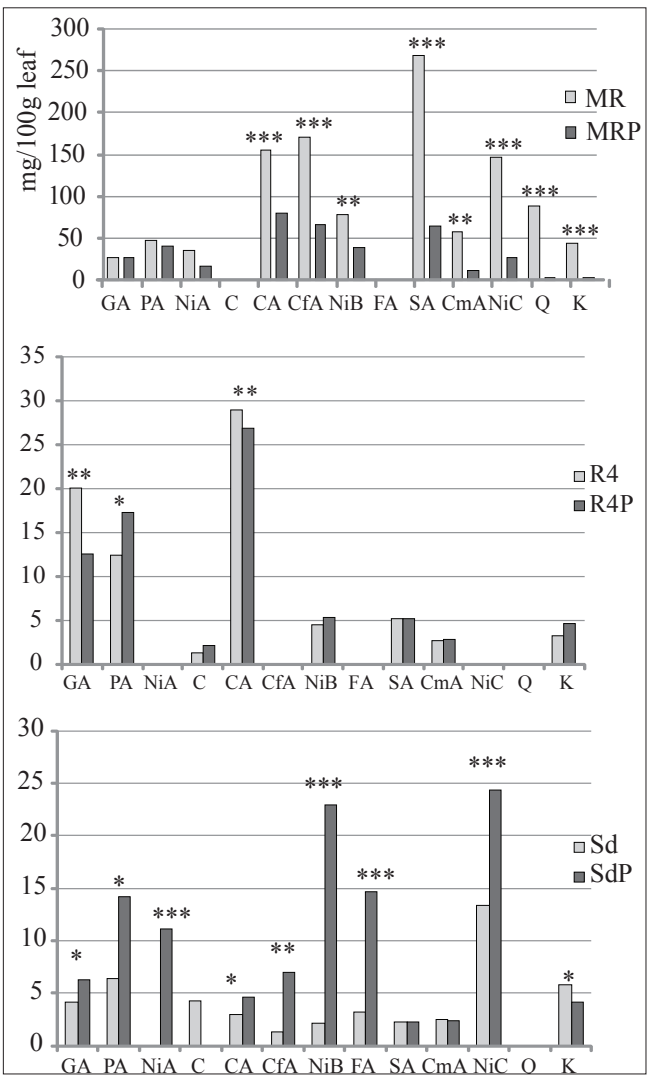


**Fig. 6. Content of potato genotypes test of different phenolic compounds. GA: gallic acid, PA: protocatecuic acid, C: catechin, CA: chlorogenic acid, CfA: caffeic acid, FA: ferulic acid, SA: sinapic acid, CMA: o-coumaric acid, Q: quercitin, K: kaempherol. *: P<0.05, **: P<0.01. ***: P<0.001**

Plants are generally resistant to the majority of pathogens. However, some pathogens developed with their evolution mechanisms for infection and colonization of plants. There are many types of resistance, going from tolerance to immunity. In this study, all potato genotypes were resistant to infection with *P. infestans*. MR genotype developed a HR response. The HR generally occurs as a rapid, localized necrosis, a form of programmed cell death (Kamoun et al., 1999). It prevents a further spread of the pathogen (Birch and Whisson, 2001). It is also possible that the HR occurred in the R4 and *S. demissum* interaction with *P. infestans* but at the microscopic level. It is (HR) considered as the last and the most effective mechanism of defense.

The differences of potato genotypes reaction to inoculation in the symptomatological level is sustained by difference at the molecular level. The total polyphenol content of the three tested genotypes differed after inoculation. Both genotypes of *S. tuberosum* shared a reduction of the polyphenol quantities detected after inoculation, while *S. demissum* we registered an augmentation. The reaction of potato at the phenolic level in accodrding with what Goodman et al. (1986) suggested, that phenolic compounds may have an important role in plant resistance to fungi when present in sufficient amounts either before or after infection. So we will consider only the phenolic compounds with a changed prior and/or post inoculation. Menden et al. (2007) found that in the pathosystem *Triticum aestivum-Puccinia graminis* f.ps. *tritici*, the resistant genotypes accumulated lesser phenolic compounds when inoculated than the control. Siranidou et al. (2002) detected a reduction of the amount of phenolic compounds with 25% in resistant wheat cultivar Frontana after inoculation with *Fusarium culmorum*. Dzhavakhiya et al. (2007) found that transgenic tobacco plants with a lower level of phenol compounds showed a much higher growth rate of *P. parasitica* compared with the control. In addition, the application of exogenous phenol increase resistance of Ulmus minor to Dutch elm disease caused by *Ophiostoma novo-ulmi* through formation of suberin-like compounds on xylem tissues (Martin et al., 2008). Andreu et al. (2001) found that beside phytoalexins and glycoalkaloids, phenolics are also involved in potato resistance to late blight.

The *S. demissum* response to inoculation was by the sunthesis of more phenolic compounds and a novo biosynthesis of an unknown Nib. The case of MR is totally the opposite. The inoculation induces generally, a diminution of the amount of phenolic compounds severely. The genotype R4 response was situated in the middle of the both MR and *S. demissum* reactions. A reduction of the amount of gallic and chlorogenic acid, but with an augmentation of the quantities of protocatecuic acid synthesized. In addition, R4 shares two detected phenolic compounds: chlorogenic acid with MR and quercitin with *S. demissum*. Beside ferulic acid, caffeicacid, and quercitin, three other compounds were not detected: caffeic acid, Nia, and Nic.

The implication of some phenolic acid in resistance is known especially; Ferulic and chlorogenic acid. Siranidou et al. (2002) found that ferulic acid reduced considerably the growth of *Fusarium graminearum*, and with *p*-coumaric acid, they have a synergistic activity in reducing the mycelial growth. Mikulic Petkovsek et al. (2003) found that *Venturia inaequalis* induces in apple when inoculated an increase on the level of chlorogenic acid. In the pathosystem *Cicer arietinum- Sclerotium rolfsii*, three major phenolic acids were identified after infection: gallic, vanillic and ferulic acids. Their quantities increased significantly after infection (Sarma and Singh, 2003). Siranidou et al. (2002) showed that the amount of *p*-coumaric acid increased significantly after inoculation of wheat by *Fusarium culmorum*. Gallic acid has the capability to render *Aspergillus flavus* and *A. parasiticus* incapable of making aflatoxin when occurring in sufficient concentrations (Wood, 2005). Gallic acid has a wide range of biological activities, including anti-oxidant, anti-inflammatory, anti-microbial, and anti-cancer activities (Kim et al., 2006 and references in). Bollina et al. (2011) identified p-coumaric acid and sinapic acid as resistance related (RR) metabolites in the pathosystem *Fusarium graminearum*-barley.

The role of phenolic compounds in resistance is not specific to infection with fungi. Thrips-resistant chrysanthemums contain higher amounts of the phenylpropanoids chlorogenic acid and feruloyl quinic acid (Leiss et al., 2009). The infection by phytoplasma of *Catharanthus roseus* leaves causes an increase of metabolites related to the biosynthetic pathways of phenylpropanoids or terpenoid indole alkaloids: chlorogenic acid, loganic acid, secologanin, and vindoline. Furthermore, higher abundance of polyphenols, and succinic acid, were detected in the phytoplasma-infected leaves (Choi et al., 2004).

We think that phenolic compounds play an important role in the both species of potato. However, the mechanism differs. In MR the preexistent phenolic compound insure the protection against the pathogen, while in the case of *S. demissum*, the protection is insured by the induction of supplementary quantities of phenolic compounds. The particular situation of MR is that carries three *R* genes in the same time, this can explain its reaction to inoculation which differs from the reaction of the other *S. tuberosum* R4. R4 genotype, did not implicate many phenolic compounds in the resistance response.

## Conclusion

Reaction of resistant potato genotypes differs according to the genotypes and the genes they carry. When in *S. demissum*, the wild relative of the cultivated potato, the inoculation with an avirulent isolate induces the synthesis of more quantities of different phenolics, the gene pyramided genotype MR with three *R* (R2, R3, R4) genes reacts differently: the inoculation induces a diminution of phenolics. For the simple genotype with a single *R* (R4) gene, the inoculation does not affect much the content of potato on phenolics, the fluctuation are in the majority of cases are not significant.

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