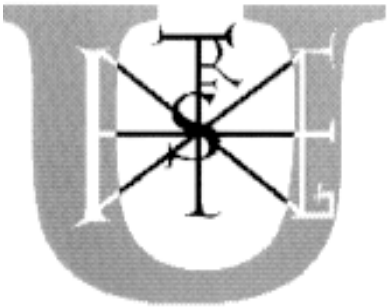

SZENT ISTVÁN UNIVERSITY

INDUCED RESISTANCE IN THE SUNFLOWER - *PLASMOPARA HALSTEDII* INTERACTION

PhD Theses

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PhD School

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1. INTRODUCTION AND OBJECTIVES

Downy mildew of sunflower caused by *Plasmopara halstedii* (Farl.) Berl. et de Toni is one of the most destructive diseases of this crop. It can be effectively controlled by using genetic resistant plants and seed treatment with fungicides. However, the traditional control strategies can be hindered by the genetic variability of the fungus. First, new pathotypes (races) appeared which can infect the resistant plants (Gulya, 2007) and another problem in the traditional control strategies arise with the appearance of tolerance to fungicides (Mouzeyar et al., 1994; Albourie et al., 1998; Gulya et al., 1999). Thus, besides the traditional control strategies, there was a need to look for alternative methods to improve the effectivity of disease control. One possible solution could be the use of induced resistance, specifically the systemic acquired resistance (SAR) that might be adapted to integrated pest management programs.

SAR may be triggered by abiotic, as well as biotic factors in plants (Sticher et al. 1997). As the former, there are chemical plant activators or resistance inducers with no direct antimicrobial activity (Kessmann et al. 1996). Instead, they activate the plant's own defense system against pathogen attack. In Hungary, the first commercial plant activator was Bion 50 WG, that were registered for use in wheat and barley against powdery mildew. Its active ingredient is benzothiadiazole (BTH: benzo (1, 2, 3) thiadiazole -7-carbothioic acid S- methyl ester), an SA analogue, so they have similar structure and mode of action (Ryals et al., 1996). In addition to BTH, two well-documented plant activators are isonicotinic acid (INA) and an aminobutyric acid enantiomer (BABA: DL-3- aminobutyric acid).

The induced resistance has been examined in several plant-pathogen interactions, however the background is still not well known. In order to use this chemically induced plant response in future practical disease management, one should investigate and characterize in more details this type of plant resistance to pathogens. For this, one of the important steps is to investigate enzyme activity changes possibly related to induced resistance in plants. For example, polyphenol oxidases (EC 1.10.3.1) are widespread copper containing proteins, which are found from bacteria to mammalian in nature. Their role in the plant defense system has been demonstrated by recent publications (Shi et al., 2002; Mayer, 2006; Lukácsy, 2006; Tegelberg at al., 2008, Nandeshkumar et al., 2008). Plant peroxidases are prevalent in plants, they catalyze several reactions (Siegel, 1993), and for example, their role was documented in stress reactions and host-parasite interactions (Low and Merida, 1996; Montalbini et al., 1995). Another enzyme catalase (EC 1.11.1.6.) is considered as one of the most important

one, which neutralizes hydrogen peroxide in plants. In case of sunflower, changes in catalase activity were observed by several abiotic stresses (Costa at al., 2002; Rios-Gonzalez et al. 2002; Azpilicueta et al., 2007). Glutathione-S-transferases (GST) (EC 2.5.1.18) are belonging to the antioxidant defense system of plants. Most of plant GST is induced by heavy metal stress, ethylene treatment, pathogen attack, wounding or ozone, so they are supposed to play a role in the defense reactions against oxidative stresses (Marrs, 1996). In addition, there are several defense mechanisms in plants against pathogens, one of these being the expression of antimicrobial peptides, like defenzin. Mauch-Mani and Métraux (1998) showed that SAR is associated with defenzin, and they found defenzin playing a role in the SAR signaling pathway.

With sunflower downy mildew, it was crucial to know what biochemical and molecular changes may occur in sunflower following infection and/or activator treatment and how far subsequent host alterations will explain the induced resistance state of such plants. Because of the high genetic variability of *P. halstedii* and of the complexity of this pathosystem, the genetically resistant cultivars may become sensitive to newly arising virulent phenotypes of the pathogen and, on the other hand, some differences in the level of genetic resistance may also exist between sunflower genotypes. In the literature, there are a few examples for such differences of resistance in plants, but no experimental data are available so far for the sunflower – downy mildew interaction. Therefore, aims of the present work were as follows:

- investigating the effect of aminobutyric acid and isonicotinic acid treatments on depressing *P. halstedii* infection in a comparison to that of benzothiadiazole including susceptible, partially resistant and completely resistant sunflower - *P. halstedii* interactions;
- comparing the plant responses to either chemical inducer or pathogen attack in relation to genetic resistance or susceptibility and characterizing the resistance expressions by
    - microscopic observations,,
    - biochemical and molecular genetic analyses of the activity of resistance related enzymes and genes;
- testing the *in vitro* effect of chemical inductors on sporangial germination.

2. MATERIALS AND METHODS

Experimental conditions

The USDA sunflower inbred lines RHA-274, RHA-340 and HA-335, as well as the pathotype 700 of *P. halstedii*, were used throughout the experiments. RHA-274 is susceptible to *P. halstedii* pathotype 700, the other two lines are resistant, but with different degree. While RHA-340 possesses hypocotyl-limited (HLI) partial resistance, namely the pathogen infects only the root and hypocotyl tissues of the plants; HA-335 is harboring complete resistance to this sunflower pathotype (Virányi and Gulya 1996).

Pre-germinated seeds were soaked in an aqueous solution of each chemical, and one day later inoculated with a sporangial suspension of the pathogen containing 50 000 sporangia /ml. The following treatments were used:

- negative control: non-treated and non-inoculated seedlings;
- chemical control, treated with one of the resistance inducers but non-inoculated;
- infected control, non-treated but inoculated with *P. halstedii*;
- treated with one of the resistance inducers and inoculated.

Disease assessment

Eight to 10 days after planting, plants were sprayed with distilled water and covered with plastic bags for inducing fungal sporulation and disease assessment was made by using a 0–4 scale described by Oros and Virányi (1987). When the plants were two-week old the number of plants showing either damping-off or chlorotic leaf symptoms were recorded and the plant height was also measured.

Microscopic observation

Hypocotyls of plants from each treatment were taken at intervals of 3, 7, 10 and 15 days after planting for histological observations. Free-hand cross sections were cut from the hypocotyl segments and examined under an Olympus BX50 fluorescence microscope to check the presence of the pathogen and its developmental structures (hyphae, haustoria), as well as the response of host tissues in relation to treatment/inoculation (Bán et al., 2004).

In vitro germination test of P. halstedii sporangia

Different concentration of activator solutions were mixed in a 1:1 ratio with the pathogen sporangial suspension. They were incubated at 16 ºC in the dark for 6 and 24 hours prior to microscopic evaluation. For this, the number of empty sporangia per treatment was recorded by counting 2 x 50 sporangia from each.

Enzyme analysis

For determining the activity changes of polyphenol oxidase (PPO) and peroxidase (POX) enzymes, plant hypocotyls were taken at 0, 3, 9, 13 and 17 dpi and homogenized to get test solutions. Measurements were made at 25 ºC, using a SmartSpec Plus Spectrophotometer (BioRad). Guaiacol-dependent POX activity was determined as described by Rathmell and Sequeira (1974), whereas PPO activity was determined by measuring the initial rate of quinone formation, as indicated by an increase in absorbance at 400 nm, using a modification of the procedure described by Fehrmann and Dimond (1967).

Gene transcript accumulation analysis

For the molecular genetic studies, sunflower plants were taken at 0, 3, 9, 13 and 17 dpi and the total RNAs were extracted using RNeasy Plant Mini Kit, followed the manufacturer's (Qiagen) instructions. The extracted RNA's concentration was adjusted to 1 µg µl^{1} and one µg of RNA was reverse transcribed using a cDNA synthesis kit (iScript cDNA Synthesis Kit, BioRad). We used the semi-quantitive PCR approach to detect the transcript accumulation of glutathione S- transferase (*Ha*-GST), sunflower defensin (*Ha*-PDF), catalase (*Ha*-CAT2), as well as of the elongation factor of *P. halstedii* (*Ph*-TEF1). The cDNA synthesis was stopped in the exponential phase, so each gene had its own number of cycles in the PCR, where the differences remained between the certain synthesis levels. Specific sunflower primers for PCR amplifications were designed according to Radwan et al. (2005) and Azpilicueta et al. (2007). The amplification program included an initial step at 94°C for 3 min and specific numbers of cycles of 15 s at 94 °C, 15 s at Tm °C, 20 s at 72°C, and then one final elongation step, 5 min at 72°C. The PCR products were then separated by electrophoresis in a 1% agarose gel, visualized with ethidium bromide and images captured in a molecular imager gel doc system (BioRad). The signals from gels were quantified using a Quantity One program with molecular mass ruler, and normalized over the signals from *Ha*-EF1α (Radwan et al., 2005). This numerical data appeared as relative transcript accumulation in our results (for example: *Ha*-PDF data/*Ha*-EF1 α data = *Ha*-PDF relative transcript accumulation).

Data analysis

All the experiments were made at least in two biological repeats and each experiment contained three replicates. Experimental data were subjected to anova ($p = 0.05$) using the MINITAB statistical package version 10.2.

3. RESULTS

Changes in disease symptom appearance

The incidence and intensity of *P. halstedii* sporulation decreased significantly on the susceptible plant cotyledons following activator treatments as compared to the untreated plants. BTH and both applied concentrations of INA (100 and 200 mg/L) gave better results than did BABA, although the latter decreased the sporulation intensity as well. In addition, activator treatments also resulted in a decrease of plants showing leaf chlorosis or damping-off symptoms. Activator treatments stimulated plant growth of the susceptible, inoculated plants and there were no differences found among the three chemicals in this respect. In case of partially resistant plants, INA treatment resulted in significantly higher plant height of the non-inoculated plants as compared to inoculated ones. No changes in the activator treated totally resistant plants were obtained.

In field experiments, where only the compatible relationship was examined, the activator treated and inoculated sunflowers became significantly higher and developed greater head than the untreated and inoculated ones.

Microscopic changes

Microscopical observations of treated susceptible hypocotyls showed a significant inhibition of colonization by the pathogen from 7 dpi onwards and, at the same time tissue necrosis expanded. As with partially resistant plants, treatments also reduced the pathogen invasion, but at the same time tissue necrosis also decreased. In the totally resistant plants we could not detect any pathogen element or tissue change.

Sporangial germination

Each activator used in this study inhibited sporangial germination *in vitro*. Inhibition by BTH was evident at all concentrations, especially with 160 mg/L, INA was effective at 100

and 200 mg/L, whereas BABA decreased the number of sporangia germinated at higher concentrations, 500, 1000 and 2000 mg/L.

BTH triggered enzyme activity changes

BTH treatment increased PPO and POX activity in both the non-inoculated and inoculated susceptible plants. All cases the treated and inoculated plants showed the highest enzyme activity. In partially resistant plants the treatment alone increased, while treatment and inoculation together decreased the activity of these enzymes, though it was not always significant. However, similarly to the susceptible interaction, the treatment and inoculation increased the enzyme activity in the totally resistant sunflowers. A comparison of the compatible and incompatible combinations showed that in partially resistant untreated but inoculated plants enzyme activity was higher as compared to the inoculated untreated ones. We also detected differences among the sunflower genotypes, when comparing POX enzyme activity of inoculated plants in relation to treatment. In case of susceptibility BTH enhanced the inductive effect of inoculation, while with partially resistance plants the effect was contrary. Already in the first sampling day (0 dpi) differences in activity were evident between inoculated and non-inoculated plants and such differences increased with time. Furthermore, in a comparison of the two differently resistant genotypes, following inoculation the enzyme activity was found higher in the partially resistant than in the totally resistant plants.

Effect of inductors on gene expression

When examining the resistance related genes, GST, CAT and PDF, we found that transcript accumulation was triggered resistance inductor treatment and/or inoculation. In case of GST, in the susceptible non-inoculated plants the activator treatments slightly enhanced transcript accumulation, while inoculation alone significantly increased it. Both, inductor treatment and inoculation further increased the accumulation of GST transcripts in those plans. In the partially resistant sunflowers the activator treatment did not cause any significant change, however, similarly to the susceptible genotype, inoculation significantly increased the gene expression and it was earlier than in the susceptible plants. In the totally resistant plants, activator treatments had no considerable effect on GST transcript accumulation whether they were inoculated or not. In a comparison of the three sunflower genotypes we found that GST transcript accumulation was the highest in the partially resistant plants and, transcript

accumulation immediately increased after inoculation in the two resistant genotypes, while in the susceptible plants this increment appeared much later.

In case of PDF, in the compatible interaction we found transcript accumulation from 9 dpi onward in the non-treated inoculated plants, and this level of accumulation was measured throughout the experiment. In contrast, in case of treated and inoculated plants we observed transcript accumulation as early as at 3 dpi, and this accumulation increased continuously up to at 13 dpi. Though all three activators caused significant increment, BTH treated plants reached their maximum level at first; INA treatment gave the maximum value of transcript accumulation. In the resistant interactions no significant changes in PDF transcript accumulation occurred after activator treatment and, similarly to GST transcript accumulation, PDF gene expression activated at 3 dpi in these resistant genotypes, while in case of susceptible plants its expression was detected later and it reached lower level. On the other hand, we found differences in gene expressions between the two resistant genotypes both in intensity and temporal change. While in the totally resistant plants we found higher gene activity at 3 dpi as compared to the partially resistant plants, in the latter case activity increased from 13 dpi to 17 dpi but not in the totally resistant plants.

As for CAT gene expression, activator treatment alone had no effect on the gene expression in the susceptible plants but inoculation increased CAT gene activity. The highest activity was measured in the treated and inoculated susceptible sunflowers. There was no difference found between the effects of the three activators. In case of partially resistant plants, similarly to susceptible sunflowers, we could not detect any change following treatment. However, inoculation alone did increase gene activity in this genotype and this effect was evident as early as from 0 dpi. Furthermore, inoculation caused higher CAT transcript accumulation in the partially resistant plants than did in the susceptible ones. Similarly to the susceptible and partially resistant sunflowers, totally resistant plants had no change the gene expression following treatment but inoculation increased gene activity and the highest values were measured following treatment and inoculation. In a comparison of the three genotypes we found that inoculation caused faster and higher transcript accumulation in the resistant genotypes as compared to the susceptible plants, and that partially resistant plants showed the highest values of this gene activity.

To prove the presence and amount of *P. halstedii* structures (biomass) within inoculated plant tissues, beside microscopic observations, we tested the appearance of. *P. halstedii*-specific (*Ph*-TEF1) gene products in the susceptible and partially resistant plants. In fact, higher transcript accumulation occurred in the susceptible untreated samples as

compared to the treated ones, and less *Ph*-TEF1 gene products were found in the partially resistant, than in the susceptible sunflowers. While we could not measure transcript accumulation in the first two sampling days in partially resistant sunflowers, a continuous increase was evident in the untreated samples up to at 13 dpi. The effect of treatment was evident in this genotype as well, since we found fewer transcripts in the treated plants than in the untreated ones.

4. NEW SCIENTIFIC FINDINGS

1. We have found that under greenhouse conditions, similarly to BTH, INA and BABA treatments also decreased in susceptible sunflowers the appearance of disease symptoms by *P. halstedii* (dwarfing, sporulation, leaf chlorosis), as well as they reduced the development of pathogen structures in the plant tissues. This latter result was also confirmed using molecular genetic techniques.
2. INA and BABA under field conditions were found to effectively enhance plant growth by counteracting the pathogen induced dwarfing and, at the same time, they were able to increase sunflower head diameter.
3. In contrast to literature data, we found a dose-dependent inhibitory effect of the three activators on the germination of *P. halstedii* sporangia *in vitro*.
4. We ascertained that activator treatments increased the defense related PPO and POX enzymes activity in both the susceptible and totally resistant sunflowers, whereas partially resistant plants responded differently. Furthermore, in genetically resistant sunflowers inoculation resulted in faster and higher activities of PPO and POX, than did in the susceptible ones.
5. We found that resistance inductors alone did not cause considerable changes in the defense related gene (GST, PDF, CAT) expression, however inoculation increased the transcript accumulation of these genes. In resistant plants transcripts accumulated significantly earlier than in susceptible ones.
6. We confirmed for the first time that the appearance and activity of induced resistance associated protein-like gene products appearance and activity are related to the intensity of tissue necrosis rather than to the degree of genetic resistance against this biotrophic pathogen.

5. DISCUSSION AND CONCLUSIONS

Our experiments demonstrated that beside BTH, two other activators, INA and BABA also decreased the symptoms of downy mildew (sporulation, damping off, leaf chlorosis, stunting) in susceptible sunflowers. It is worth mentioning that in case of BABA only the highest concentration (2000 mg/L) gave good result and this concentration was effective against grape downy mildew in a study by Cohen et al. (1999).

The activator treatments, that were effective under greenhouse conditions, gave similarly good results in the field by counteracting the downy mildew-associated stunting and by enhancing sunflower capitulum size. These results are in good accordance with those of Cole (1999) and Bubici et al. (2006) who successfully applied BTH against different pathogens.

Our microscopic observations in the activator treated susceptible plants showed that fewer pathogen structures were present and tissue necrosis occurred at and near infection sites. These findings highly correlated with the macroscopically found withdrawal of disease symptoms in treated and infected plants. It seems that these changes in the susceptible plants closely resemble those defense responses which are known to occur in sunflower plants carrying *P. halstedii* resistance genes (Mouzeyar et al., 1993). In case of partially resistant interaction, the BTH and INA treatment reduced the tissue necrosis in the inoculated plants. The question arises, whether activator treatment was against the manifestation of resistance in this partially resistant genotype. Since of this type worked well in these plants by reducing the pathogen development, it is assumed that in this relationship the resistance may not be closely associated with host necrosis, but with an other hitherto unknown mechanism.

In our *in vitro* germination test the activators slightly inhibited the zoospore release from *P. halstedii* sporangia. These results partly correlate well with other findings (Lopez et al., 2002; Bengtsson et al., 2006; Meyer et al., 2006); partly contradict them (Cohen et al., 1999 Kessmann et al., 1996). However, it is important to note, that these chemical inductors will not come into direct contact with *P. halstedii* sporangia in the practice.

Similarly to our results, several workers found that BTH treatment enhanced enzyme activities in sunflower. Thus, Nandeshkumar and coworkers (2008) showed that chitosan and inoculation improved the activity of peroxidase and polyphenol oxidase, while Serráno and coworkers (2007) measured increased activity of chitinase and peroxidase after BTH treatment in sunflower hypocotyls. They also examined the sunflower downy mildew pathosystem but used fewer host – parasite combinations and they included one sampling

only. We did not find any information in the literature about the partially resistance of sunflower related to induced resistance, so further examinations of this phenomenon are crucial by involving more partially resistant and possibly non-race resistant genotypes. It would be important to find the key factors, which are responsible for the relationship between induced and genetic resistance either supporting or counteract each other.

We considered as one of the important result that both enzymes activity was found more rapid and intensive in the resistant plants than in the susceptible ones. This result contradicts the findings of Harrach and coworkers (2008) working on barley powdery mildew but interestingly, Sedlarova and coworkers (2007) were unable to determine whether susceptible or resistant lettuce plants are capable of higher enzyme activity following inoculation with the downy mildew pathogen, *Bremia lactucae*.

Our experiments clearly showed that the chemically induced increase of activity of a few genes studied here are associated with the effective mobilization of sunflower defense system. In fact, treated sunflowers became, to some extent, resistant against *P. halstedii* infection and this condition manifested itself in the withdrawal of disease symptoms and a considerable reduction of pathogen development. At the moment it is not yet clear which factor or factors are in the background of these favorable host responses. Further efforts are required to see, for example, how chemical inductors act or counteract with the different Pl genes in sunflower upon pathogen attack, or what kind of resistance mechanism at all exists in the partially resistant interaction that obviously differs from the immune-like, total resistance. Finally, an additional question to be answer relates to the prospect of practical use of these resistance inductors in sunflower production. In any case, our field experiments are promising but should be continued.

6. REFERENCES

ALBOURIE, J. M., TOURVIEILLE, J., LABROUHE, D.T. (1998): Resistance to metalaxyl in isolates of the sunflower pathogen *Plasmopara halstedii*. European Journal of Plant Pathology 104: 235-242.

AZPILICUETA E. C., BENAVIDES P. M., TOMARO L. M., GALLEGO S. M. (2007): Mechanism of CATA3 induction by cadmium in sunflower leaves. Plant Physiology and Biochemistry 45: 589-595.

BÁN R., VIRÁNYI F., KÖRÖSI K., NAGY S. (2004): Indukált rezisztencia a napraforgó-peronoszpórával szemben. Növényvédelem, 40: 545–550.

BENGTSSON M., JORGENSEN H.J.L., PHAM A., WULFF E., HOCKENHULL J. (2006): Screening of organically based fungicides for apple scab (*Venturia inaequalis*) control and a histopathological study of the mode of action of a resistance inducer. Pome Fruit Diseases IOBC/wprs Bull. 29: 123 – 127.

BUBICI G., AMENDUNI M., COLELLA C., D'AMICO M., CIRULLI M. (2006): Efficacy of acibenzolar-S-methyl and two strobilurins, azoxystrobin and trifloxystrobin, for the control of corky root of tomato and verticillium wilt of eggplant. Crop Protection, 25: 814–820.

COHEN Y., REUVENI M., BAIDER A. (1999): Local and systemic activity of BABA (DL-3-aminobutyric acid) against *Plasmopara viticola* in grapevines. European Journal of Plant Pathology, 105: 351–361.

COLE D.L. (1999): The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance, against bacterial and fungal diseases of tobacco. Crop Protection, 18: 267-273.

COSTA H., GALLEGO S.M., TOMARO M. (2002): Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons. Plant Science, 162: 939-945.

FEHRMANN H., DIMOND A.E. (1967): Peroxidase activity and phytophthora resistance in different organs of the potato plant. Phytopathology, 57: 69-72.

GULYA T. (2007). Distribution of *Plasmopara halstedii* races from sunflower around the world. In: Advances in Downy Mildew Research, eds A Lebeda & P T N Spencer-Phillips, Vol. 3., pp. 121-134.

GULYA T., DRAPER M., HARBOUR J., HOLEN C., KNODEL J., LAMELY A., MANSON P. (1999): Metalaxyl resistance in sunflower downy mildew in North America. Proceedings of the 21st Sunflower Research Workshop, January 14-15, pp. 118-123.

HARRACH B., FODOR J., POGÁNY M., PREUSS J., BARNA B. (2008): Antioxidant, ethylene and membrane leakage responses to powdery mildew infection of near-isogenic barley lines with various types of resistance. European Journal of Plant Pathology, 121:21–33.

KESSMANN H., OOSTENDORP M., STAUB T., GOERLACH J., FRIEDRICH L., LAWTON K., RYALS J. (1996): CGA 245704, mode of action of a new plant activator. Brighton Crop Protection Conference- Pest and Diseases, pp. 961-966.

LOPEZ A.M.Q, LUCAS J.A. (2002): Effects of plant defence activators on anthracnose disease of cashew. European Journal of Plant Pathology, 108: 409–420.

LOW P.S., MERIDA J.R. (1996): The oxidative burst in plant defense: Function and signal transduction. Physiologia Plantarum, 96:532-542.

LUKÁCSY GY. (2006): A fürtritkítás idejének és mértékének hatása a „furmint" és „hárslevelű" fajták vegetatív és generatív teljesítményére Tokaj-Hegyalján. Doktori értekezés. Budapesti Corvinus Egyetem.

MARRS K. A. (1996): The functions and regulation of glutathione S-transferases in plants. Annual Review of Plant Physiology and Plant Molecular Biology, 47: 127–158.

MAUCH-MANI B., MÉTRAUX J.-P. (1998): Salicylic acid and systemic acquired resistance to pathogen attack. Annals of Botany, 82: 535-540.

MAYER A. (2006): Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry, 67: 2318-2331.

MOUZEYAR, S., LABROUHE, D.T., VEAR, F. (1993): Histopathological studies of resistance of sunflower (*Helianthus annuus* L.) to downy mildew (*Plasmopara halstedii*). Journal of Phytopathology, 139: 289-297.

MOUZEYAR, S., LABROUHE, D.T., VEAR, F. (1994): Effect of host-race combination on resistance of sunflower, *Helianthus annuus* L., to downy mildew *Plasmopara halstedii*. Journal of Phytopathology, 141: 249-258.

MONTALBINI P., BUONAURIO R., UMESH-KUMAR N.N. (1995): Peroxidase activity and isoperoxidase pattern in tobacco leaves infected with tobacco necrosis virus and other viruses inducing necrotic and non necrotic alterations. Journal of Phytopathology, 143:295-301.

NANDESHKUMAR P., SUDISHA J., RAMACHANDRA K.K., PRAKASH H.S., NIRANJANA S.R., SHEKAR S.H. (2008): Chitosan induced resistance to downy mildew in sunflower caused by *Plasmopara halstedii*. Physiological and Molecular Plant Pathology, 72: 188-194.

OROS G., VIRÁNYI F. (1987): Glasshouse evaluation of fungicides for the control of sunflower downy mildew *(Plasmopara halstedii*). Annals os Applied Biology, 110: 53–63.

RADWAN O., MOUZEYAR S., VENISSE J S., NICOLAS P., BOUZIDI M. F. (2005): Resistance of sunflower to the biotrophic oomycete *Plasmopara halstedii* is associated with a delayed hypersensitive response within the hypocotyls. Journal of Experimental Botany, 56: 1683-2693.

RATHMELL W. G., SEQUEIRA L. (1974): Soluble peroxidase in fluid from the intercellular spaces of tobacco leaves. Plant Physiology, 53: 317-318.

RIOS-GONZALEZ K., ERDEI L., LIPS H. (2002): The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources. Plant Science, 162: 923-930.

RYALS J.A., NEUENSCHWANDER U.H., WILLITS M.G., MOLINA A., STEINER H-Y., HUNT M.D. (1996): Systemic acquired resistance. Plant Cell, 8: 1808-1819.

SEDLAROVA M., LUHOVA L., PETRIVALSKY M., LEBEDA A. (2007): Localisation and metabolism of reactive oxygen species during *Bremia lactucae* pathogenesis in *Lactuca sativa* and wild *Lactuca* spp. Plant Physiology and Biochemistry, 45: 607-616.

SERRÁNO A.R., DEL CASTILLO J.L., NOVO J.J., OCAÑA A.F., RODRÍGUEZ M.V.G. (2007): Chitinase and peroxidase activities in sunflower hypocotyls: effects of BTH and inoculation with *Plasmopara halstedii*. Biologia Plantarum, 51: 149-152.

STICHER L.B., MAUCH-MANI B., MÉTRAUX J.P. (1997): Systemic acquired resistance. Annual review of Phytopathology, 35: 235-270.

SHI C., DAI Y., XU X., XIE Y., LIU Q. (2002): The purification of polyphenol oxidase from tobacco. Protein Expression and Purification, 24: 51-55.

SIEGEL, B.Z. (1993): Plant peroxidases-an organismic perspective. Plant Growth Regulation, 12: 303-312.

TEGELBERG R., JULKUNEN-TIITTO R., VARTIAINEN M., PAUNONEN R., ROUSI M., KELLOMAKI S. (2008): Exposures to elevated CO_2, elevated temperature and enhanced UV-B radiation modify activities of polyphenol oxidase and guaiacol peroxidase and concentrations of chlorophylls, polyamines and soluble proteins in the leaves of *Betula pendula* seedlings. Environmental and Experimental Botany, 62: 308-315.

VIRÁNYI F., GULYA T. (1996): Expression of resistance in the *Plasmopara halstedii*-sunflower pathosystem. ISA Symposium I. Disease Tolerance in Sunflower, Bejing, 13 June 1996, pp. 14–21.

7. PUBLICATIONS IN RELATION TO THIS STUDY

Scientific articles in English

K. Körösi, R. Bán, B. Barna, F. Virányi (2011): Biochemical and molecular changes in downy mildew-infected sunflower triggered by resistance inducers. Journal of Phytopathology (in press)

K. Körösi, N. Lázár, F.Virányi (2009): Resistance to downy mildew in sunflower induced by chemical activators. Acta Phytopathologica et Entomologica Hungarica 44, 1-9.

Scientific articles in Hungarian

Körösi K., Virányi F., Bán R. (2007): Növényi aktivátorok hatása a napraforgó peronoszpórás betegségére. Növényvédelem 43, 597-602.

Bán R., Virányi F., **Körösi K.**, Nagy S. (2004): Indukált rezisztencia a napraforgó-peronoszpórával szemben. Növényvédelem 40, 545-550.

Conference Proceedings and Abstracts in English

K. Körösi, F. Virányi (2010): Antioxidant activity changes in downy mildew-infected sunflower triggered by BTH. Proceedings of the ISA-VNIIMK International Symposium "Sunflower Breeding on Resistance to Diseases" 23-24. June, 2010, Krasnodar, Russia, p. 167.

K. Körösi, F. Virányi (2009): Induction of defense-related genes in downy-mildew infected sunflower plants treated with resistance inducer. In: F. Feldmann, D.V. Alford, C. Furk (Eds.) Crop Plant Resistance to Biotic and Abiotic Factors: Current Potential and Future Demands, Proceedings of the 3rd International Symposium on Plant Protection and Plant Health in Europe, Berlin, pp. 465-472. ISBN 978-941261-05-1

K. Körösi, F. Virányi (2008): Molecular changes in downy mildew-infected sunflower triggered by resistance inducers. In: L. Velasco (Ed.) Proceedings of the 17th International Sunflower Conference, 8-12. June, 2008, Cordoba, Spain, Vol.1, 157-161.

K. Körösi, N. Lázár, F. Virányi (2007): Resistance response to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*) activated by chemical inducers. In: A.Lebeda, P.T.N. Spencer-Phillips (Eds.) Advances in Downy Mildew Research, Vol. 3, 237-241. ISBN 80-86636-19-4

Bán, R., Virányi, F., Nagy, S., **Körösi, K.** (2004): Investigations of the induced resistance to *Plasmopara halstedii*. In: G. J. Seiler (Ed.) Proceedings of the 16th International Sunflower Conference, 29 August – 2 September, 2004, Fargo, USA, Vol. 1, 89-91.

Conference Abstracts in Hungarian

Körösi K., Virányi F. (2009): Védekezésben szerepet játszó gének indukált rezisztenciával kapcsolatos aktiválódása peronoszpórával fertőzött napraforgó növényekben 55. Növényvédelmi Tudományos Napok, Budapest, p. 27.

Lázár N., **Körösi K**., Virányi F. (2008): Növényi aktivátorok hatása a napraforgó peronoszpórás betegségére. XVIII. Keszthelyi Növényvédelmi Fórum, pp. 92-95.

Kegye Zs., **Körösi K**., Virányi F. (2008): A napraforgó- peronoszpórával szemben kiváltott indukált rezisztencia hatására végbemenő változások napraforgóban 54. Növényvédelmi Tudományos Napok, Budapest, p. 24.

Körösi K., Virányi F., Bán R. (2007): Három immunaktivátor hatásának vizsgálata a napraforgó peronoszpórás betegségére. 53. Növényvédelmi Tudományos Napok, Budapest, p. 43.

Körösi K., Bán R., Virányi F. (2005): Újabb adatok a napraforgó-peronoszpórával szemben indukált rezisztencia vizsgálatához. 51. Növényvédelmi Tudományos Napok, Budapest, p. 26.

Bán R., Virányi F., Nagy S., **Körösi K**. (2004): A napraforgó-peronoszpórával szembeni indukált rezisztencia vizsgálata. 50. Növényvédelmi Tudományos Napok, Budapest, p. 74