Acta Phytopathologica et Entomologica Hungarica 59 (2024) 1, 17–28
DOI: 10.1556/038.2024.00208

AKADÉMIAI KIADÓ

Changes in RNase activities and in expression of two RNase genes in powdery mildew infected barley, wheat and *Brachypodium distachyon* leaves

Csilla Juhász, Gábor Gullner and Balázs Barna*

Plant Protection Institute, HUN-REN Centre for Agricultural Research, Herman Ottó Str. 15, H-1022 Budapest, Hungary

RESEARCH ARTICLE

Received: April 11, 2024 • Accepted: June 8, 2024
Published online: July 1, 2024

ABSTRACT

Changes in RNase activities were investigated in extracts from barley near isogenic lines without or with various powdery mildew resistance genes and were compared to changes in wheat and *Brachypodium distachyon* leaves after powdery mildew infections. In barley, the compatible interaction with powdery mildew induced the highest increase in RNase activity as measured spectrophotometrically. The incompatible interaction that accompanied with hypersensitive reaction in Mla leaves gave less increase, whereas incompatible interactions in Mlg and mlo barley leaves without visible symptoms gave the least increase of RNase activity. In wheat, the largest RNase activity was found in leaves infected with the compatible wheat powdery mildew or wheat stem and leaf rusts. RNase activity in *B. distachyon* was higher than that in healthy wheat and especially barley leaves. The electrophoretic RNase enzyme activity patterns were different in barley, wheat and *B. distachyon* plants, but showed similar activities as determined spectrophotometrically. Barley genes encoding endonuclease 2 and ribonuclease 3-like protein X3 showed the highest expression in the compatible barley - barley powdery mildew interaction as measured by RT-qPCR. This correlated with RNase activities in leaf extracts suggesting that RNases in barley and wheat may act as susceptibility factors of powdery mildew and rust diseases.

KEYWORDS

barley Mla, Mlg and mlo near-isogenic lines, RNase enzymes, ion leakage, RT-qPCR

* Corresponding author. E-mail: barna.balazs@atk.hun-ren.hu

INTRODUCTION

Powdery mildews and rusts are among the most devasting plant pathogens all over the world. It is especially true for cereal powdery mildews (Dracatos et al., 2018). Resistance breeding is an effective and environmentally safe, although time consuming method to control these pathogens. However to improve resistance breeding we have to understand the mechanisms of plant susceptibility and resistance against these pathogens in detail.

Non-host type of resistance is the resistance of plants against so-called non-adapted pathogens, and it is considered the most durable and efficient type of resistance in plants. Therefore non-host plant-pathogen interactions are increasingly in the focus of research (Heath, 2000; Cheng et al., 2014; Huang et al., 2016; Lee et al., 2017). Recently we found that pre-inoculation of various wheat genotypes with barley powdery mildew induced resistance against a challenge infection with wheat leaf rust (Barna et al., 2022). Hence we also included non-host resistance in our study.

Plant ribonucleases (RNases) participate in many physiological processes like pre- mRNA splicing, RNA modification (Wilson, 1975; Hillwig et al., 2011; MacIntosh and Castandet, 2020), gene silencing, leaf senescence (Blank and McKeon, 1989) and abscission, nutrient remobilization, self-incompatibility (Green, 1994; Sugiyama et al., 2000). On the other hand, RNases are not only typical stress enzymes reacting to many abiotic stresses (Kim et al., 2008) like ozone (Booker, 2004), wounding (Kariu et al., 1998; LeBrasseur et al., 2002) physical injury (Kariu et al., 1998), salinity, water stress (Gomes-Filho et al., 2008), chilling stress (Kazmierczak and Knypl, 1994), but they have been proposed to participate also in plant-pathogen interactions (Barna et al., 1989; Galiana et al., 1997; Hugot, 2002; Sugavara et al., 2016; Graeme et al., 2018; Gobert et al., 2021). In addition, recently it was published that an antimicrobial and phytotoxic ribonuclease is secreted by the fungal wheat pathogen Zymoseptoria tritici (Kettles et al., 2018).

In our earlier work we found that rust infection of wheat leaves induced RNase activity, especially in the case of compatible interactions (Barna et al., 1989). Although non-host resistance has been investigated intensively for several decades, there are still many open questions regarding the underlying mechanisms (Heath, 2000; Cheng et al., 2014; Lee et al., 2017; Barna et al., 2022). Therefore, the aim of this work was to investigate RNase activities in barley near isogenic lines without or with various powdery mildew resistance genes and in wheat and *Brachypodium distachyon* leaves after during host and non-host interactions with powdery mildew. Furthermore, we analysed the expression of two RNase encoding genes in susceptible and resistant near-isogenic barley lines after powdery mildew infection.

MATERIAL AND METHODS

Plants and pathogens

The barley cultivar Pallas and its near-isogenic lines with Mla, Mlg and mlo resistance genes, the cultivar Ingrid and its Mla near isogenic line, the wheat cultivar Winzi, as well as the Brachypodium distachion inbred lines 21 and 21-3 were used in the experiments. The plants were grown in greenhouse pots containing 0.5 l potting soil and incubated in a growth chamber at

20 °C with 60 % relative humidity, and a 16 h light/8 h dark photoperiod. Barley powdery mildew was maintained on barley cultivar Ingrid under the same conditions.

For inoculation, barley powdery mildew fungus *Blumeria hordei* (*Bh*) race A6 (Wiberg, 1974), wheat stem rust (*Puccinia. graminis* f. sp. *tritici*) pathotype Pgt-MCC, wheat leaf rust (*Puccinia triticina*) race 12, and a Hungarian isolate of wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) were used. Leaf rust races were isolated, identified and maintained according to Manninger (2000).

Urediniospore suspension (25 mg in 100 ml of 1% w/v starch solution) was applied on the adaxial side of leaves using a fine brush (Ádám et al., 2000). The inoculated plants were incubated in a moist chamber for 24 h and then kept in greenhouse conditions (18−25 °C). During winter, a 16-h photoperiod was maintained using supplemental lighting (160 µE m−2 s−1).

Powdery mildew conidia and uredinospore suspension of rusts were inoculated onto primary leaves of 9–10-day-old barley and wheat, as well as 14-days-old B. distachyion plants as described earlier (Ádám et al., 2000; Harrach et al., 2008).

Ribonuclease activity tests

Ribonuclease enzyme activity was determined spectrophotometrically at 260 nm using low molecular weight yeast RNA (Sigma-Aldrich) as substrate, while RNase activity of leaf extracts from control and infected leaves was determined after electrophoresis in 10 % native polyacrylamide gel and specific in-gel staining as described earlier (Barna et al., 1989, 2014).

Ion leakage test

Leakage of electrolytes was measured as described previously (Barna et al., 2011). Briefly, eight 1-cm-long segments from the middle parts of the first leaves of seedlings were placed on distilled water. Changes in conductivity (µS) of bathing solutions were measured by a conductivity meter and were calculated on a fresh weight basis.

Gene expression measurements by real-time, quantitative PCR (RT-qPCR)

For gene expression measurement 0.1 g of powdery mildew-inoculated or control (non-inoculated) barley leaves were ground in liquid nitrogen and total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen Hilden, Germany). Reverse transcription (RT) of 1.5 µg total RNA was carried out in a total volume of 20 µl with a RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Fisher, Waltham, MA, USA) using an oligo(dT)18 primer. The expression of barley RNase genes was measured by quantitative real-time RT-PCR (RT-qPCR). The qPCR reaction mixture contained 10 µl KAPA SYBR FAST qPCR Master Mix (2X) (KAPA Biosystems, USA), 0.8 µl of 5 µM forward and reverse primers, 6.4 µl PCR-grade water and 2 µl of 20-fold diluted cDNA in 20 µl total reaction volume. The primer sequences are shown in Table 1. DNA amplifications were performed in a Bio-Rad CFX-96 real-time thermocycler (Bio-Rad, USA) with the following program: 95 °C for 3 min, 40 cycles at 95 °C for 10 s, 60 °C for 20 s and 72 °C for 10 s, followed by a melting curve analysis to determine amplicon specificity using a temperature range from 65 to 95 °C with increments of 0.5 °C. Gene expression was normalized to a barley ubiquitin (HvUBI-1) gene as a reference (Table 1).

Table 1. Sequences of gene-specific primer pairs used for the detection of two barley RNase genes and the barley UBI-1 reference gene in 5′ to 3′ direction. Accession numbers of nucleotide sequences in NCBI GenBank: XM_045114837.1 (HvNucl-2); XM_045101965.1 (HvRnucl-3) and M60175 (HvUBI-1)

Target gene	Forward primer	Reverse primer	Product length (bp)
HvNucl-2	gccggttgtcgagaagagga	cgcgaattcgtgattgtggtg	215
HvRnucl-3	aggcagcccttgacaaactaa	tccagaacggtccaattcgt	101
HvUBI-1	cggcaaggtaaccaggctca	aggcgatcaaatatccaggaaa	257

RESULTS

Symptoms of inoculated plants

Powdery mildew grew vigorously on wild-type Pallas and Ingrid barley cultivars 7 days after inoculation, while it caused visible hypersensitive reaction (HR) on the Mla lines, and no visible symptoms on Mlg and mlo lines in accordance with earlier observations (Harrach et al., 2008).

The Winzi wheat cultivar proved to be susceptible to both of the applied leaf and stem rust races and showed sporulation on the leaves, while *B. distachyon* genotypes showed no visible symptoms in the non-host interactions (data are not shown).

RNase enzyme activities in leaf extracts

In order to investigate the effect of powdery mildew infection on RNase activities in barley near-isogenic lines, in wheat and in *B. distachyon* genotypes, a spectrophotometric assay was carried out. The largest increase in RNase activity was observed in the susceptible wild-type Pallas at 7 days after inoculation with *Bh* race A6. A significantly smaller increase was found in extracts of the inoculated Mla near isogenic line, and even less in extracts from inoculated Mlg and mlo lines, but still higher than the activity in extracts from control, uninfected uninoculated Pallas leaves (Fig. 1).

As with barley, we found larger increase in RNase activity in the case of wheat extracts from the compatible interaction with wheat powdery mildew than after inoculation with barley powdery mildew, which is a non-adapted pathogen. In order to compare with other compatible biotrophic interactions, the RNase activities were determined also in stem rust and leaf rust-infected wheat leaves. Both rust species induced a significant increase in RNase activities in the infected leaves (Fig. 1).

We also investigated the RNase activity in leaves of the cereal model plant *B. distachyon* during non-host interactions with powdery mildew and leaf rust. Interestingly, extracts from healthy leaves of *B. distachyon* genotypes showed much higher activity than extracts from healthy leaves of wheat and even higher than that of barley. In addition, the RNase activities markedly increased in all non-host plant-pathogen interactions (Fig. 1).

RNase isoenzyme patterns after polyacrylamide gel electrophoresis

Since we wanted to compare RNase isoenzyme patterns of powdery mildew-inoculated barley lines with or without mildew resistance genes, as well as isoenzyme patterns of wheat and *B. distachyon* genotypes before and after inoculation, polyacrylamide gel electrophoresis

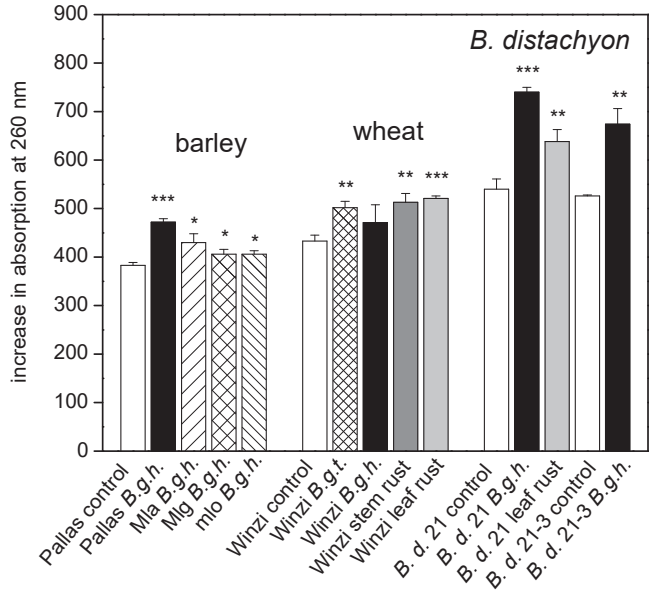

Fig. 1. Spectrophotometrically measured RNase enzyme activities in leaf extracts from healthy and infected barley cv. Pallas, its near-isogenic Mla, Mlg and mlo lines, from wheat cv. Winzi and *Brachypodium distachyon* genotypes 21 and 21-3. Samples were taken 7 days after inoculation with *Blumeria hordei* race A6 (*Bh*), *B. graminis* f. sp. *tritici* (*Bgt*), stem rust race 11, or leaf rust race 12. Mean values of three replicates of a representative experiment from at least three independent experiments with similar results ±SD are shown. The symbols *, ** and *** show significant differences between inoculated and respective control leaves at $P < 5\%$, $P < 1\%$ and $P < 0.1\%$, respectively

and specific in-gel staining were carried out. Although there was no quantifications of the RNase patterns of leaf extracts, it showed similar tendency as the spectrophotometric assays; leaf extracts from non-infected barley had the weakest, and *B. distachyon* leaf extracts had the strongest RNase band (Fig. 2). Barley extracts showed one distinct fast moving band, in wheat extracts there were an even faster and a somewhat slower band, while extracts from *B. distachyon* showed a slower moving and strong band with RNase activity. All the extracts from the 3 different plant species showed slower diffuse bands (Fig. 2).

Furthermore, infection of wheat with the compatible wheat powdery mildew induced higher RNase activity than inoculation with the non-adapted barley powdery mildew. On the other hand, inoculation of both *B. distachyon* genotypes with the non-adapted barley powdery mildew increased RNase activity as it was shown by the spectrophotometric assay as well. It is noteworthy, that RNase isoenzyme patterns were markedly different even between barley and wheat, but especially in the case of *B. distachyon* leaves (Fig. 2).

Leakage of electrolytes from healthy and infected leaves

We also wanted to know also if cell and tissue damage was at least partly, responsible for the increase in RNase activities. Leakage of electrolytes from control and infected barley, wheat and

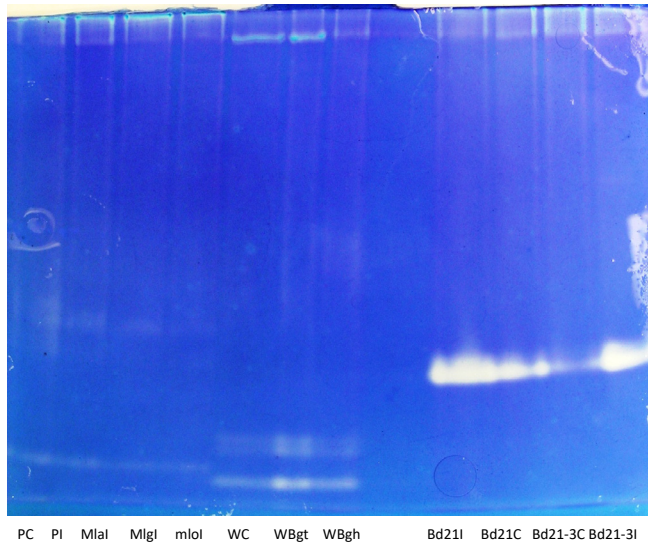

Fig. 2. RNase enzyme activities in 10% polyacrylamide gel after electrophoresis of barley, wheat and *B. distachyon* leaf extracts. PC: Pallas control, PI: Pallas infected with *Bh*, MlaI: Mla barley infected with *Bh*, MlgI: Mlg barley infected with *Bh*, mloI: mlo barley infected with *Bh*, WC: Winzi wheat control, WBgt: Winzi wheat infected with *Bgt*, and W*Bh*: Winzi wheat infected with *Bh*. Bd21I: *B. distachyon* line 21 infected with *Bh*, Bd21C: *B. distachyon* line 21 control, Bd21-3C: *B. distachyon* line 21-3 control, Bd21-3I: *B. distachyon* line 21-3 infected with *Bh*. Samples were taken 7 days after inoculation with the pathogens

B. distachyon leaves were measured by a conductivity meter. The leakage of electrolytes from barley near isogenic lines showed similar tendency as RNase activities; the highest leakage was found from the susceptible Pallas, and less from the hypersensitive resistant Mla line after infection with barley powdery mildew race A6 (Fig. 3). Infection of Mlg and mlolines caused very slight or no increase of electrolytes (data not shown). Similarly to RNase activities, infection of wheat with Bgt induced larger, while infection with *Bh* induced smaller increase in the leakage of electrolytes. Furthermore, infection of *B. distachyon* lines caused slight or no increase in ion leakage (Fig. 3).

Gene expression measurements by RT-qPCR

We also tested the expression of the barley *HvNucl-2* and *HvRnucl-3* genes that encoded the endonuclease 2 and ribonuclease 3-like protein 3 isoform X3, respectively. These RNase-like genes were identified in a recently performed RNA-Seq project carried out with healthy and barley powdery mildew race A6 infected leaves of Ingrid and its Mla near-isogenic barley line (data not shown). The expression of these two genes was measured by quantitative RT-qPCR in total RNA extracts of leaves of two wild-type barley cultivars (Pallas and Ingrid) as well as their near-isogenic Mla lines with and without inoculation with barley powdery mildew race A6. Both genes showed the highest expression in the susceptible interaction 7 days after infection (Fig. 4).

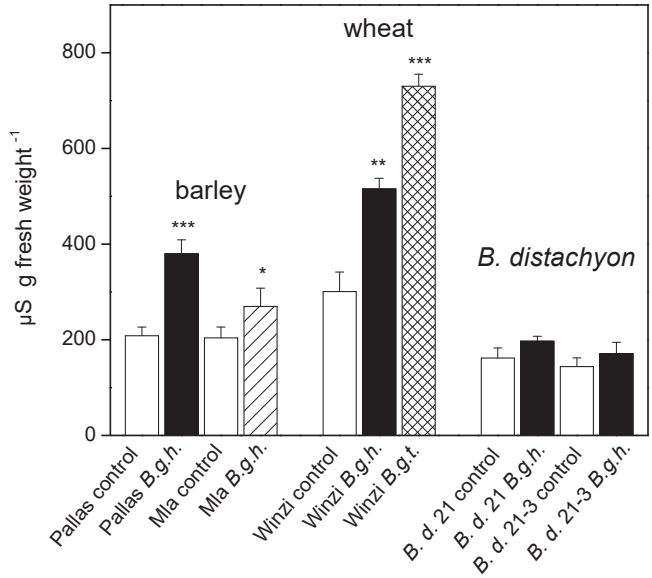

Fig. 3. Leakage of electrolytes from leaves of healthy and barley powdery mildew (*Bh*) infected Pallas and Mla barley near isogenic lines, and from leaves of healthy and barley or wheat powdery mildew (*Bgt*) infected Winzi wheat and *Brachypodium distachyon* lines 21 and 21-3 inoculated with *Bh*. Samples were taken 7 days after inoculation with the pathogens. Mean values of three replicates of a representative experiment from at least three independent experiments with similar results ±SD are shown. The symbols *, ** and *** show significant differences between inoculated and respective control leaves at $P < 5\%$, $P < 1\%$ and $P < 0.1\%$, respectively

DISCUSSION

Powdery mildews and rusts are among the most important pathogens of cereals all over the world. These typical biotrophs have very narrow host specificity, they form races and pathotypes. The physiological and biochemical changes during their interactions with wheat and barley have been investigated as a model since a long time (Dracatos et al., 2018).

The importance of research on the non-host plant-pathogen interactions has become generally accepted in the last decades (Heath, 2000; Cheng et al., 2014; Huang et al., 2016; Lee et al., 2017). Since we found that pre-inoculation of various wheat genotypes with barley powdery mildew induced certain degree of resistance against a challenge infection with wheat leaf rust (Barna et al., 2022), we were interested in the effects of various non-host powdery mildew interactions with barley, wheat or *B. distachyon*, especially on RNase activity.

Earlier we showed that stem and stripe rust infection of wheat leaves induce RNase activity especially in the case of compatible interactions (Barna et al., 1989). Furthermore, it was reported, that a superfamily of barley powdery mildew effector candidates shows structural similarities to ribonucleases (Pennington et al., 2019a,b). Changes in RNase activities were also investigated by PAGE and spectrophotometric assays during maize response to *Meloidogyne arenaria* infection (Przybylska, 2021). Furthermore, the pathogenesis related proteins PR4 and

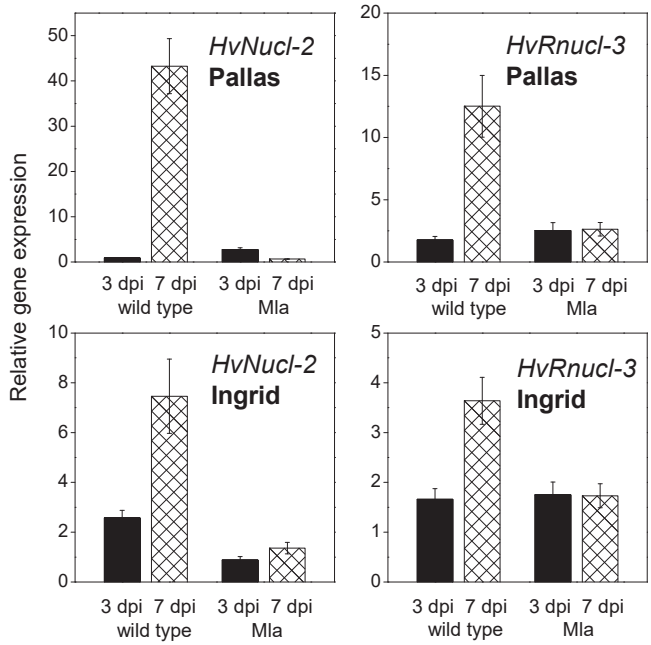

Fig. 4. Changes in the expression of barley HvNucl-2 and HvRnucl-3 genes encoding the endonuclease 2 and ribonuclease 3-like protein 3 isoform X3, respectively, following powdery mildew (Blumeria hordei, race A6) inoculation. Experiments were carried out with two barley cultivars (Pallas and Ingrid) as well as with their Mla near-isogenic lines. Total RNA was extracted at 3 and 7 days post-inoculation (dpi) from inoculated and control (non-inoculated) barley leaves. Gene expression levels were determined by real-time RT-qPCR and normalized by using a barley ubiquitin gene. Relative gene expression levels were calculated by comparing the expression of inoculated leaves with those of control leaves. Mean values of three replicates of a representative experiment from at least three independent experiments with similar results ±SD are shown

PR10 proved to have RNase activity (Guevara-Morato et al., 2010; Choi et al., 2012; Filipenko et al., 2013). Even RNases are suggested to play a role in systemic signaling (LeBrasseur et al., 2002; Lusso and Kuc, 1995). It is also noteworthy that transgenic expression of ribonucleases increases resistance to viruses (Zhang et al., 2001; Trifonova et al., 2007; Sugawara et al., 2016).

When we compared the RNase activities with the various symptoms on barley, wheat and *B. distachyon* plants after powdery mildew and rust infection, we found that the compatible interaction of barley with powdery mildew induced the highest increase in RNase activity as measured spectrophotometrically. The hypersensitive interaction on Mla leaves resulted in less while the strongly incompatible interactions on Mlg and mlo barley leaves without visible symptoms resulted in the least increase in RNase activities (Fig. 1). Similarly, the largest RNase activity was shown by wheat leaves infected with the compatible wheat powdery mildew, wheat stem and leaf rusts, and less activity was found in the leaves infected with the incompatible non-host barley powdery mildew. In addition, we showed that heat stress-induced susceptibility of barley near isogenic lines to powdery mildew was correlated with elevated RNase

activity (Barna et al., 2014). It is noteworthy that Shivakumar et al. (2000) reported on ribonucleases in pearl millet and their involvement in resistance against downy mildew disease. However, in their work they used a very ealy (24 h) samplings when the disease could not develop totaly. Furthermore, we found that powdery mildew infection led to the appearance of a new RNase band in various tobacco lines, and to an increase in total RNase activity (Gullner et al., 2017).

Interestingly, the RNase activity of leaves of healthy *B. distachyon* lines 21 and 21-3 was significantly higher than that of leaves from wheat and especially barley plants. Although we did not have compatible pathogen for these host plants, even the incompatible non-host interaction with barley powdery mildew or leaf rust strongly increased RNase activities (Fig. 1).

It is noteworthy, that the electrophoretic RNase enzyme patterns of extracts from barley, wheat and *B. distachyon* are different from each other (Fig. 2).

The question arises whether the increase in RNase activity corresponds to the degree of cell and tissue damage of plants? When the degree of ion leakage was compared to the increase in RNase activity, we found that in the case of barley the compatible interaction showed (at later stage of the disease) the most severe damage as we showed earlier (Harrach et al., 2008), and the largest RNase activity (Fig. 3). Similarly, the largest increase in leakage and RNase activity was measured in the compatible wheat-wheat powdery mildew interaction. However, in the non-host *B. distachyon*-powdery mildew interactions there were slight increases in conductivity (weak cell damage), but strong increase in RNase activities (Fig. 3) indicating that in this case cell damage did not correlate with RNase activity, although we did not have compatible interaction with *B. distachyon*, when an even stronger RNase activity can not be excluded.

We also investigated the changes in gene expression of two barley RNase-like genes following powdery mildew inoculation. These genes, which encode the endonuclease 2 and the ribonuclease 3-like protein 3 isoform X3 were identified by a recent RNA-Seq analysis of powdery mildew infected barley leaves (data not shown). Gene expression levels were measured in leaves of two barley cultivars (Pallas and Ingrid) as well as in their near-isogenic Mla lines. Both genes showed the highest expression in the compatible Pallas - barley powdery mildew interaction at 7 dpi. Furthermore, the analysis showed similar tendencies in both cultivars, the gene expressions were most pronounced during compatible interactions at 7 days post-inoculation (dpi) (Fig. 4). Thus, gene expressions of these two RNase-like genes were in correlation with the RNase activities of leaf extracts. In accordance, Zhang et al. (2014) reported that a large-scale transcriptome analysis revealed distinct gene activations in wheat responding to stripe rust and powdery mildew, as we found different gel patterns for mildew and rust infected wheat.

In conclusion, it seems that RNase activities are may be a kind of susceptibility factors in the interactions of barley and wheat with the biotrophic powdery mildew and rust pathogens.

ABBREVIATIONS

Bh	*Blumeria hordei*
Bgt	*Blumeria graminis* f. sp. *tritici*
HR	hypersensitive reaction
PAGE	polyacrylamide gel electrophoresis
Pgt	*Puccinia graminis* f. sp. *tritici*

Pt	*Puccinia triticina*
RNase	ribonuclease
RT-qPCR	real-time, quantitative PCR

REFERENCES

Ádám, A.L., Galal, A.A., Manninger, K., and Barna, B. (2000). Inhibition of the development of leaf rust (*Puccinia recondita*) by treatment of wheat with allopurinol and production of a hypersensitive-like reaction in a compatible host. *Plant Pathology*, 49: 317–323.

Barna, B., Ibenthal, W.D., and Heitefuss, R. (1989). Extracellular RNase activity in healthy and rust infected wheat laves. *Physiological and Molecular Plant Pathology*, 35: 151–160.

Barna, B., Jansen, C., and Kogel, K.H. (2011). Sensitivity of barley leaves and roots to fusaric acid but not to H2O2 is associated with susceptibility to fusarium infections. *Journal of Phytopathology*, 159: 720–725.

Barna, B., Harrach, B.D., Viczián, O., and Fodor, J. (2014). Heat induced susceptibility of barley lines with various types of resistance genes to powdery mildew. *Acta Phytopathologica et Entomologica Hungarica*, 49: 177–188.

Barna, B., Máté, G., Preuss, J., Harrach, B.D., Gullner, G., Manninger, K., and Fodor, J. (2022). Defence responses triggered by Blumeria graminis f.sp. hordei in non-host wheat genotypes results in a decrease in *Puccinia triticina* infection. *Journal of Phytopathology*, 170: 82–90.

Blank, A. and McKeon, T.A. (1989). Single-strand-preferring nuclease activity in wheat leaves is increased in senescence and is negatively photoregulated. *Proceeding of the National Academy of Sciences of the USA*, 86: 3169–3173.

Booker, F.L. (2004). Influence of ozone on ribonuclease activity in wheat (Triticum aestivum) leaves. *Physiologia Plantarum*, 120: 249–255.

Cheng, Y., Yao, J., Zhang, H., Huang, L, and Kang, Z. (2014). Cytological and molecular analysis of nonhost resistance in rice to wheat powdery mildew and leaf rust pathogens. *Protoplasma*, 252: 1167–1179.

Choi, D.S, Hwang, I.S., and Hwang, B.K. (2012). Requirement of the cytosolic interaction between Pathogenesis related Protein 10 and Leucine-Rich Repeat Protein 1 for cell death and defense signalling n pepper. *Plant Cell*, 24: 1675–1690.

Dracatos, P.M., Haghdoust, R., Singh, D., and Park, R.F. (2018). Exploring and exploiting the boundaries of host specificity using the cereal rust and mildew models. *New Phytologist*, 218: 453–462.

Filipenko, E.A., Kochetov, A.V., Kanayama, Y., Malinovsky, V.I., and Shumny, V.K. (2013). PR-proteins with ribonuclease activity and plant resistance against pathogenic fungi. *Russian Journal of Genetics and Applied Research*, 3: 474–480.

Galiana, E., Bonet, P., Conrod, S., Keller, H., Panabieres, F., Ponchet, M., Poupet, A., and Ricci, P. (1997). RNase activity prevents the growth of a fungal pathogen in tobacco leaves and increases upon induction of systemic acquired resistance with elicitin. *Plant Physiology*, 115: 1557–1567.

Gobert, A., Quan, Y., Arrivé, M., Waltz, F., Da Silva, N., Jomat, L., Cohen, M., Jupin, I., and Giegé, P. (2021). Towards plant resistance to viruses using protein-only RNase P. *Nature Communications*, 12(1): 1007.

Gomes-Filho, E., Lima, C.R.F M., Costa, J.H., da Silva, A.C.M., Lima, M.G.S., de Lacerda, C.F., and Prisco, J.T. (2008). Cowpea ribonuclease, properties and effect of NaCl-salinity on its activation during seed germination and seedling establishment. *Plant Cell Reports*, 27: 147–157.

Graeme, J.K., Bayon, C., Sparks, C.A., Canning, G., Kanyuka, K., and Rudd, J.J. (2018). Characterization of an antimicrobial and phytotoxic ribonuclease secreted by the fungal wheat pathogen *Zymoseptoria tritici*. *New Phytologist*, 217: 320–331.

Green, P.J. (1994). The ribonucleases of higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 45: 421–445.

Guevara-Morato, M., Garcia de Lacoba, M., Garcia-Luque, I., and Serra, M.T. (2010). Characterization of a pathogenesis-related protein 4 (PR-4) induced in Capsicum chinense L-3 plants with dual RNase and DNase activities. *Journal of Experimental Botany*, 61: 3259–3271.

Gullner, G., Juhász, Cs., Németh, A., and Barna, B. (2017). Reactions of tobacco genotypes with different antioxidant capacities to powdery mildew and Tobacco mosaic virus infections. *Plant Physiology and Biochemistry*, 119: 232–239.

Harrach, B.D., Fodor, J., Pogány, M., Preuss, J., and 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.

Heath, M.C. (2000). Nonhost resistance and nonspecific plant defenses. *Current Opinion in Plant Biology*, 3: 315–319.

Hillwig, M.S., Contento, A.L., Meyer, A., Ebany, D., Bassham, D.C., and MacIntosh, G.C. (2011). RNS2, a conserved member of the RNase T2 family, is necessary for ribosomal RNA decay in plants. *Proceeding of the National Academy of Sciences of the USA*, 108: 31093–31098.

Huang, L.F., Lin, K.H., He, S.L., Chen, J.L., Jiang, J.Z., Chen, B.H., Hou, Y.S., Chen, R.S., Hong, C.Y., and Ho, S.L. (2016). Multiple patterns of regrulation and overexpression of a ribonuclease-like pathogenesis-related protein gene, *OsPR10a*, conferring disease resistance in rice and arabidopsis. *Plos One*, 11(6): e0156414.

Hugot, K., Ponchet, M., Marais, A., Ricci, P., and Galiana, E. (2002). A tobacco S-like RNase inhibits hyphal elongation of plant pathogens. *Molecular Plant Microbe Interactions*, 15: 243–250.

Kariu, T., Sano, K., Shimokawa, H., Itoh, R., Yamasaki, N., and Kimura, M. (1998). Isolation and characterization of a wound-inducible ribonuclease from *Nicotiana glutinosa* leaves. *Bioscience and Biotechnology*, 62: 1144–1151.

Kazmierczak, A. and Knypl, J.S. (1994). Endoribonuclease of *Vicia faba* ssp. minor radicles, and enhancement of its activity by chilling stress or abscissic acid. *Physiologia Plantarum*, 91: 722–728.

Kettles, G., Bayon, C., Sparks, C.A., Canning, G., Kanyuka, K., and Rudd, J.J. (2018). Characterization of an antimicrobial and phytotoxic ribonuclease secreted by the fungal wheat pathogen *Zymoseptoria tritici*. *New Phytologist*, 217(1): 320–331.

Kim, S.T., Yu, S., Kang, Y.H., Kim, S.G., Kim, J.Y., Kim, S.H., and Kang, K.Y. (2008). The rice pathogen-related protein 10 (JIOsPR10) is induced by abiotic and biotic stresses and exhibits ribonuclease activity. *Plant Cell Reports*, 27: 593–603.

LeBrasseur, N.D., MacIntosh, G.C., Perez-Amador, M.A., Saitoh, M., and Green, P.J. (2002). Local and systemic wound-induction of RNase and nuclease activities in Arabidopsis: RNS1 as a marker for a JA-independent systemic signaling pathway. *Plant Journal*, 29: 393–403.

Lee, H.A., Lee, H.Y., Seo, E., Lee, J., Kim, S.B., Oh, S., Choi, E., Choi, E., Lee, S.E., and Choi, D. (2017). Current understandings of plant nonhost resistance. *Molecular Plant-Microbe Interactions*, 30: 5–15.

Lusso, M. and Kuc, J. (1995). Increased activities of ribonuclease and protease after challenge in tobacco plants with induced systemic resistance. *Physiological Molecular Plant Pathology*, 47: 419–428.

MacIntosh, G.C. and Castandet, B. (2020). Organellar and secretory ribonucleases: major players in plant RNA homeostasis. *Plant Physiology*, 183: 1438–1452.

Manninger, K. (2000). Virulence survey of wheat leaf rust in Hungary: races/pathotypes in 1999. *Acta Phytopathologica et Entomologica Hungarica*, 35: 421–428.

Pennington, H.G., Jones, R., Kwon, S., Bonciani, G., Thieron, H., Chandler, T, Luong, P., Morgan, S.N., Przydacz, M., Bozkurt, T., Bowden, S., Craze, M., Wallington, E.J., Garnett, J., Kwaaitaa, M., Panstruga, R., Cota, E., and Spanu, P.D. (2019a). The fungal ribonuclease-like effector protein CSEP0064/BEC1054 represses plant immunity and interferes with degradation of host ribosomal RNA. *PLoS Pathology*, 15(3): e1007620.

Pennington, H.G., Jones, R., Kwon, S., Bonciani, G., Thieron, H., Chandler, T., Luong, P., Morgan, S.N., Przydacz, M., Bozkurt, T., Bowden, A., Craze, M., Wallington, E., Garnett, J., Kwaaitaal, M., Panstruga, R., Cota, E., and Spanu, P.D. (2019b). A fungal ribonuclease-like effector protein inhibits plant host ribosomal RNA degradation. *Plos Pathogens*, 15(3): e1007620. https://doi.org/10.1371/journal.ppat.1007620.

Przybylska, A. (2021). Analysis of ribonuclease and peroxidase activities during maize (*Zea mays*) response to *Meloidogyne arenaria* infection. *Journal of Plant Protection Research*, 61: 371–376.

Shivakumar, P.D., Vasanthi, N.-S., Shetty, H.S., and Smedegaard-Petersen, V. (2000). Ribonucleases in the seedlings of pearl millet and their involvement in resistance against downy mildew disease. *European Journal of Plant Pathology*, 106: 825–836.

Sugawara, T., Trifonova, E.A., Kochetov, A.V., and Kanayama, Y. (2016). Expression of an extracellular ribonuclease gene increases resistance to Cucumber mosaic virus in tobacco. *BMC Plant Biology*, 16(Suppl 3): 246.

Sugiyama, M., Ito, J., Aoyagi, S., and Fukuda, H (2000). Endonucleases. *Plant Molecular Biology*, 44: 387–397.

Trifonova, E.A., Sapotsky, M.V., Komarova, M.L., Scherban, A.B., Shumny, V.K., Polyakova, A.M., Lapshina, L.A., Kochetov, A.V., and Malinovsky, V.I. (2007). Protection of transgenic tobacco plants expressing bovine pancreatic ribonuclease against tobacco mosaic virus. *Plant Cell Reports*, 26: 1121–1126.

Wiberg, A. (1974). Genetical studies of spontaneous sources of resistance to powdery mildew in barley. *Hereditas*, 77: 89–148.

Wilson, C.M. (1975). Plant nucleases. *Annuual Review of Plant Physiology*, 26: 187–208.

Zhang, L., French, R.C., Langenberg, W.G., and Mitra, A. (2001). Accumulation of barley stripe mosaic virus is significantly reduced in transgenic wheat plants expressing a bacterial ribonuclease. *Transgenic Research*, 10: 13–19.

Zhang, H., Yang, Y., Wang, C., Liu, M., Li, H., Fu, Y., Wang, Y., Nie, Y., Liu, X., and Ji, W. (2014). Large-scale transcriptome comparison reveals distinct gene activations in wheat responding to stripe rust and powdery mildew. *BMC Genomics*, 15: 898.