View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Repository of the Academy's Library

Cereal Research Communications 45(1), pp. 104–113 (2017)
DOI: 10.1556/0806.44.2016.047

Changes in Transcript and Protein Expression Levels in the Barley – *Cochliobolus sativus* Interaction

M. JAWHAR*, A. SHOAIB, M.I.E. ARABI and A. ALDAOUDE

Department of Molecular Biology and Biotechnology, AECS, P.O.Box 6091 Damascus, Syrian Arab Republic

(Received 26 April 2016; Accepted 8 August 2016;
Communicated by J. Kolmer)

Spot blotch, caused by *Cochliobolus sativus*, is an important barley disease which causes extensive grain yield losses worldwide. In order to investigate the molecular responses to the *C. sativus* infection, leaf transcriptome and proteome before and after fungus inoculation in a resistant barley genotype, were compared using cDNA-AFLP and 2-D PAGE techniques. A notable number of transcripts and proteins exhibiting significant differential accumulations were detected compared to the non-inoculated controls. Functional annotation of the transcripts and proteins revealed a wide range of pathways including cell wall fortification, metabolism, signal transduction and defence. Spearman correlations of the relative abundances for those genes represented by both an mRNA and a protein showed a weak ($r_s = 0.4$; $P<0.001$) relationship, indicating that post-transcriptional processes play a critical role in regulating the protein level during infection. Taken together, our study suggested that a joint analysis of the transcriptomic and proteomic of barley data can provide useful insights that may not be deciphered from individual analysis of mRNA or protein expressions.

Keywords: barley, *Cochliobolus sativus*, defense response, transcriptomics, proteomics

Introduction

Spot blotch (SB), caused by the necrotrophic fungus *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur [anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.], is a common foliar disease of barley, wheat and other cereals in warmer parts of the world. It reduces yield as well as quality of barley grain (Mathre 1997; Ghazvini and Tekauz 2008). SB infection on barley plants results in numerous host-specific biochemical responses which show strategies for the ability of the plant to withstand disease (Kumar et al. 2002). However, to fight agronomic losses due to SB, a better understanding of molecular mechanisms of disease resistance is required.

Studies on the inheritance of SB resistance in barley have indicated that both monogenic and oligogenic as well as polygenic resistance have been reported in barley genotypes (Arabi 2005; Bilgic et al. 2006). Oligogenic inheritance of genes and several genes/QTLs on chromosomes 1H, 3H and 7H have been also recently identified in barley (Ghazvini 2014; Haas et al. 2016). Furthermore, hypersensitive responses were detected

*Corresponding author; E-mail: ascientific@aec.org.sy

0133-3720/$20.00 © 2017 Akadémiai Kiadó, Budapest

in epidermal and mesophyll cells associated with either pre-penetration or post-penetration stages of fungal infection of epidermis tissue of resistant and susceptible (Rodríguez-Decuadro et al. 2014). However, it is highly challenging to control this disease in barley due to a poor understanding of the mechanisms of plant resistance and since no highly resistant barley cultivar is yet available.

Recent advances in functional genomics studies have facilitated understanding of global metabolic and regulatory alterations caused by genotypic and/or environmental changes. CDNA-AFLP has proven to be a successful tool for monitoring genome-wide expression profiles at the mRNA level. Similarly, proteomics can be used to compare changes in levels of many proteins under particular genetic and environmental conditions. However, proteomic studies provide information on post-translational modifications, which cannot be obtained from mRNA expression profiles; these have proven critical to our understanding of proper physiological protein function, translocation, and subcellular localization (Bastiaanse et al. 2014; Liu and Aebersold 2016). Understanding the mutual regulatory interactions between transcriptome and proteome necessitates an approach that encompasses the simultaneous monitoring of expression at both the RNA and protein levels (Cheng et al. 2016).

On the other hand, the resistant SB cultivar 'Banteng' is an old German cultivar that is used intensively in SB breeding programs at the AECS to broaden genetic SB resistance, and therefore reduce the risk of resistance breakdown. Under heavy SB infection, this cultivar shows typical resistance symptoms of chlorosis and necrosis. Its resistance against *C. sativus* has been durable for several years in SB evaluation in different fields in Syria without any fungicide treatment (Arabi and Jawhar 2004). The partial resistance has been shown to be polygenic, but highly heritable (Arabi 2005).

To complete a picture of gene activity drawn by Al-Daoude et al. (2013; 2015), we investigated in the current study the defense response of 'Banteng' at the transcriptome (cDNA-AFLP) and proteome (2-D PAGE; MALDI-TOF/MS analysis) levels for enhancing our genetic and molecular understanding of plant fungal resistance.

Materials and Methods

Experimental design

Plants of cv. 'Banteng' were grown in plastic flats (60 × 40 × 8 cm) filled with sterilized peatmoss, and arranged in a randomized complete block design with three replicates. Each experimental unit consisted of 10 seedlings. A full replicate consisted of 10 pots inoculated with Pt4 isolate. Pots were placed in a growth chamber at 22 ± 1 °C (day) and 17 ± 1 °C (night) with a day length of 12 h and a relative humidity of 80–90%.

Inoculum preparation

The most virulent isolate (Pt4) to all barley genotypes available so far (Arabi and Jawhar 2004) was used in this study. The fungal mycelia were transferred from a stock culture

into Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) with 13 mg/L kanamycin sulphate and incubated for 10 days at 21 ± 1 °C in the dark. Then, conidia were collected with 10 mL of sterile distilled water. The conidial suspension was adjusted to 2×10^4 conidia/mL using hemacytometer. A surfactant (polyoxyethylene-20-sorbitan monolaurate) was added (100 µL/L) to the conidial suspension to facilitate dispersion of the inoculum over leaf surfaces. Infections were initiated by spraying the third and fourth barley leaves with the conidial suspension, and leaves covered for one night with plastic bags to increase humidity and the plants were kept in the same greenhouse at 20 °C with a 16 h photoperiod. Non-inoculated control plants were sprayed with distilled water and surfactant.

mRNA isolation

For mRNA isolation, primary leaves from 20 experimental plants were collected at 1, 2, 4 and 6 days post-inoculation and were immediately frozen in liquid nitrogen. At the same time points samples from mock inoculated plants were collected as control. RNA isolation was achieved as described previously by Al-Daoude et al. (2013). mRNA was extracted from samples (100–200 mg) with the Nucleotrap mRNA mini kit (Macherey-Nagel, MN, Germany) following the manufacturer's protocol. RNA was used for cDNA synthesis with the QuantiTect Reverse Transcription Kit (Qiagen) following the manufacturer's instructions. cDNA was stored at −20 °C.

cDNA-AFLP analysis

The cDNA-AFLP protocol was performed according to the method described by Breyne et al. (2002), with minor modifications which permit the visualization of one single cDNA fragment for each messenger originally present in the sample, thus reducing the redundancy of sequences obtained (Al-Daoude et al. 2013). PCR products were purified with MultiScreen PCR µ96 plates (Millipore) and sequenced directly (BMR Genomics). Prior to sequencing, PCR products were purified with QIAgen gel extraction kit according to the manufacturer´s recommendations.

Protein extraction and 2-D PAGE

Samples were prepared for 2-DE as described previously (Al-Daoude et al. 2015). The first dimensional electrophoresis (isoelectric focusing) was carried out with a Protein Isoelectric Focusing Unit (Bio-Rad) according to the manufacturer's instructions. The second dimensional electrophoresis was conducted on 12% polyacrylamide LDS (lithium-dodecyl sulphate) gel using Bio-Rad Protein II XL Gel Cell (Bio-Rad). The second electrophoresis running conditions were as follows: constant 16 mA for 30 min at 6 °C followed by constant 30 mA per gel until the BPB dye reached the bottom of the gel. The 2-D gels were stained with Sypro-Ruby (Bio-Rad), and images were acquired using an FX scanner (Bio-Rad).

In-gel digestion and MALDI-TOF mass spectrometry

Proteins were identified from tryptic peptides by mass spectrometry as described by Koller et al. (2002). Each excised gel containing the protein of interest was placed in a protein low binding tube (Eppendorf) containing 500 µl of MilliQ ddH_2O at 4 °C for 24 h. Water was discarded and 300 µl of 50 mM triethylammonium bicarbonate buffer (TEAB, Sigma) was added. Tubes were left on gentle shaker at room temperature (RT) for 15 min before supernatant was replaced with 50 mM TEAB/50% acetonitrile solution (CH_3CN) twice each for 15 min at RT with gentle agitation. Supernatant was removed; 100 µl of CH_3CN was added to rehydrate the protein band for 5 min at RT. Gel pieces were dried in a speed vacuum before they were reduced with 10 mM DTT, 50 mM TEAB for 1 h at 56 °C and alkynated with 55 mM iodoacetamide, 50 mM TEAB for 45 min at RT in the dark. After this treatment, each gel piece was minced and lyophilized, then re-hydrated at 37 °C overnight in 5 µl of 50 mM TEAB containing 50 ng of modified trypsin (Promega, Madison, USA). After digestion, the protein peptides were collected, and the gels were washed with 0.1% trifluoroacetic acid in 50% CH_3CN three times to collect the remaining peptides. Peptides were cleaned using C18 resin ready packed tips and diluted into freshly prepared saturated sinapinic acid dissolved in 50% acetonitrile, 0.3% trif-luoroacetic acid. Two µl samples were spotted onto a stainless steel plate and spectra were collected by averaging three shots each for 200–300 lazer shots. Samples were irradiated using Bruker Microflex MALDI/TOF mass spectrometer (Bruker Daltonics, Germany) with a 377-nm nitrogen lazer, attenuated and focused on the sample target using the built-in software (Microflex package). Ions were accelerated with a deflection voltage of 30 kV and differentiated according to their mass-to-charge ratio (m/z) using a time-of-flight mass analyzer. Proteins showed significant variation in relative abundance after pathogen challenges were selected. This consisted of three sub-replications of a protein sample, two treatments of infection (inoculation vs. non-inoculation) and two biological replications. The first step involved the separation of theses protein according to their pH using IPG strips with pH values from 3–10. The second dimension was carried out using 12% acrylamide gels. Selected protein spots from 2D gels with two changes in spot volume after *C. sativus* infection were excised from gel, digested with sequencing grade trypsin (Promega, USA) and subjected to MALDI-TOF MS and MS/MS combined with database searching to assign putative identities to the proteins, as previously described (Ferry et al. 2011).

Database searches

Transcripts Sequencing was carried out on a Genetic Analyzer (ABI 310, Perklin-elmer, Applied Biosystems, USA). Each sequence was identified by homology search using the Basic Local Alignment Search Tool (BLAST) program (Altschul et al. 1997) against the GenBank no redundant public sequence database using an *E*-value (BLASTX expectation values [*E*] of $<10^{-5}$) to database entries with assigned identities.

Peptide masses (mass list) generated from the peptide mass fingerprint (PMFs) was used to search the NCBI database with the MASCOT search engine (Matrix Science, UK) for protein identification. The search parameters were set according to Kerim et al. (2003). Mascot use a probability-based molecular weight search (MOWSE) score to evaluate data obtained from tandem mass spectra. Protein identification was accepted based on a significant MOWSE score and at least four matched peptide masses. Matching ESTs were queried to the nrNCBI database with a significance cut-off value of 1e-5 using BLASTX searches. The identity of these spots was further confirmed by peptide fragmentation and MS/MS analysis.

To compare the transcriptome vs. the proteome, a ranked Spearman correlation was used to compare the abundances of the overlapping expressed features.

Results

The patterns of transcript and protein expressed genes at the beginning of the SB inoculation test represents the "normal" set of active genes in barley plant after four hours of being sprayed with water (Al-Daoude et al. 2015). Based on the assumption that disease infection involves the early recognition of the invading pathogen, the 2D-PAGE and cDNA-AFLP patterns of resistant plants were screened for newly expressed patterns to wrap up barley response to *C. sativus* invasion. Differentially-expressed protein and transcripts

Table 1. Defense-related gene classes containing protein spots and transcripts detected at $P<0.001$ in the resistant barley cultivar Banteng

	Total	Common	Trans-criptome	Proteome
All classes	62	7	28	34
Common classes				
Oxidative burst-related general	1	0	1	0
PR protein general	8	2	4	4
R genes	6	1	4	2
MAPK related	4	0	3	1
Transcription	4	0	1	3
Protease inhibitor	2	0	0	2
Antifungal	2	0	2	0
Transport	3	0	1	2
PR protein	10	0	3	7
Defense-related	8	2	5	3
Unknown	14	2	4	10

were visually scored relative to the first sampling time point which was arbitrarily attributed a zero value. A total of 62 unique proteins and transcripts were detected in the resistant cultivar, seven (11%) of them were in common (Table 1).

Spearman correlations of the relative abundances for those genes represented by both an mRNA and a protein showed a weak ($r_s = 0.4$; $P < 0.001$) connection. When protein

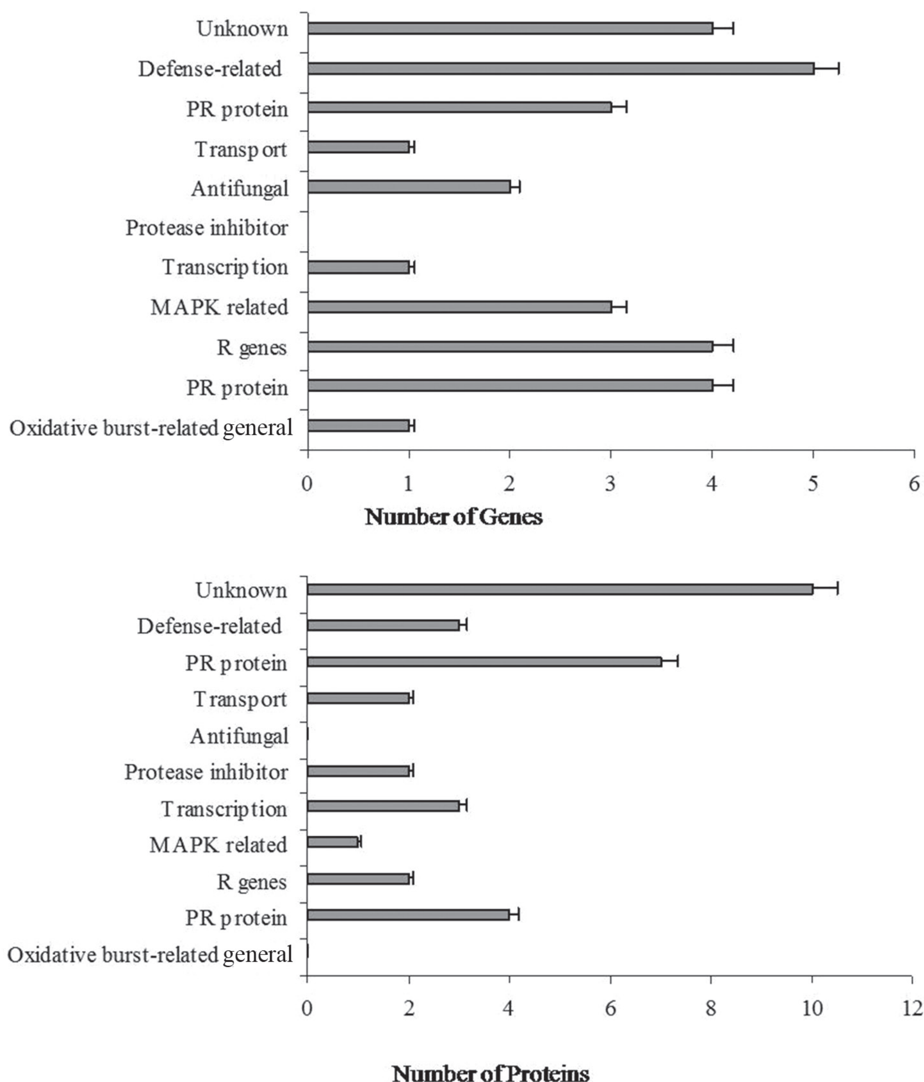

Figure 1. Differentially expressed proteins with the corresponding transcripts expression grouped by functions in resistant barley cultivar infected with *C. sativus*

and transcripts were divided into one of five general categories (defense, regulatory, transport, metabolism and unknown function), the unknown group had the greatest number of common transcripts and proteins (Table 1).

The comparison of the functional annotation of the major differential observed proteins and transcripts suggests that transcript and protein detection methods reveal functional categories with different preference (Fig. 1). We identified transcripts and proteins that specifically responded to *C. sativus* in the resistant cultivar 'Banteng'. Due to their well-known function in response to pathogens, we focused on the transcripts categorized as having a possible role in the defense response. The GTP-binding protein (pRac1 gene) was common between transcriptome and proteome (Table 2). Similarly, a NBS-LRR like protein, which is one of the most numerous gene families in plants involved in signaling, and they are organized either as isolated genes or as linked clusters of varying sizes that are thought to facilitate rapid *R*-gene evolution. Additionally, serine-threonine protein kinase (NPK2) was a common transcriptome and proteomics detected gene which plays a role in the regulation of cell proliferation, programmed cell death (apoptosis), cell differentiation, and embryonic development (Table 2).

Table 2. Common transcripts and proteins expressed genes detected after *C. sativus* inoculation

Gene category	Transcriptome			Proteome		
	Accession no.	Length (bp)	Blast X score	Accession no.	MS/MS score	E-value
Serine-threonine protein kinase (RK1)	DQ285022	148	e-28	gl|223452304	82	0.2
Oryza sativa hypothetical protein	EAZ29972	146	e-17	gl|222636517	81	0.0001
GTP-binding protein (pRac1gene)	AJ276225	157	e-27	gl|15238542	94	1.1
Oryza sativa hypothetical protein	EAY96996	172	e-11	gl|115469492	81	1.2
NBS-LRR like protein	AAK93796	198	e-30	gl|20378923	92	0.92
Oryza sativa putative glucan synthase	AAM19120	129	e-09	gl|462435593	79	0.009
Oryza sativa hypothetical protein	AJ251717	123	e-11	gl|218199970	73	0.7

Discussion

This study sheds some light on the transcript and protein expression changes that occur during *C. sativus*–barley interaction, taking into account the findings of Gayad (1961) on the production of barley SB symptoms and the observations of Wisniewska et al. (1998) with barley susceptibility to *Bipolaris sorokiniana.* Results demonstrated that there was a low correlation between the proteome and transcriptome data, suggesting that post-transcriptional gene regulation influences different biological pathways and secondary metabolite gene clusters. Liu and Aebersold (2016) reported that it is highly unlikely that cells would divert resources towards such elaborate regulatory mechanisms at both the transcript and protein levels if they could have simply controlled protein synthesis via strict transcriptional regulation, as predicted by the central dogma.

A low correlation between transcriptomic and proteomic barley data was detected in this study, as such, no one tool is expected to provide full coverage for the system and with equal accuracy throughout. Jovanovic et al. (2015) reported that many proteins whose concentration changed in response to pathogen were encoded by corresponding RNA transcripts whose expression did not appear to change. We have observed transcripts of a disease resistance protein similar to NBS-LRR protein which is an R gene that confers resistance to various plant fungal pathogens (Gao et al. 2005; Kohler et al. 2008) and has also been shown to impart barley leaf stripe resistance (Bulgarelli et al. 2010). The GTP-binding protein (pRac1 gene) was common between transcriptome and proteome, which is well known to play a significant role in barley resistance to powdery mildew (Hückelhoven et al. 2001).

In conclusion, this study illustrated that only 11% of genes encoding differentially expressed proteins had transcript accumulation. Such poor correlation could either be attributed to the post-transcriptional regulation of the identified genes or be the experimental limitations associated with the transcript and protein turnover measurements. Differentially expressed protein and transcript genes generated in the present study revealed that the true mRNA-protein discordance would not have been revealed without such integrated analyses and are arguably of greater scientific interest than concordance as they open up additional post-transcriptional intervention points for the development of therapeutics. However, these observations may possibly reveal genes and proteins, which might be useful in allowing the host plant to cope up with the invading pathogen and provide new insights into the molecular mechanism of plant–fungal interaction.

Acknowledgements

The authors thank the Director General of AECS and the Head of Biotechnology Department for their help throughout the period of this research. Thanks are also extended to Mr. E. Al-Shehadah for his help in the Spearman test analysis.

References

Al-Daoude, A., Jawhar, M., Shoaib, A., Arabi, M.I.E. 2013. Proteomic analysis of barley response during early spot blotch infection**.** J. Plant Pathol. **95**:313–319.

Al-Daoude, A., Arabi, M.I.E. Shoaib, A., Jawhar, M. 2015. Protein variation identified in resistant and susceptible barley genotypes infected with *Cochliobolus sativus*. Phyton. **55:**1–9.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. **25**:3389–3402.

Arabi, M.I.E. 2005. Inheritance of partial resistance to spot blotch in barley. Plant Breed. **127**:605–607.

Arabi, M.I.E., Jawhar, M. 2004. Identification of *Cochliobolus sativus* (spot blotch) isolates expressing differential virulence on barley genotypes in Syria. J. of Phytopathol. **152**:461–464.

Bastiaanse, H., Muhovski, Y., Parisi, O., Paris, R., Mingeot, D., Lateur, M. 2014. Gene expression profiling by cDNA-AFLP reveals potential candidate genes for partial resistance of 'Président Roulin' against *Venturia inaequalis*. BMC Genomics **15**:1043.

Bilgic, H., Steffenson, B.J., Hayes, P.M. 2006. Molecular mapping of loci conferring resistance to different pathotypes of the spot blotch pathogen in barley. Phytopathol. **96**:699–708.

Breyne, P., Dreesen, R., Vandepoele, K. 2002. Transcriptome analysis during cell division in plants. Proc. Natl Acad. of Sci., USA **99**:14825–14830.

Bulgarelli, D., Biselli, C., Collins, N.C., Consonni, G. et al. 2010. The CC-NB-LRR- Type Rdg2a resistance gene confers immunity to the seed-borne barley leaf stripe pathogen in the absence of hypersensitive cell death. PLoS ONE **5**:12599.

Cheng, Z., Teo, G., Krueger, S., Rock, T.M., Koh, H.W.L., Choi, H., Vogel, C. 2016. Differential dynamics of the mammalian mRNA and protein expression response to misfolding stress. Mol. Syst. Biol. **12**:855.

Ferry, N., Stavroulakis, S., Guan, W., Davison, G.M., Bell, H.A., Weaver, R.J., Down, R.E., Gatehouse, J.A., Gatehouse, A.M.R. 2011. Molecular interactions between wheat and cereal aphid (*Sitobion avenae*); analysis of changes to the wheat proteome. Proteomics **11**:1985–2002.

Gao, H., Narayanan, N.N., Ellison, L., Bhattacharyya, M.K. 2005. Two classes of highly similar coiled coil-nucleotide binding-leucine rich repeat genes isolated from the Rps1-k locus encode *Phytophthora* resistance in soybean. Mol. Plant Microbe Interact. **18**:1035–1045.

Gayad, S.K. 1961. Production of symptoms of barley leaf spot disease by cultural filtrates of *Helminthosporium sativum.* Nature **191**:725–726.

Ghazvini, H., Tekauz, A. 2008. Host pathogen interactions among barley genotypes and *Bipolaris sorokiniana* isolates. Plant Dis. **92**:225–233.

Ghazvini, H. 2014. Identification, by selective genotyping, of quantitative trait loci conferring resistance to *Cochliobolus sativus* in barley line TR 251. Crop Breed. J. **4**:35–45.

Gygi, S.P., Rochon, Y., Franza, B.R., Aebersold, R. 1999. Correlation between protein and mRNA abundance in yeast. Mol. Cell. Biol. **19**:1720–1730.

Haas, M., Menke, J., Chao, S., Steffenson, B.J. 2016. Mapping quantitative trait loci conferring resistance to a widely virulent isolate of *Cochliobolus sativus* in wild barley accession PI 466423. Theor. Appl. Genet. 2016 June 17. DOI: 10.1007/s00122-016-2742-y

Hückelhoven, R., Dechert, C., Trujillo, M., Kogel, K.H. 2001. Differential expression of putative cell death regulator genes in near-isogenic, resistant and susceptible barley lines during interaction with the powdery mildew fungus. Plant Mol. Biol. **47**:739–748.

Jovanovic, M., Rooney, M.S., Mertins, P., Przybylski, D., Chevrier, N. et al. 2015. Dynamic profiling of the protein life cycle in response to pathogens. Science **347**:1259038

Kerim, T., Imin, N., Weinman, J.J., Rolfe, B.G. 2003. Proteome analysis of male gametophyte development in rice anthers. Proteomics **3**:738–751.

Kohler, A., Rinaldi, C., Duplessis, S., Baucher, M., Geelen, D., Duchaussoy, F., Meyers, B.C., Boerjan, W., Martin, F. 2008. Genome-wide identification of NBS resistance genes in *Populus trichocarpa*. Plant. Mol. Biol. **66**:619–636.

Kumar, J., Schafer, P., Huckelhoven, R., Langen, G., Baltruschat, H., Stein, E., Nagarajan, S., Kogel, H.K. 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Mol. Plant Pathol. **3**:185–195.

Liu, Y., Aebersold, R. 2016. The interdependence of transcript and protein abundance: new data–new complexities. Mol. Syst. Biol. **12**:856.

Mathre, D.E. 1997. Compendium of Barley Disease, 2nd Ed. APS Press. St. Paul, MN, USA.

Rodríguez-Decuadro, S., Silva, P., Bentancur, O., Gamba, F., Pritsch, C. 2014. Histochemical characterization of early response to *Cochliobolus sativus* infection in selected barley genotypes. Phytopathol. **104**:715–723.

Wisniewska, H., Wakulinski, W., Chelkowski, J. 1998. Susceptibility of barleys to *Bipolaris sorokiniana* seedling blight determined by disease scoring and electrolyte. J. Phytopathol. **146**:563–566.