Small RNA bidirectional crosstalk during the interaction between wheat and *Zymoseptoria tritici*

Xin Ma[1], Nicolas Bologna[2], Javier Palma-Guerrero[1*]

[1] Plant Pathology, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland

[2] Department of Biology, Swiss Federal Institute of Technology (ETH), Zürich 8092, Switzerland.

* Corresponding Authors:

Javier Palma-Guerrero

javier.palma@usys.ethz.ch

+41 44 632 57 89

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Abstract

Cross-kingdom RNAi has been shown to play important roles during plant pathogen interactions. But this cross-kingdom RNAi was still unexplored in the wheat-*Zymoseptoria tritici* pathosystem. Here we performed a detailed analysis of the sRNA bidirectional crosstalk between wheat and *Z. tritici*. Using a combination of sRNA-seq and mRNA-seq we were able to identify known and novel sRNAs and study their expression and their action on putative targets in both wheat and *Z. tritici*. We predicted the target genes of all the sRNAs in either wheat or *Z. tritici* transcriptome and used degradome analysis to validate the cleavage of these gene transcripts. We could not find any clear evidence of a cross-kingdom RNAi in this pathosystem. We also found that the fungal sRNA enrichment was lower *in planta* than during *in vitro* growth, probably due to the lower expression of the only Dicer gene of the fungus during plant infection. However, we found a downregulation of specific wheat sRNAs during the fungal infection, leading to a boost expression of wheat defense related genes, which may be enhancing the plant defense ability against the pathogen. Additionally, the fungal infection also induced sRNAs regulating the expression of specific wheat genes, including auxin related genes, as an immune response. These results confirm the role of sRNAs in the regulation of wheat defenses during *Z. tritici* infection. Our findings contribute to improve our understanding of the interactions between wheat and *Z. tritici*.

Introduction

Small RNAs (sRNAs) are short non-coding RNAs (approximately 20 to 30 nucleotides in length) that play important roles in the regulation of gene expression and genome stability in eukaryotic organisms (Moazed, 2009). The three major classes of sRNAs are microRNAs (miRNAs), small interfering RNAs (siRNAs) and piwi-associated RNAs (piRNAs) (Carthew and Sontheimer, 2009; Moazed, 2009). While miRNA and siRNA are highly extended among plants, piRNAs have so far only been found in animal cells and are necessary in the development of germ cells by silencing transposons or other genetic elements (Vagin et al., 2006; Siomi et al., 2011).

In plants, miRNAs (~21nt) are produced from imperfect fold-back structures of self-complementary non-coding transcripts, originated from endogenous loci, and they regulate target mRNAs by Post-Transcriptional Gene Silencing (PTGS). In contrast, siRNAs derive from double strand RNA (dsRNA) with near-perfect complementarity, and they can be both of *endogenous origin*, e.g. inverted repeat (IR) and dsRNA synthesized by endogenous RNA-dependent RNA polymerases (RDRs) (Allen et al., 2005; Felippes and Weigel, 2009; Fei et al., 2013; Creasey et al., 2014), or *exogenous origin*, e.g. viral intermolecular replication intermediates or intramolecular secondary structures. siRNAs guide either PTGS (mediated by 21/22nt siRNAs) or RNA-directed DNA methylation (RdDM) often resulting in transcriptional gene silencing (TGS; mediated by 24nt siRNAs) (Matzke and Mosher, 2014; Borges and Martienssen, 2015). While each sRNA pathways possess specific characteristics in terms of biogenesis and accessory proteins, the basic mechanism of RNA silencing share few consensus biochemical steps: *(i)* initiation by dsRNA, *(ii)* dsRNA processing to generate the mature sRNAs by Dicer (or Dicer-like, DCL) proteins, and *(iii)* sRNA incorporation into Argonaute (AGO) proteins to interact with target mRNA or DNA in order to execute their silencing functions (Liu et al., 2004; Hutvagner and Simard, 2008; Bologna and Voinnet, 2014).

sRNAs are involved in plant development, reproduction, genome reprogramming, and contribute to the phenotypic plasticity of plants (Borges and Martienssen, 2015). Besides, plant sRNAs also mediate the response to abiotic stresses, such as water stress, sulfate stress, phosphate starvation, and cold stress (Sunkar and Zhu, 2004; Sunkar et al., 2007; Park et al., 2010; Guleria et al., 2011; Kamthan et al., 2015). Plants as well employ RNAi as defense strategies against biotic stress (Ruiz-Ferrer and Voinnet, 2009; Zhang et al., 2011; Weiberg et al., 2014). In Arabidopsis, miR393 is induced by the bacteria elicitor flg22 promoting the pathogen-associated molecular patterns (PAMPs) triggered immunity (PTI) (Navarro et al., 2008). MiR393 suppresses the auxin signaling by targeting auxin receptors TIR1 and three related F-box proteins, this results in increased plant resistance against the pathogen (Achard et al., 2007; Chen et al., 2007; Grant and Jones, 2009). MiR482, which can target numerous NBS-LRR genes in tomato, reduces its levels in response to bacterial pathogens and some viruses, resulting in increased target NBS-LRR transcripts in the immune system (Shivaprasad et al., 2012). This miRNA mediated NB-LRR regulation is considered to be important for plant immunity to pathogens (Zhai et al., 2011; Li et al., 2012; Shivaprasad et al., 2012; Fei et al., 2013).

In fungi, RNAi is an ancient host defense mechanism against viruses and transposons invasion (Chang et al., 2012; Nicolas and Ruiz-Vazquez, 2013; Torres-Martinez and Ruiz-Vazquez, 2017). However, no miRNAs, but only miRNA-like RNAs (milRNAs) have been identified in fungi (Lee et al., 2010; Goodwin et al., 2011), which share similarities with conventional miRNAs, but are synthesized by different mechanisms (Lee et al., 2010). The first RNAi

process in fungi, called quelling, was found in *Neurospora crassa*. Quelling is triggered by repetitive transgenes during mitotic growth, and it is essential for suppressing transposons replication (Romano and Macino, 1992; Nolan et al., 2005). QiRNAs, a class of Quelling-Deficient 2 (QDE2) interacting sRNAs, are specifically induced when *Neurospora* is treated with a DNA-damaging agent (Lee et al., 2009). Additionally, sRNAs are also required for fungal growth and pathogenesis in *Magnaporthe oryzae* (Raman et al., 2017).

RNAi has recently been reported to play important roles in plant and pathogen interactions. In host-induced gene silencing (HIGS), accumulation of double-stranded or antisense RNAs in barley and wheat can target and silence genes in the fungal pathogen *Blumeria graminis* as an effective defense strategy (Nowara et al., 2010). This cross-kingdom RNAi has also been shown to happen in a natural way. A fungal pathogen, *Botrytis cinerea*, can hijack host plant defenses by using small RNAs that bind to the plant AGO1 and silence plant defense related genes (Weiberg et al., 2013). A novel milRNA has been identified in *Puccinia striiformis f. sp.tritici* (Pst) that silences a wheat pathogenesis-related 2 gene and influences the plant immunity (Wang et al., 2017). Plants can also use the same RNAi mechanisms to defend against pathogenic fungi, e.g. cotton plants can export their miRNAs into the fungal pathogen *Verticillium dahliae*, and inhibit the expression of virulence genes during the infection cycle (Wang et al., 2016; Zhang et al., 2016). In addition, a recent study has shown that *Arabidopsis* can send sRNAs in extracellular vesicles to fungal pathogens, revealing how sRNAs travel from hosts to fungal pathogens (Cai et al., 2018).

However, no previous studies have reported the roles of sRNAs in the pathosystem of wheat and the fungal pathogen *Zymoseptoria tritici*, even though this fungus is the causal agent of the septoria tritici blotch, the most damaging disease on wheat in Europe (Hardwick et al., 2001; O'Driscoll et al., 2014) and an important disease of wheat worldwide. After penetrating wheat leaves through stomata, *Z. tritici* starts the complex infection cycle characterized by a long period of asymptomatic growth known as a latent period that can up to two weeks (Sanchez-Vallet et al., 2015). This latent period is followed by a rapid switch to necrotrophic growth in which a large number of plant cells are killed in a two to three days period, and it is accompanied by a rapid accumulation of fungal biomass inside the plants (Rudd et al., 2015). So far, only one effector gene, LysM, has been identified in *Z. tritici*, which is required for virulence and can suppress PTI during the infection (Marshall et al., 2011). Therefore, the mechanisms underlying *Z. tritici* pathogenicity still remain largely unknown. In recent studies we have analyzed the transcriptomes of both wheat and *Z. tritici* during the infection (Palma-Guerrero et al., 2017; Ma et al., 2018). These studies revealed the mutual transcriptomic responses between wheat and *Z. tritici* during the interactions, which, together with other transcriptomics studies in this pathogen, shows that a whole genome wide reprogramming of gene expressions happens in both plant and fungus (Rudd et al., 2015; Palma-Guerrero et al., 2017; Ma et al., 2018). But the role of sRNAs and the RNAi in the wheat-*Z. tritici* pathosystem remains largely unknown. Besides, no previous study described this natural bidirectional RNAi between plant and pathogen at the same time in the same samples, which could provide a detailed scenario of the communications between plant and pathogens.

Here we analyzed the small RNAs produced during wheat infection by *Z. tritici* at key stages of the disease cycle to identify both the *Z. tritici* sRNAs and the wheat sRNAs and their degraded targets. We found fungal sRNAs induced during infection and predicted their targeted genes in the wheat transcriptome. But cleavage of these predicted targets could not be confirmed by degradome analysis. Additionally, the expression of the fungal dicer gene was downregulated and barely expressed *in planta*. At the same time, we found that wheat could

138 downregulate sRNA expressions and lead to an increase defense ability against *Z. tritici*.
139 Additionally, wheat sRNAs involved in the regulation of auxin signaling were also induced in
140 response to *Z. tritici*. Thus, our results suggest that the cross-kingdom RNAi in wheat and *Z.*
141 *tritici* pathosystem does not lead do detectable mRNA degradation by PTGS during infection.
142 However, RNAi can be used as an immune response in plants cells.
143
144 **Materials and Methods**
145
146 **Samples collection and RNA extractions**
147
148 The total RNAs from leaf samples of wheat cultivar drifter infected with the *Z. tritici* 3D7
149 strain at 7, 12 and 14dpi (days post infection) were obtained in a previous study from our group
150 (Palma-Guerrero et al., 2017). The RNAs from uninfected mock samples at 7, 12 and 14dpi
151 that serve as control were obtained in this study, by using the control samples generated in the
152 same previous study (Palma-Guerrero et al., 2017). The RNA samples of the fungus growing
153 *in vitro* were generated in a different previous study (Francisco et al., 2018). Three replicates
154 per sample with the highest RNA quality according to Bioanalyzer 2100 (Agilent) were used
155 as biological replicates for library preparation and sequencing.
156
157 **Small-RNA-seq analysis**
158
159 The small-RNA-seq libraries were constructed using the TruSeq small-RNA kit and sequenced
160 by HiSeq4000 by the Functional Genomic Center of Zurich (FGCZ). Reads were trimmed to
161 remove sequencing adapters and keep only insert with length >=15 using Trimmomatic-0.36
162 (Bolger et al., 2014) with the settings "ILLUMINACLIP:smallRNA-SE.fa:2:30:10
163 LEADING:2 TRAILING:2 SLIDINGWINDOW:4:15 MINLEN:15". The adapter sequence in
164 smallRNA-SE.fa is "TGGAATTCTCGGGTGCCAAGG".
165
166 **Wheat sRNA predictions**
167
168 All the clean reads were collapsed using fastx-ToolKit (Gordon and Hannon, 2010). Wheat
169 tRNAs, rRNAs, and small nucleolar RNAs (snoRNAs) were removed. The sequences of
170 tRNAs, rRNAs and snoRNAs were extracted from Triticum_aestivum.TGACv1.ncrna.fa on
171 Ensembl plant (Kersey et al., 2018).
172
173 We used miRDeep-p (Yang and Li, 2011) and ShortStack (Shahid and Axtell, 2014; Johnson
174 et al., 2016) for the wheat miRNA predictions. For miRDeep-p, all the filtered reads were
175 mapped against wheat genome IWGSC RefSeq v1.0 (Appels et al., 2018) using bowtie
176 (Langmead et al., 2009) allowing no mismatches. The mapped reads from all the samples
177 including infected samples and mocks were merged together and collapsed. We used the
178 mapped reads to retrieve miRNAs annotated in IWGSC RefSeq v1.0 annotations (Appels et
179 al., 2018). We used miRDeep-P (Yang and Li, 2011) to predict wheat miRNAs setting the
180 length of candidate precursors as 300nt. A customized Perl script was used to filter the miRNAs
181 based on the new criteria of plant miRNA annotation (Axtell and Meyers, 2018). Basically, 1)
182 both the miRNA and miRNA* are predicted and expressed in the sequencing data; 2) The
183 expression of miRNA is more than 10 reads per million in at least one of the samples; 3) The
184 length of mature miRNAs should be between 20nt and 24nt; 4) The mismatches between
185 miRNA and miRNA* are under 5; 5) The asymmetric mismatches between miRNA and
186 miRNA* are under 3; 6) The length of precursor should be under 300nt.
187

We as well used ShortStack (Shahid and Axtell, 2014; Johnson et al., 2016) to predict the wheat sRNA loci with the settings "—mismatches 0 –mincov 0.5rpm". Three replicates of each conditions were merged together for the ShortStack alignment and annotation. Only the sRNAs candidate locus with expressions of at least 10 rpm in at least two replicates of each condition were kept. Then the major siRNAs and miRNAs from every condition were summarized together. We named every unique siRNA and miRNA reads as miRNA-uniq or siRNA, and with ascending order numbers. We combined the annotations of miRNAs from Arabidopsis, rice and wheat from miRBase and the annotations of wheat miRNA from IWGSC together to make a customized database of the known plant miRNAs (Griffiths-Jones, 2004; Griffiths-Jones et al., 2006; Griffiths-Jones et al., 2008; Kozomara and Griffiths-Jones, 2011; 2014). All the unique mature sequences of predicted miRNAs were used to blast against the customized database to detect if the miRNAs were previously annotated (Camacho et al., 2009).

All the sequences of mature sRNAs were used to count sRNAs expression in each sample. We used TMM (Robinson and Oshlack, 2010) to normalize the library sizes and RPM (reads per million) to describe the sRNAs expression. The differentially expressed sRNAs were called by edgeR (Robinson et al., 2010) using log(Fold change) >=1 (or <=-1) and FDR<0.05.

Fungal sRNA predictions

The fungal tRNAs, rRNAs and snoRNAs were removed from clean reads. The annotations of tRNAs, rRNAs and snoRNAs were extracted from Ensembl fungi (Goodwin et al., 2011; Kersey et al., 2018). The published genome reference of 3D7 was used in the analysis (Plissonneau et al., 2016). Then the filtered reads were used to predict fungal sRNAs with ShortStack, using the same strategy as described above for the plant. The expression of the fungal sRNAs were obtained as described above for the plant sRNAs.

SRNA target predictions and degradome analysis

The wheat and *Z. tritici* sRNAs upregulated or highly expressed (RPM>=100) at 7dpi or 12dpi were used to predict the target genes in wheat and *Z. tritici* transcriptome using psRNATarget (Dai et al., 2018) Default parameters from Schema V2 (2017 release) were used, but the expectation value was set at 4.5. Only the targeted genes that were significantly downregulated while the sRNAs showed upregulation or were constantly highly expressed were considered as the predicted target genes.

To confirm the cleavage of the predicted target genes, we generated modified PARE libraries with RNAs from the fungus (*in vitro*), infected wheat leaves (7dpi and 12dpi), and mock leaves at the same time points. The samples for the modified PARE libraries were the same ones used for the sRNA-seq libraries. Poly(A)+ RNA was isolated from the RNA samples. Illumina TruSeq sequencing adapter was ligated to the 5'P ends of the degraded mRNA. Next, first-strand cDNA synthesis was performed using a N6 randomized primer. After RNA hydrolysis, the 3' Illumina TruSeq sequencing adapter was ligated to the 5' ends of the antisense 1st-strand cDNA fragments. The 5' cDNA fragments were finally amplified with PCR using the Illumina TruSeq sequencing adapters as primers. The libraries were sequenced on an Illumina Next Seq 500 single end at 1x75bp using a Truseq SBS kit v3-HS (Illumina, Inc. San Diego, CA, USA).

The raw sequencing data were trimmed to remove adapters and trimmed length>=15 using Trimmomatic-0.36 (Bolger et al., 2014) with the settings "ILLUMINACLIP:TruSeq3-SE.fa:2:30:10 LEADING:10 TRAILING:10 SLIDINGWINDOW:4:15 MINLEN:15". The

238 clean reads were analyzed using CleaveLand4 (Brousse et al., 2014) with the parameters "-c 3
239 -p 0.05 -r 0.7" for every possible conditions. Only the results with a p value under 0.05 and a
240 minimum free energy (MFE) ratio higher than 0.7 were regarded as significant results. We only
241 considered the degradome peaks in Category 0, 1, 2 and 3. These categories are described in
242 Cleaveland 4.
243
244 **mRNA-seq sequencing and analysis**
245
246 The mRNA-seq data from the wheat samples infected with *Z. tritici* were generated in a
247 previous study (Palma-Guerrero et al., 2017) and they are available at the National Center for
248 Biotechnology Information (NCBI) Short Read Archive (SRA) under the project SRP077418.
249 The new mRNA-seq data from the 3D7 isolate grown *in vitro* and plant mocks at 7dpi, 12dpi
250 were generated in this study using Illumina HiSeq2500 at 2 X 125 bp (NCBI-SRA project
251 number SRP154808). All the raw sequencing reads were trimmed using Trimmomatic-0.36 as
252 described before. The clean reads were aligned against wheat genome and *Z. tritici* genome
253 separately using tophat v2.1.1 (Kim et al., 2013). HTSeq-count v0.6.1 was used to calculate
254 the gene counts (Anders et al., 2015). We used TMM (trimmed mean of M values)
255 implemented in Bioconductor EdgeR v3.12.0 to normalize the library sizes and gene
256 expressions (Robinson et al., 2010; Robinson and Oshlack, 2010). Differentially expressed
257 genes were defined using EdgeR. The Benjamin-Hochberg false discovery rate correction was
258 used to adjust P values based on the exact Fisher test (Robinson et al., 2010). The genes with
259 a log fold change >=1 (or <=-1) and adjusted p value <0.05 were determined as the
260 differentially expressed genes. The GO analysis were performed with TopGO v2.32.0 (Alexa
261 and Rahnenfuhrer, 2016).
262
263 **Results**
264
265 ***Z. tritici* produces sRNAs during wheat infection**
266
267 To explore the roles of RNAi in the wheat-*Z. tritici* pathosystem, we analyzed sRNA-seq
268 libraries from samples of wheat leaves infected by *Z. tritici* after 7dpi, 12dpi and 14dpi. The
269 corresponding wheat mock leaves at the same points, and *Z. tritici* grown *in vitro*, were used
270 as controls. Three biological replicates were used for each condition. According to the
271 description of the disease cycle (Rudd et al., 2015; Sanchez-Vallet et al., 2015), our dataset
272 covered the latent period (7dpi), transition period (12dpi) and the early necrotrophic period
273 (14dpi) of the 3D7 strain disease cycle (Palma-Guerrero et al., 2017).
274
275 Reads with similarity to tRNAs, rRNAs and snoRNAs of wheat and *Z. tritici* were filtered and
276 the remaining reads were aligned against the wheat and the *Z. tritici* genome separately to
277 distinguish the plant and fungal sRNA reads (Table 1). In total, we obtained 0.3 to 1.1 million
278 fungal sRNA reads in the infected samples and 1.8 to 2.8 million fungal sRNA reads in the *in*
279 *vitro* samples. Between 3.4 to 18.1 million plant sRNA reads were obtained in the infected
280 samples and 1.9 to 6.5 million plant sRNA reads in the mock samples. The number of fungal
281 sRNA reads increased during the infection process while the number of plant sRNA reads
282 decreased, which is consistent with the disease progression on the infected leaves.
283
284 Using a customized bioinformatic analysis (see the methods section), we predicted 662 unique
285 fungal sRNAs in total (Table S1) (Shahid and Axtell, 2014; Johnson et al., 2016). We
286 considered all the major RNAs predicted by ShortStack as the fungal sRNAs. The most
287 abundant fungal sRNAs were of 20 and 21 nucleotides (nt) in length (Figure 1A). The fungal

288 sRNAs were originated across the fungal genome, including both core and accessory
289 chromosomes, although most sRNAs came from chromosomes 1 and 3 (Figure 1B). Among
290 the total number of fungal sRNAs, 160 were originated from gene exon regions, 19 were from
291 gene intron regions, 343 were from transposable elements and 212 were from the intergenic
292 regions (Figure 1C).
293
294 **The composition of fungal sRNAs changes during the infection cycle**
295
296 We analyzed the differentially expressed fungal sRNAs between infected samples and *in vitro*
297 samples to identify the infection induced fungal sRNAs. We detected that 66 fungal sRNAs
298 were significantly upregulated in the infected samples compared to the *in vitro* samples (Figure
299 2A). These fungal sRNAs were considered as the infection induced fungal sRNAs and
300 therefore they may play important roles during plant infection. The number of induced fungal
301 sRNAs was only 16 at 7dpi, when the fungal pathogen was in the latent period and no
302 symptoms were detected on the leaves. But this number increased to 30 at 12dpi, when the first
303 chlorotic symptoms appeared on the leaves, and 59 at 14dpi, when necrotic lesions are clearly
304 observed on the leaves (Palma-Guerrero et al., 2017). Besides, there were 174 fungal sRNAs
305 significantly downregulated in the infection samples (Figure 2A).
306
307 The length and the nucleotide at 5' end of the sRNAs is considered to determine to which AGO
308 proteins are they binding, as it has been shown for Arabidopsis, and subsequently the sRNA
309 final function (Mi et al., 2008). In our analysis, the length distribution of the differentially
310 expressed fungal sRNAs between *in vitro* and infected samples were similar being 20/21nt
311 (Figure 1A, 2B). At the same time, most of the fungal sRNAs shared uracil (U) at 5' end, which
312 have been shown to bind preferentially to AGO1 in Arabidopsis (Figure 2C). Interestingly, the
313 proportion of U at 5' end changed dramatically, from 87% among all the sRNAs to 48% among
314 the infection induced sRNAs. Correspondingly, 23% of the infection induced fungal sRNAs
315 started with C, binding preferentially to Ago 5 in Arabidopsis, and 16.7% started with A,
316 binding preferentially to Ago2 and Ago3 in Arabidopsis (Figure 2C). This change indicates
317 that there is different fungal RNA accumulation between growth *in vitro* and *in planta*, and
318 that the sRNAs induced during infection could have different functions than the sRNAs
319 produced during *in vitro* growth being part of different AGOs/sRNA complexes.
320
321 ***Z. tritici* sRNAs were not detected to cleave wheat transcripts**
322
323 The cross-kingdom RNAi between plants and fungal pathogens has been shown in different
324 pathosystems (Zhang et al., 2011; Weiberg et al., 2013; Wang et al., 2016; Zhang et al., 2016;
325 Wang et al., 2017). To investigate the presence of cross-kingdom RNAi in the wheat-*Z. tritici*
326 pathosystem, we used the predicted fungal sRNAs to detect target genes in the wheat
327 transcriptome. We found that 33 fungal sRNAs were either highly expressed (above 100 RPM)
328 or upregulated at 7dpi or 12dpi, which were predicted to target wheat genes. Among these
329 targeted wheat genes, 139 were significantly downregulated during the infection (Table S2).
330
331 These 139 wheat genes were significantly enriched in chlorophyll related processes and
332 pigment biosynthesis (Figure 3A), which would affect photosynthesis, the leaf color, and the
333 plant defense ability (Menzies et al., 2016). This suggests that fungal sRNAs may regulate
334 plant chlorophyll related genes to reduce the plant defense ability. Interestingly, these targeted
335 wheat genes were enriched in "microtubule-based movement" biological process, which could
336 affect the plant intracellular transport and secretion, and therefore affect the plant response to
337 the pathogen infection (Lee et al., 2012). Besides these, fungal sRNAs were also predicted to

target wheat RLK genes and resistance genes, which also suggests that fungal sRNAs were trying to disturb the plant defense system (Table S3).

Next, we performed a degradome analysis to validate the cleavage of the candidate targeted genes. Three conditions were used in this analysis: *Z. tritici* grown in vitro, wheat leaves infected by *Z. tritici* (7dpi and 12dpi) and uninfected mock leaves at the same time points. To determine the quality of the degradome sequencing, we first analyzed several plant miRNAs that are already known to target plant genes. In our analysis, these plant miRNAs were predicted to target and cleave plant transcripts as previously reported, which confirmed the good quality of the degradome sequencing. For example, miRNA-uniq-21 (known as miR393) and miRNA-uniq-46 (known as miR156) were correctly found to cleave TIR1 genes and Squamosa promoter-binding-like (SPL) transcription factor genes respectively in our data (Figure 3B,C), as it has been previously reported (Addo-Quaye et al., 2009).

In the degradome analysis, only one wheat gene, TraesCS3B01G136500, was found to be cleaved and downregulated by fungal sRNA56 (Figure 3D). This wheat gene encodes a "Remodeling and spacing factor 1 (RSF-1)" protein, which is known to be involved in DNA double strand break repair in mammals (Pessina & Lowndes, 2014). SRNA56 was induced during infection, but being low expressed (Figure 3E). Additionally, the significantly reduced target, TraesCS3B01G136500, was constantly very low expressed (below 3 RPKM) and the degradome coverage of the cleaved transcripts was also low, which raises doubt about it being a real target (Figure 3D,F).

We also investigated the *RNAi* in *Z. tritici* during the disease cycle. In total, 39 fungal sRNAs were predicted to target and downregulate the fungal own genes during the infection (Table S4). But none of these genes were detected to be cleaved in the degradome sequencing. Finally, we analyzed the expressions of genes involved in RNAi in *Z. tritici*. There is only one dicer gene in the *Z. tritici* genome (g9428 from the 3D7 genome), which is consistent across five different sequenced *Z. tritici* genomes (Goodwin et al., 2011; Plissonneau et al., 2016; Plissonneau et al., 2018). Surprisingly, this dicer gene was significantly downregulated *in planta* compared with *in vitro* (Figure 4A). Additionally, the sRNAs were more abundant *in vitro* than *in planta*, which could be caused by the low expression of the fungal Dicer gene (Figure 4B). There are four Ago genes in the *Z. tritici* genome (g4308, g1839, g10449, and g2094 of the 3D7 genome), among which the Ago gene g2094 was not expressed in any condition tested. We as well did not find any significant upregulation of AGO genes in *Z. tritici* during infection (Figure 4A).

Wheat regulates sRNAs in response to fungal infections

We used the same dataset to identify the plant sRNAs and to study the roles of plant sRNAs in the disease cycle. In total, we identified 158 wheat miRNAs and 1120 wheat siRNA loci (Table S5). By blasting these predicted miRNAs against the known wheat miRNAs, we found 69 miRNAs that were already annotated in the wheat genome. The other 89 miRNAs were considered as novel wheat miRNAs (Table S5). Most wheat predicted miRNAs were 21nt in length, consistent with previous studies (Figure 5A) (Rajagopalan et al., 2006; Axtell, 2013). The majority of these plant miRNAs start with U at 5' end (Figure 5B) which should favor binding the plant AGO1 proteins (Mi et al., 2008). Unlike the miRNAs, most of the wheat siRNAs were 24nt in length and started with A (Figure 5A,B). These 24nt siRNAs have been shown to act by RdDM in Arabidopsis (Matzke and Mosher, 2014), being preferentially loaded in AGO4 (Mi et al., 2008).

Plants can regulate the sRNAs, especially miRNAs, to respond to pathogens, which is thought to be a fast and efficient immune response (Fei et al., 2013). In order to determine if the infection induces variations in sRNAs accumulation, we compared the expression of all the plant sRNAs between infected and mock samples at every time point. Like for the fungus, the infection induced plant sRNAs also exhibited different frequencies of nucleotides at 5'end (Figure 5B), which suggests a different sRNA production during infections. We found 191 plant sRNAs (27 miRNAs and 164 siRNAs) significantly downregulated during the infections (Figure 5C, Table S6). Interestingly, these downregulated plant sRNAs were predicted to target 122 disease resistance genes and 214 genes encoded RLKs in mocks (Table S7). Two RLK genes were detected to be cleaved by siRNA191 (Figure 6).

Conversely, 191 plant sRNAs were significantly upregulated in the infections, among which 39 were miRNAs (15 novel miRNAs) and 152 were siRNAs (Figure 5C, Table S6). Among these induced or extremely highly expressed (RPM above 100) plant sRNAs, 176 sRNAs (47 miRNAs and 129 siRNAs) were predicted to target and silence 690 wheat genes (Table S8). Interestingly, 20 (3%) of these candidates targeted genes were resistance genes and 77 (11.2%) were receptor like kinases (RLKs), which are considered to play central roles in plant immunity. Besides, 12 (2%) genes encode ABC transporters and 11 (1.5%) genes encode Auxin response factor which are also genes involved in the plant immune system (Figure 7A).

We confirmed the cleavage of 5 transcripts among these 690 genes (Figure 7, S1). MiRNA-uniq-133 (miRNA160 family) was detected to silence TraesCS6A01G222300.1 which encodes an auxin response factor (Figure 7B). Two Squamosa promoter-binding-like gene (SPL) were negatively regulated by miRNA-uniq-113 (belonging to miRNA156 family), which was dramatically upregulated at 7dpi and 12dpi (Figure 7C,D). Jasmonate zim-domain (JAZ) protein, which negatively regulates Jasmonate (JA) signaling in plants, was silenced by siRNA180 at 7dpi (Figure 7E). SiRNA60 was also detected to target TraesCS3D01G339300.1, which encodes potassium transporter. But the low expression of siRNA60 and the high category of the degradome peak (in Category 3) suggests that it was not a real target (Figure S2). These findings suggest that wheat induced sRNAs to regulate plant gene expressions as a response to *Z. tritici* infections.

In plant-pathogen interactions, plants can as well export miRNAs into pathogen cells to influence pathogen virulence (Wang et al., 2016). We predicted 190 wheat siRNAs that were upregulated during the infections or extremely highly expressed (RPM above 100), and could target and downregulate 1115 fungal genes (Table S9). Interestingly, these targeted fungal genes were significantly enriched in "vesicle-mediated transport" GO term (Figure 8). We detected that two of these 1115 fungal genes could be cleaved by wheat sRNAs (Figure S3). One gene is g1915, which encodes an Antibiotic biosynthesis monooxygenase domain (ABM) protein. The other gene is g9791, which encodes FAD dependent oxidoreductase. These two fungal genes were only downregulated at the beginning of the disease cycle and recovered their expressions from 14dpi (Figure S3). However, it has to be noted that the degradome coverage over those genes was very low and the slice site of g1915 transcript belong to Category 3 (Figure S3), which suggests that is not a real target.

Discussion

sRNAs have been shown to play important roles in plant-pathogen interactions, where RNAi is triggered naturally in a cross-kingdom manner (Nowara et al., 2010; Weiberg et al., 2013;

438 Wang et al., 2016; Zhang et al., 2016; Wang et al., 2017; Cai et al., 2018). Here we combined
439 sRNA-Seq, mRNA-Seq and degradome sequencing from samples covering key stages during
440 the infection cycle to analyze the bidirectional RNAi in the wheat and *Z. tritici* pathosystem.
441 We analyzed all fungal sRNAs and plant sRNAs in the infection samples, as well as in the
442 fungus growing *in vitro* and non-infected plant mocks. By comparing the expression of sRNAs
443 between infection and control samples, we found the infection induced sRNAs for both the
444 fungus and the plant. The composition of these infection induced sRNAs changed during
445 infections, suggesting that different sRNAs were induced or that they are loaded into different
446 Ago proteins. By using degradome sequencing to validate the target predictions, we did not
447 find a clear evidence that fungal sRNAs could cleave wheat defense related genes. However,
448 our results suggest that wheat can regulate sRNAs in response to *Z. tritici* infections.
449
450 **RNAi does not play important roles in *Z. tritici* during wheat infection**
451 Fungal sRNAs can hijack the plant host RNAi machinery to silence plant defense related genes
452 and facilitate fungal infection (Weiberg et al., 2013; Wang et al., 2017). In our analysis, the
453 predicted targets of the fungal sRNAs induced during wheat infections were significantly
454 enriched in chlorophyll biosynthesis (Figure 3A). The inhibition of chlorophyll biosynthesis is
455 consistent with the chlorotic symptoms observed from 12dpi. Chloroplasts are essential for the
456 response to multiple environmental stimuli and for the biosynthesis of plant hormones
457 (Shapiguzov et al., 2012). Besides, chloroplast can also produce reactive oxygen species (ROS)
458 as an immune response to pathogens (Galvez-Valdivieso and Mullineaux, 2010). To
459 successfully infect plants, pathogens can export effectors to target chloroplast to prevent
460 chloroplastic ROS (Zabala et al., 2015). Here, we suggest that fungal sRNAs could target
461 chlorophyll genes to disturb plant immunity. Also, the wheat genes targeted by the induced
462 fungal sRNAs were also enriched in "microtubule-based movement" (Figure 3A). Plant
463 microtubules need to be reorganized in response to symbiotic and pathogenic organisms to
464 allow the proper intracellular trafficking activated in response to pathogen infection (Hardham,
465 2013). By disturbing the plant microtubules reorganization, may facilitate the fungal infection.
466 Additionally, we found that plant RLKs were predicted to be targeted by fungal sRNAs (Table
467 S3). These findings provide the possibility that the fungal sRNAs may be transported into plant
468 cells and affect the plant immunity system. But all of these wheat genes mentioned above were
469 not detected to be cleaved in the degradome analysis. We only detected one wheat gene that
470 may be cleaved by fungal sRNAs. The wheat gene was TraesCS3B01G136500 and encodes a
471 RSF-1 protein. (Figure 3D). But the expression of the corresponding fungal sRNA56 was very
472 low (Figure 3E,F). Additionally, the frequency of the cleaved transcript from
473 TraesCS3B01G136500 was very low. All these results suggest that this wheat gene was not a
474 real target, at least in the canonical way.
475
476 We also predicted 39 fungal sRNA that could regulate fungal genes *in planta*. But none of
477 these targeted fungal genes were detected to be cleaved in the degradome sequencing. The
478 fungal Dicer gene was barely expressed in the infections, which leaded to a decrease of sRNA
479 genesis by the fungus (Figure 4). This is supported by the lower expressions of sRNAs
480 observed *in planta* than *in vitro*. In addition, deletion of the *Z. tritici* Dicer gene has been found
481 to have no effect on fungal virulence, and it failed to block the disease (Kettles et al., 2018).
482 These results, together with our findings, suggest minor roles of the *Z. tritici* Dicer for fungal
483 virulence. Moreover, we did not find any strong evidence that fungal sRNAs could cleave plant
484 transcripts. These data suggest that fungal sRNAs are not playing important roles in PTGS
485 during the wheat-*Z. tritici* interaction. However, even if the *Z. tritici* sRNAs do not play a direct
486 role in the interaction with wheat, they may play a role at specific developmental stages of the
487 fungus, as it has been shown for other plant pathogenic fungi (Kim et al., 2015; Raman et al.,

488 2017; Zeng et al., 2018). Also, we cannot discard the possibility that *Z. tritici* sRNAs may be
489 acting at the transcriptional or translational level.
490
491 **Wheat sRNAs were induced in response to *Z. tritici***
492 Previous studies have reported that plants can employ sRNAs as a rapid defense response
493 against pathogens. MiRNA393 is induced to target auxin related genes and repress auxin
494 signaling in response to bacterial pathogens (Achard et al., 2007; Chen et al., 2007; Navarro et
495 al., 2008; Grant and Jones, 2009). MYB transcription factors, which are involved largely in
496 plant development and defense against abiotic and biotic stress, can also be targeted and
497 regulated by the plant sRNAs (Samad et al., 2017). Additionally, plants also regulate the
498 expressions of resistance genes by sRNAs under the pathogen attack (Zhai et al., 2011;
499 Shivaprasad et al., 2012; Fei et al., 2013).
500
501 Here we found that 191 wheat sRNAs were downregulated during the fungal infections (Table
502 S6). These downregulated wheat sRNAs were predicted to target R genes and RLKs in mocks
503 (Table S7). Additionally, the cleavage of the transcripts from two RLKs were confirmed in the
504 degradome analysis (Figure 6). These results suggest that during the infections, wheat could
505 downregulate specific sRNAs triggering the upregulation of the target genes, especially the R
506 genes and RLKs, and increase the plant defense ability in response to *Z. tritici* infections.
507
508 We also found that 191 wheat sRNAs were induced during the fungal infection (Figure 5C,
509 Table S6). The composition of these induced sRNAs were quite different to the plant sRNAs
510 expressed in the mocks (Figure 5B). Among these, 176 sRNAs (47miRNAs and 129 siRNAs)
511 were predicted to target and silence 690 wheat genes (Table S8). These wheat genes included
512 R genes, RLKs, ABC transporters and Auxin response factors, which are known to play central
513 roles in the plant immunity (Figure 7A). These induced sRNA regulation of defense related
514 genes suggests an immune response in wheat against *Z. tritici*. Besides, many transcription
515 factor genes were also predicted to be targeted by these induced sRNAs, including SPL
516 transcription factors, F-box domain proteins, MYB transcription factors and basic/helix-loop-
517 helix (bHLH) proteins (Figure 7A). These results suggest that transcription factors can also be
518 regulated by plant sRNAs as a response to the fungal pathogens. We confirmed the cleavage
519 of five transcripts among these 690 wheat genes. They encode one auxin response factor, two
520 SPL proteins, one JAZ protein and one potassium transporter (Figure 7). The induced sRNA
521 mediated auxin related gene here is consistent with the response observed during plant
522 infection by bacterial pathogens (Navarro et al, 2008). When plants are under biotic stress,
523 sRNAs are induced to regulate transcription factors as an immune response (Tsuda and
524 Somssich, 2015). Here we confirmed that SPL transcription factors are mainly involved in this
525 sRNA mediated immune response. Besides, by silencing the JAZ gene with the induced sRNAs,
526 the plant can trigger an enhance JA signaling as an immune response against the fungal
527 pathogen. Thus, we show clear evidence that wheat will induce a group of specific sRNAs to
528 regulate wheat genes as an immune response to *Z. tritici*.
529
530 In HIGS, plants can transport RNA modules and target pathogen genes to reduce pathogen
531 virulence (Nowara et al., 2010; Baulcombe, 2015). It has been proved recently that plants use
532 extracellular vesicles to send sRNAs into fungal cells (Cai et al., 2018). These finding suggest
533 that the RNA molecules can be transported to fungal pathogens naturally. We predicted 1115
534 fungal genes that could be targeted by 190 wheat sRNAs. These wheat sRNAs were either
535 upregulated or extremely highly expressed during the infections (Table S9). Interestingly, the
536 targeted fungal genes were enriched in the "vesicle-mediated transport" GO term (Figure 8).
537 Fungal Extracellular Vesicles (EVs) are essential to transport proteins, glycans pigments,

538 nucleic acids and lipids (Rodrigues et al., 2015; Rodrigues and Casadevall, 2018). Fungal EVs
539 are also responsible to pathogenesis during the infection (Rodrigues et al., 2007; Albuquerque
540 et al., 2008; Rodrigues et al., 2008; Vallejo et al., 2011; Vargas et al., 2015), including
541 mediating the transport of virulent effectors into plant cells (Rodrigues et al., 2008). A recent
542 study also proved that plant miRNAs can be transported by EVs into fungal cells to affect
543 fungal virulence (Cai et al., 2018). Here our results suggest that wheat sRNAs may also target
544 fungal EVs related genes to interfere fungal virulence. However, we only detected two fungal
545 genes that were cleaved by the wheat sRNAs (Figure S3). Fungal g1915, which encodes an
546 ABM protein, involved in diverse biological processes, including metabolism, transcription,
547 translation and biosynthesis of secondary metabolites was cleaved by plant siRNA180. The
548 other one is fungal g9791, which encodes FAD dependent oxidoreductase, was targeted by
549 plant miRNA-uniq-21. These two fungal genes play fundamental roles in fungal growth and
550 they are downregulated at the beginning of the disease cycle. However, these two fungal genes
551 were not completely silenced, and the expression of these two genes was recovered at 14dpi,
552 when the fungus started the necrotrophic growth phase. But as we discussed before, the
553 degradome results did not provide support for the cleavage of the transcripts from these two
554 genes. These results suggest that wheat is not able to use sRNAs to silence fungal genes. These
555 results are supported by the finding that dsRNAs generated from RNA virus vectors *in planta*
556 are not effective in triggering gene silencing in *Z. tritici* during the fungal infection (Kettles et
557 al., 2018).
558
559 In conclusion, our results suggest that there is no natural cross-kingdom RNAi by PTGS
560 between these two interacting species. But we found that wheat can use sRNAs to regulate the
561 plant defenses during *Z. tritici* infection. These findings contribute to improve our
562 understanding of this pathosystem.
563
564
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577
578 **Authors' contribution**
579
580 X.M., N.B., and J.P.-G. conceived and designed experiments. X.M. was responsible for the
581 bioinformatics analysis. X.M., N.B. and J.P.-G. wrote the paper.
582

Conditions	Library ID	Total clean reads	Fungal sRNA reads	Fungal sRNA ratios	Plant sRNA reads	Plant sRNA ratios
Infection samples	3D7-7dpi-2	71,764,255	339,262	0.47%	18,186,405	25.34%
	3D7-7dpi-8	75,365,538	385,127	0.51%	17,763,333	23.57%
	3D7-7dpi-10	48,440,536	306,834	0.63%	9,039,173	18.66%
	3D7-12dpi-2	65,969,573	460,119	0.70%	12,378,083	18.76%
	3D7-12dpi-5	65,138,243	653,052	1.00%	10,109,163	15.52%
	3D7-12dpi-6	45,790,107	317,915	0.69%	10,725,249	23.42%
	3D7-14dpi-4	31,090,290	901,839	2.90%	3,783,930	12.17%
	3D7-14dpi-5	34,372,386	577,053	1.68%	5,365,083	15.61%
	3D7-14dpi-10	30,549,120	1,108,689	3.63%	3,406,298	11.15%
Fungal *in vitro*	3D7-vitro-1	16,547,092	2,865,958	17.32%	----------	----------
	3D7-vitro-2	12,318,911	2,191,788	17.79%	----------	----------
	3D7-vitro-3	9,515,263	1,819,328	19.12%	----------	----------
Plant mocks	mock-7dpi-C1	17,166,095	----------	----------	4,586,381	26.72%
	mock-7dpi-C2	21,565,083	----------	----------	5,321,432	24.68%
	mock-7dpi-C3	24,379,228	----------	----------	6,568,124	26.94%
	mock-12dpi-C1	20,048,211	----------	----------	3,884,980	19.38%
	mock-12dpi-C2	23,466,121	----------	----------	4,787,936	20.40%
	mock-12dpi-C4	14,114,494	----------	----------	3,065,456	21.72%
	mock-14dpi-C1	8,993,244	----------	----------	1,902,557	21.16%
	mock-14dpi-C2	28,168,941	----------	----------	6,222,211	22.09%
	mock-14dpi-C4	11,895,554	----------	----------	2,884,932	24.25%

583

584 Table 1. SRNA-seq data summary. sRNA ratios represent the percentage of fungal or plant

585 sRNAs among the total amount of clean reads.

586

587

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Figure legends

Figure 1. Predictions of the fungal sRNAs. (A) Length distribution of the predicted fungal sRNAs. The x-axis indicates the lengths of the sRNAs and the y-axis indicates the counts of the sRNAs. (B) Distribution of the fungal sRNAs across the fungal genome. The outer layer shows the chromosomes of the fungal genome. The red colors are the core chromosomes and the blue colors are the accessory chromosomes. Accumulation of fungal sRNAs (log2 transformed) are shown in the middle layer. The inner layer shows the genomic elements where fungal sRNAs come from. The green ones reveal the sRNAs comes from gene region, the orange ones from TE and the pink ones from the overlap of gene and TE region. (C) Origin of the fungal sRNAs. The different colors reveal the genomic elements where the fungal sRNAs come from. The sense means the sRNAs come from the same strand with the genomic elements while the antisense means the opposite strand.

Figure 2. Upregulated sRNAs during the infection cycle. (A) Number of the differentially expressed (DE) fungal sRNAs during the infection cycle. "Total" indicates the total number of DE fungal sRNAs across all the time points. (B) Length distributions of the DE fungal sRNAs. (C) Frequency of nucleotides at 5' end of the fungal sRNAs.

Figure 3. Degradome analysis of the fungal sRNAs. (A) GO enrichment of biological process of wheat genes that were downregulated and predicted to be targeted by fungal induced sRNAs during the infections. (B, C) Positive controls of degradome sequencing results. The x-axis indicates the position of the target transcript and the y-axis indicates the degraded frequency of the target transcript. The red point and red arrow indicate the slice site from the transcript and the sRNA. The P value indicates the significance of the target. (D) Degradome results of fungal sRNA56 targeting plant gene. (E,F) Expressions of fungal sRNA56 and its target gene in plant. The asterisk indicates that the sRNA or the target genes are differentially expressed compared to the *in vitro* or the Mock conditions respectively.

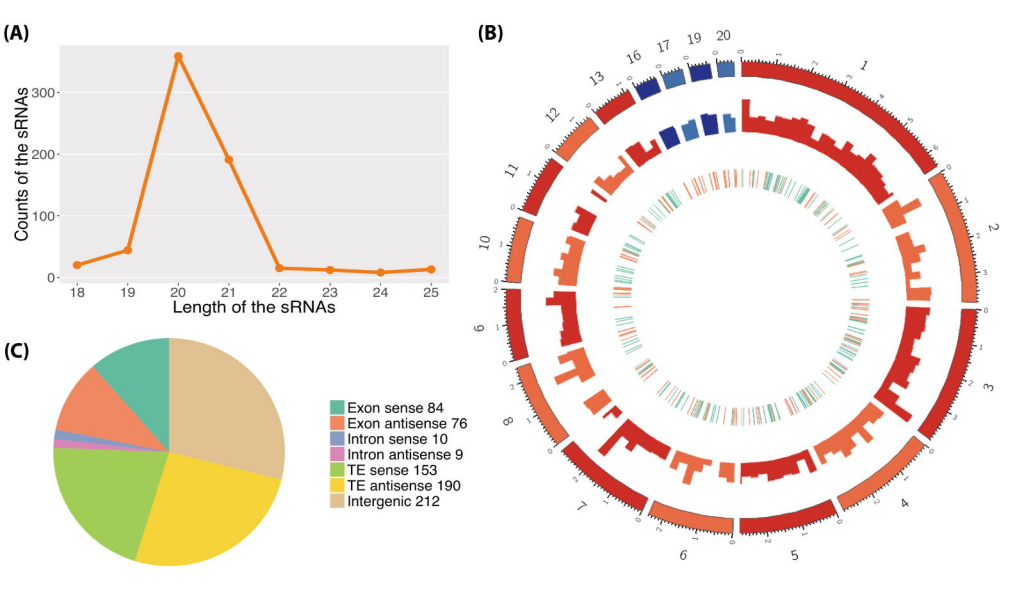
Figure 4. Fungal sRNAs are more abundant *in vitro* than *in planta*. (A) Expression of the fungal Dicer gene and three AGO genes. One asterisk indicates that the adjusted p value is under 0.05 and three asterisks indicates that the p value is under 0.001. ns indicates no significant difference. (B) Average expression of all the fungal sRNAs *in planta* and *in vitro*.

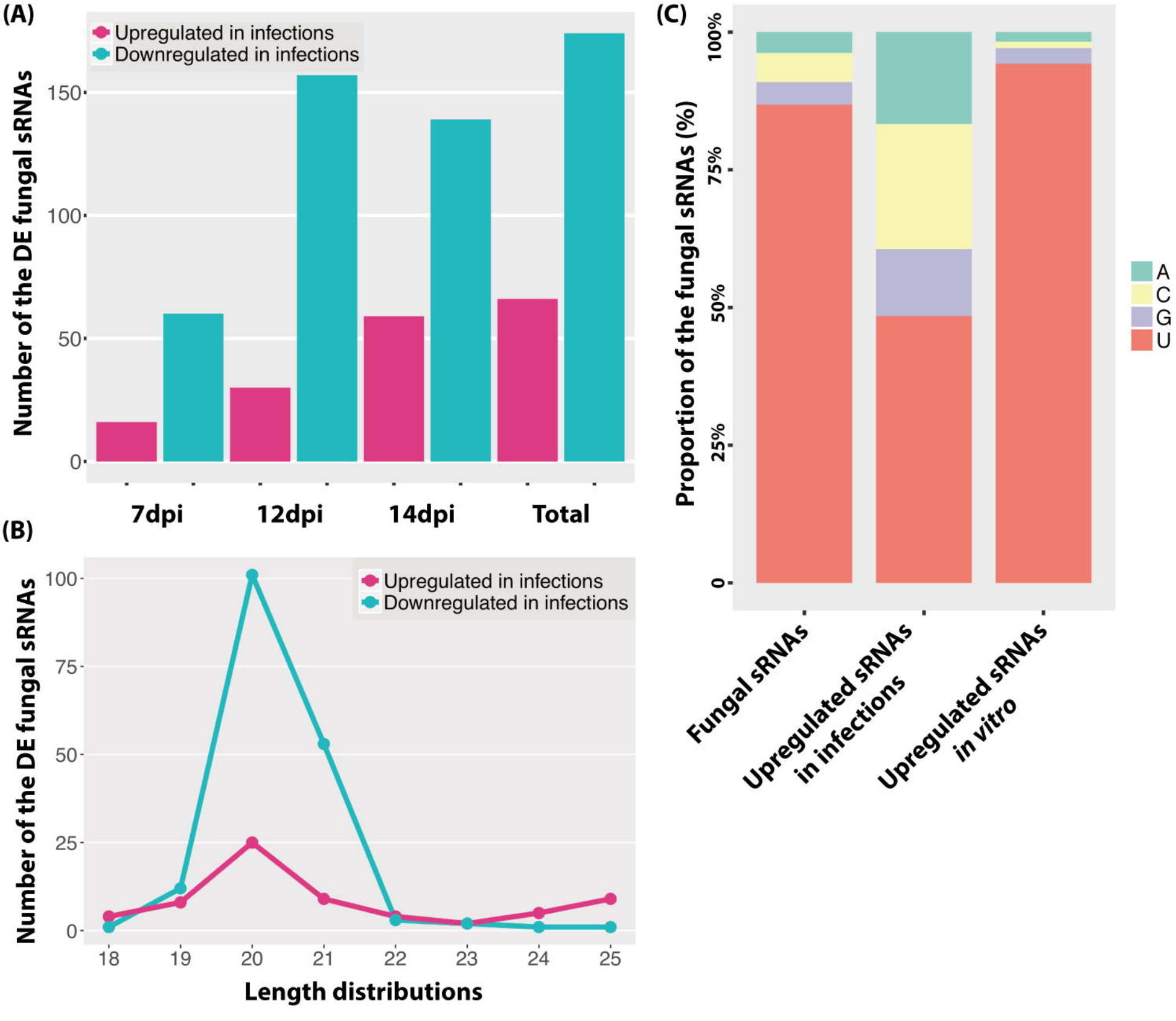
Figure 5. Predicted plant sRNAs. (A) Length distribution of the predicted plant sRNAs. (B) Frequency of nucleotides at 5' end of plant sRNAs. Different colors indicate different nucleotides. (C) Number of the DE sRNAs between infections and mocks. Upregulated indicates the sRNAs are upregulated in the infections and Downregulated indicates the sRNAs are downregulated in the infections.

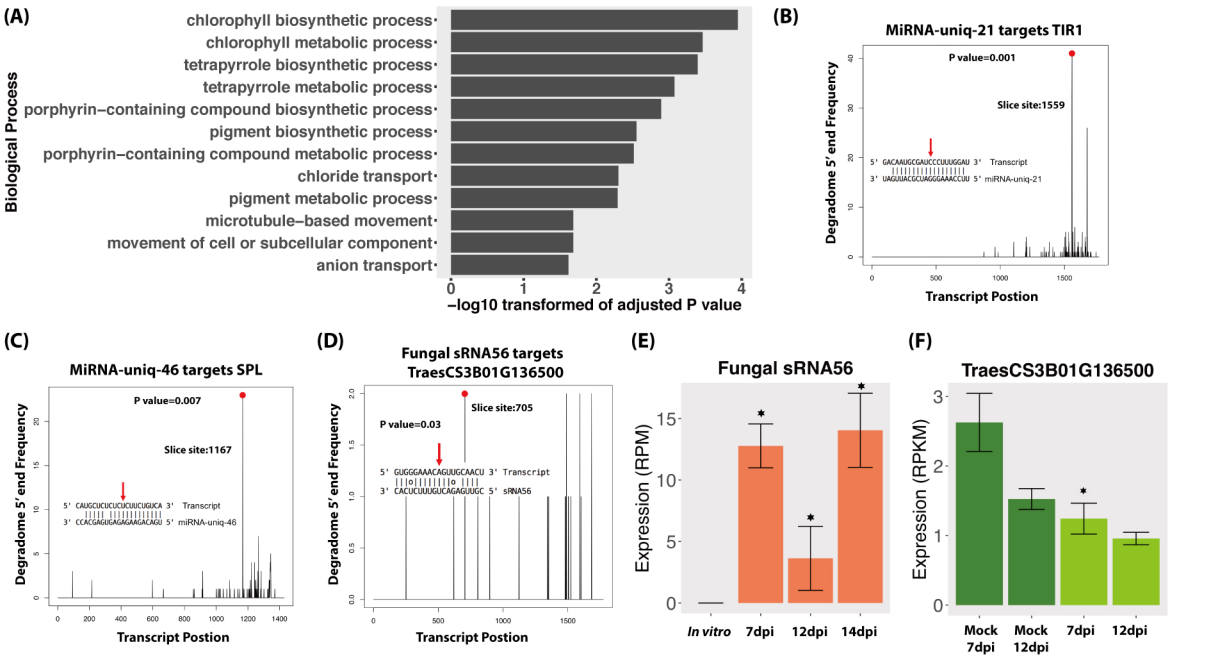
Figure 6. **Wheat downregulates siRNA191 to upregulate two wheat RLK during the infections.** (A) Degradome results of siRNA191 and its target genes in plant. (B) Expressions of siRNA191 and its target genes in plant. The asterisk indicates that the adjusted p value is under 0.05.

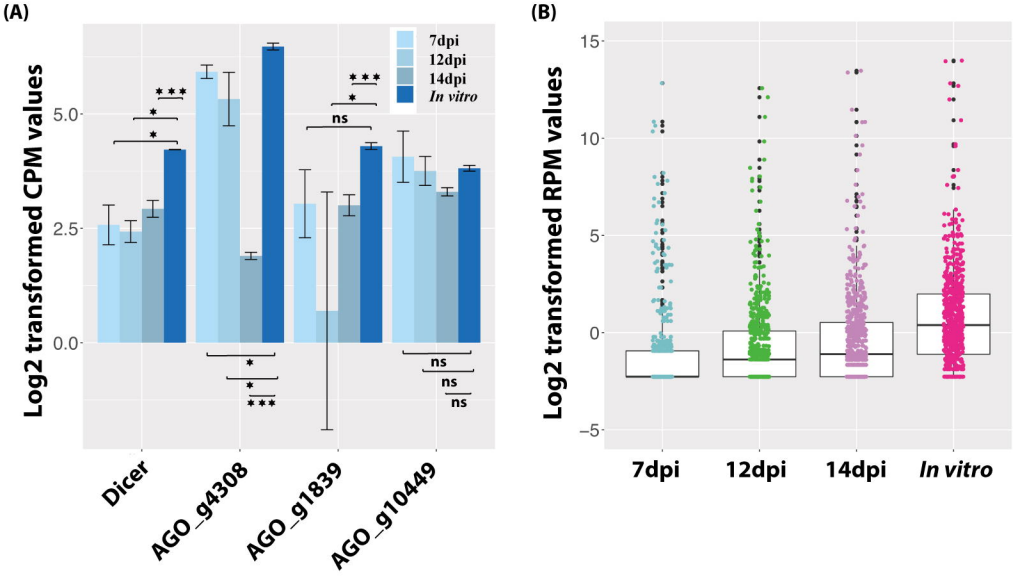
Figure 7. Wheat induces sRNAs to regulate wheat genes as an immune response against *Z. tritici*. (A) The functions of the wheat genes, which were downregulated and predicted to be targeted by wheat induced sRNAs. (B,C,D,E) Degradome analysis of the wheat genes targeted by miRNA-uniq-133, miRNA-uniq-113 and siRNA180.

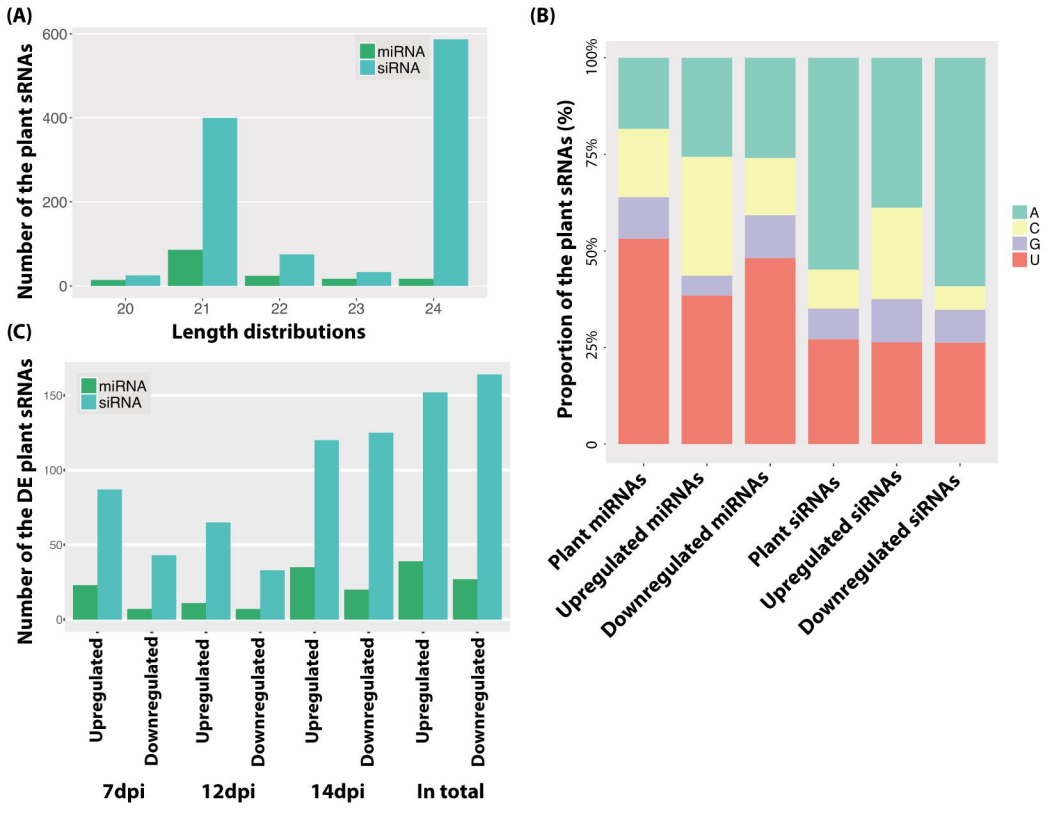
943 **Figure 8.** GO enrichment of fungal genes, which were downregulated and predicted to be
944 targeted by induced plant sRNAs during the infection.
945
946 **Supplementary information**
947
948 **Figure S1. The expressions of wheat miRNA-uniq-133, miRNA-uniq-113, siRNA180 and**
949 **siRNA60 and their target genes in wheat genome**.
950
951 **Figure S2. Degradome results of wheat siRNA60 and its target gene in plant.**
952
953 **Figure S3. Degradome result and expressions of two wheat genes and their target genes**
954 **in fungi.**
955
956 **Table S1. Predicted sRNAs in *Z. tritici.***
957
958 **Table S2. Differentially expressed fungal sRNAs between *in planta* and *in vitro* growth.**
959
960 **Table S3. Induced or highly expressed fungal sRNAs that target and downregulate wheat**
961 **genes.**
962
963 **Table S4. Induced or highly expressed fungal sRNAs that target and downregulate fungal**
964 **genes *in planta.***
965
966 **Table S5. Predicted miRNAs and siRNAs in wheat.**
967
968 **Table S6. Differentially expressed wheat sRNAs.**
969
970 **Table S7. The downregulated wheat sRNAs and their target upregulated wheat genes in**
971 **the infections.**
972
973 **Table S8. Induced or highly expressed wheat sRNAs that target and downregulate wheat**
974 **genes in the infections.**
975
976 **Table S8. Induced or highly expressed wheat sRNAs that target and downregulate fungal**
977 **genes in the infections.**

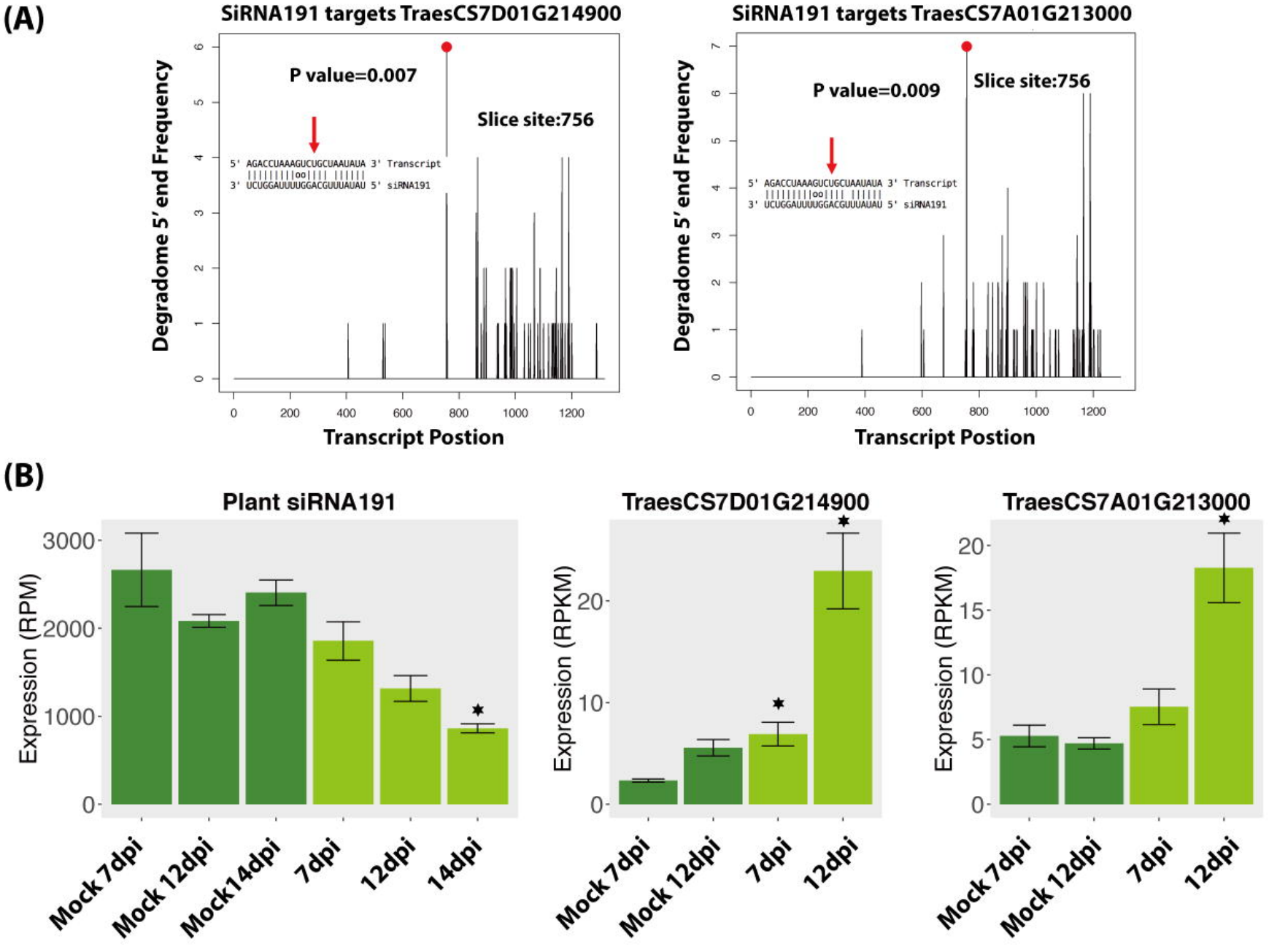

(A)
Counts of the sRNAs
300
200
100
0
18
19
20
21
22
23
24
25
Length of the sRNAs
(B)
(C)
Exon sense 84
Exon antisense 76
Intron sense 10
Intron antisense 9
TE sense 153
TE antisense 190
Intergenic 212


(A)
Upregulated in infections
Downregulated in infections
Number of the DE fungal sRNAs
0
50
100
150
7dpi
12dpi
14dpi
Total
(B)
Upregulated in infections
Downregulated in infections
Number of the DE fungal sRNAs
0
25
50
75
100
18
19
20
21
22
23
24
25
Length distributions
(C)
Proportion of the fungal sRNAs (%)
0
25%
50%
75%
100%
A
C
G
U
Fungal sRNAs
Upregulated sRNAs in infections
Upregulated sRNAs in vitro


(A)
Biological Process
chlorophyll biosynthetic process
chlorophyll metabolic process
tetrapyrrole biosynthetic process
tetrapyrrole metabolic process
porphyrin−containing compound biosynthetic process
pigment biosynthetic process
porphyrin−containing compound metabolic process
chloride transport
pigment metabolic process
microtubule−based movement
movement of cell or subcellular component
anion transport
0
1
2
3
4
−log10 transformed of adjusted P value
(B)
MiRNA-uniq-21 targets TIR1
P value=0.001
Slice site:1559
5' GACAAUGCGGAUCCCUUUGGAU 3' Transcript
3' UAGGUAGCGCUAGGGAAACCUU 5' miRNA-uniq-21
Degradome 5' end Frequency
Transcript Postion
(C)
MiRNA-uniq-46 targets SPL
P value=0.007
Slice site:1167
5' CAUGCUCUCUCUCUUCUGUCA 3' Transcript
3' CCACGAGUGAGGAAGACAGU 5' miRNA-uniq-46
Degradome 5' end Frequency
Transcript Postion
(D)
Fungal sRNA56 targets
TraesCS3B01G136500
P value=0.03
Slice site:705
5' GUGGGAAACAGUUGCAACU 3' Transcript
3' CACUCUUUGUCAGAGUUGC 5' sRNA56
Degradome 5' end Frequency
Transcript Postion
(E)
Fungal sRNA56
Expression (RPM)
In vitro
7dpi
12dpi
14dpi
(F)
TraesCS3B01G136500
Expression (RPKM)
Mock 7dpi
Mock 12dpi
7dpi
12dpi


(A)
Log2 transformed CPM values
7dpi
12dpi
14dpi
In vitro
5.0
2.5
0.0
***
*
*
*
***
ns
*
*
***
ns
ns
ns
Dicer
AGO_g4308
AGO_g1839
AGO_g10449
(B)
Log2 transformed RPM values
15
10
5
0
−5
7dpi
12dpi
14dpi
In vitro


(A)
Number of the plant sRNAs
600
400
200
0
miRNA
siRNA
20
21
22
23
24
Length distributions
(B)
Proportion of the plant sRNAs (%)
100%
75%
50%
25%
0
A
C
G
U
Plant miRNAs
Upregulated miRNAs
Downregulated miRNAs
Plant siRNAs
Upregulated siRNAs
Downregulated siRNAs
(C)
Number of the DE plant sRNAs
150
100
50
0
miRNA
siRNA
Upregulated
Downregulated
Upregulated
Downregulated
Upregulated
Downregulated
Upregulated
Downregulated
7dpi
12dpi
14dpi
In total


(A)
SiRNA191 targets TraesCS7D01G214900
P value=0.007
Slice site:756
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3' UCUGGAUUUUGGACGUUUAUAU 5' siRNA191
Degradome 5' end Frequency
Transcript Postion
SiRNA191 targets TraesCS7A01G213000
P value=0.009
Slice site:756
5' AGACCUAAAGUCUGCUAAUAUA 3' Transcript
3' UCUGGAUUUUGGACGUUUAUAU 5' siRNA191
Degradome 5' end Frequency
Transcript Postion
(B)
Plant siRNA191
Expression (RPM)
Mock 7dpi
Mock 12dpi
Mock14dpi
7dpi
12dpi
14dpi
TraesCS7D01G214900
Expression (RPKM)
Mock 7dpi
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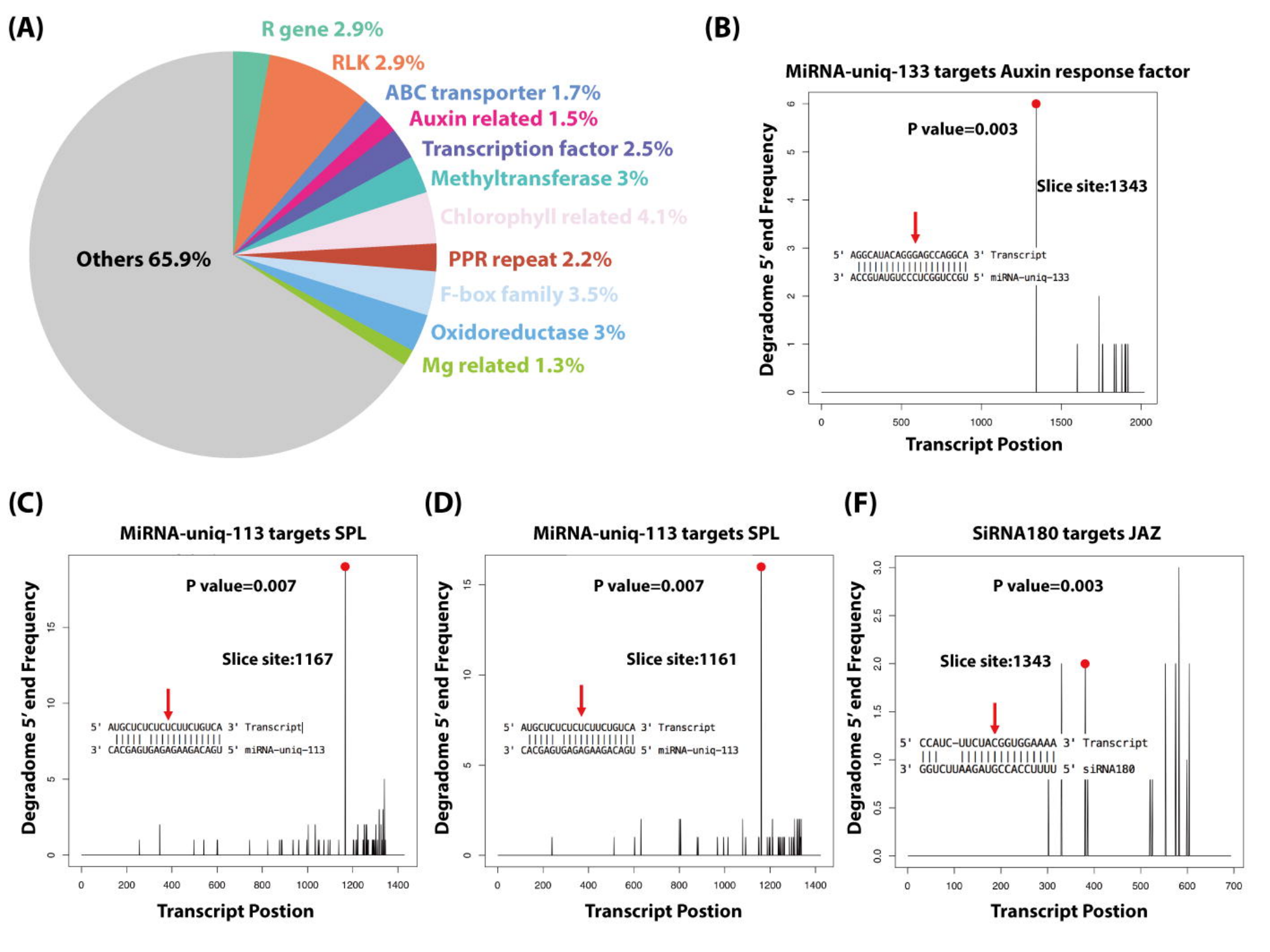
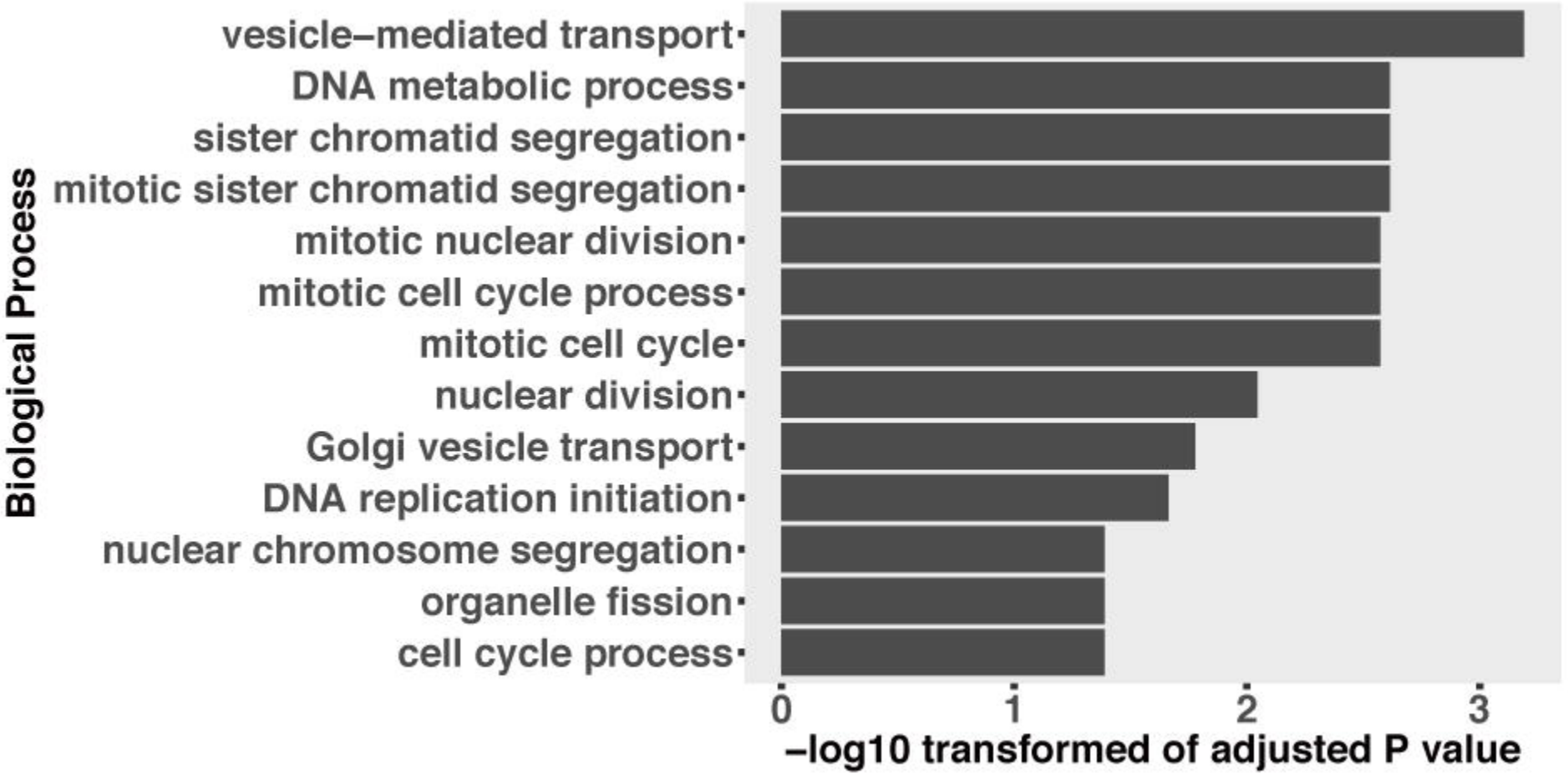
Mock 12dpi
7dpi
12dpi


(A)
R gene 2.9%
RLK 2.9%
ABC transporter 1.7%
Auxin related 1.5%
Transcription factor 2.5%
Methyltransferase 3%
Chlorophyll related 4.1%
PPR repeat 2.2%
F-box family 3.5%
Oxidoreductase 3%
Mg related 1.3%
Others 65.9%
(B)
MiRNA-uniq-133 targets Auxin response factor
P value=0.003
Slice site:1343
5' AGGCAUACAGGGAGCCAGGCA 3' Transcript
3' ACCGUAUGUCCCUCGGUCCGU 5' miRNA-uniq-133
Degradome 5' end Frequency
Transcript Postion
(C)
MiRNA-uniq-113 targets SPL
P value=0.007
Slice site:1167
5' AUGCUCUCUCUCUUCUGUCA 3' Transcript
3' CACGAGUGAGAGAAGACAGU 5' miRNA-uniq-113
Degradome 5' end Frequency
Transcript Postion
(D)
MiRNA-uniq-113 targets SPL
P value=0.007
Slice site:1161
5' AUGCUCUCUCUCUUCUGUCA 3' Transcript
3' CACGAGUGAGAGAAGACAGU 5' miRNA-uniq-113
Degradome 5' end Frequency
Transcript Postion
(F)
SiRNA180 targets JAZ
P value=0.003
Slice site:1343
5' CCAUC-UUCUACGGUGGAAAA 3' Transcript
3' GGUCUUAAGAUGCCACCUUUU 5' siRNA180
Degradome 5' end Frequency
Transcript Postion


Biological Process
vesicle−mediated transport
DNA metabolic process
sister chromatid segregation
mitotic sister chromatid segregation
mitotic nuclear division
mitotic cell cycle process
mitotic cell cycle
nuclear division
Golgi vesicle transport
DNA replication initiation
nuclear chromosome segregation
organelle fission
cell cycle process
0
1
2
3
−log10 transformed of adjusted P value