Potential mechanisms of quantitative resistance to *Leptosphaeria maculans* (blackleg) on cotyledons of canola (*Brassica napus*)

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Abstract

**Background:** Blackleg disease, caused by *Leptosphaeria maculans* (*Lm*), can lead to significant losses of canola/rapeseed crops. Growing resistant canola cultivars can be an effective and environmentally friendly way to manage blackleg. Major resistance genes may stop infection, but can also be rapidly overcome by shifts in pathogen population towards virulence. Thus, using race-nonspecific or quantitative resistance (QR) is of interest because it is potentially more durable.

However, the mechanisms and genes underlying QR are mostly unknown. In this study, we explored QR in “74-44 BL”, a Canadian canola cultivar carrying a moderate level of race nonspecific resistance, based on cotyledon inoculation (Supple. Fig.1) . The susceptible cultivar “Westar” was used as a control. Lesions developed more slowly on 74-44BL than on Westar. We used RNA-Seq to identify genes and gene functions putatively involved in the QR.

**Results:** Relative to inoculated Westar, some of the *B. napus* genes that were differentially expressed strongly in inoculated 74-44 BL included those putatively involved in programmed cell death (PCD), reactive oxygen species (ROS) generation, signal transduction and/or intracellular endomembrane transport. Examples included genes annotated as a Bax inhibitor 1, a development/cell death (DCD) domain containing proteinases and peptidases, all of which could play a role in PCD and a zinc-finger Sec23/Sec24 and five small GTPases likely involved in endoplasmic reticulum (ER) to Golgi vesicle traffic and/or signal transduction. Further experiments, however, did not confirm changes in genomic DNA degradation, a potential marker for PCD, between the two cultivars. In addition, infection progression in cotyledons was not altered by applying protease inhibitors directly to cotyledons.

Additional testing was done using green fluorescent protein (GFP)-tagged *Lm* for cotyledon colonization as well as ROS production, in relation to the lesion development. The results showed that ROS production occurred beyond the area colonized by *Lm* hyphae in 74-44 BL.

**Conclusions:** ROS may also be involved in signal transduction and/or intracellular endomembrane transport. These results provide a starting point for a better understanding of the mechanisms behind QR against *Lm* in canola and developing new host-resistance strategies for management of blackleg.

Background
Canola or rapeseed (*Brassica napus* L.) is an economically important oilseed crop cultivated worldwide. Blackleg, caused by *Leptosphaeria maculans* (*Lm*) Ces. & de Not. is a serious disease of canola, especially in Australia, Europe and Canada [1]. Genetic resistance is a cornerstone for blackleg management and is usually classified as either qualitative or quantitative. The former is controlled by single resistance (R) genes, while the latter is thought to be polygenic [2]. While many of the R genes have been identified [3-8], quantitative resistance (QR) is much less well understood.

QR can be attributed to multiple genomic regions in *B. napus* [9], with many of the same loci found in multiple canola cultivars [10]. QR to blackleg in canola is believed to be expressed primarily in adult plants. However, “74-44 BL”, a Canadian canola cultivar used in this study, has been shown to have QR to stem canker [11], as well as to infection in cotyledons by *Lm* [12]. Poland et al. [13] postulates that plant QR may be due to weaker versions of R-genes, alterations in plant morphology and/or development, phytoalexin production, variants of innate immunity or signal transduction associated genes.

RNA sequencing (RNA-seq) has provided valuable insights into the interactions between canola and blackleg in the initial stages of cotyledon infection in the absence of QR [14], in canola with and without major resistance genes [15, 16], as well as the genes potentially involved in other plant-pathogen interactions. For example Hao et al. [17] used RNA-seq to explore QR to rust in wheat. In addition, Joshi et al. [18] used RNA-seq to identify genes involved in resistance to *Sclerotinia* in *B. napus*. Haddadi et al. [14] found that, in the absence of any known resistance, genes related to initial lignin biosynthesis, biosynthesis and breakdown of glucosinolates and cell surface receptors (PAMP and effector recognition) were upregulated. In contrast, transcription factors, proteases and protease inhibitors, peroxidases and chitinases were less highly expressed within blackleg lesions. However, it is not known if any of these host responses can be induced in seedlings carrying QR. Larkan et al. [19] found evidence that a cluster of receptor-like kinases could be involved in QR to blackleg in adult canola plants. Consistently, one of the cell surface receptors found to be differentially expressed in blackleg-infected seedlings by Haddadi et al. [14] was also a receptor-like kinase. Thus it makes sense to also use RNA-Seq to explore the modes of action for QR against *Lm*. 
Fluorescent microscopy of proteins tagged with fluorophores, such as green fluorescent protein (GFP), provides valuable information about plant colonization by microbes, including the canola-blackleg pathosystem [12, 20]. Next generation sequencing approaches may help relate phenotypic observations, such as those obtained from microscopy, to molecular mechanisms. Here we present data on the colonization and lesion formation in Westar (susceptible) and 74-44 BL (expressing QR) cotyledons inoculated with a GFP-expressing isolate of _L. maculans_. This work also aimed to explore the genes differentially expressed at the seedling stage between canola cultivars in order to gain insights into the potential mechanisms of QR in 74-44 BL.

**Methods**

This manuscript includes the following experiments on cotyledons of canola cultivars without (Westar) and with QR (74-44 BL): 1) RNA-Seq and corresponding infection severity, 2) time series evaluation of lesion size and the corresponding area colonized by _Lm_ hyphae, 3) staining for the reactive oxygen species (ROS), hydrogen peroxide, via 3,3-diaminobenzidine (DAB), 4) a protease inhibitor study and 5) an assessment of the level of fragmentation of genomic DNA as a proxy for programmed cell death (PCD).

**Fungal and plant material**

Inoculum was prepared from _L. maculans_ isolates 12CC09 carrying _AvrLm6,7_ and 12CC09-GFP, grown on V8 agar until pycnidia were visible. Isolate 12CC09-GFP was generated by transforming the isolate 12CC09 with a binary vector containing the GFP gene via _Agrobacterium_ -mediated transformation. Pycnidiospores were harvested in sterile water, filtered through a Falcon™ Cell Strainer (70 μm pore size), diluted to 2 ×10⁷ spores / mL and stored at -20°C until use. One week after planting, cotyledons were wounded on each lobe with modified tweezers before being inoculated with 10μl droplets of water or pycnidiospore suspension.

74-44 BL is DEKALB® hybrid with multi-genic _Lm_ resistance and R genes _Rlm1, Rlm3_ and _RlmS_ (Saskatchewan Seed Guide, 2019). This cultivar also carries a level of QR against multiple _Lm_ races in cotyledons [11, 12] found that 74-44 BL carried both race nonspecific resistance and specific R genes _Rlm1, Rlm3_ and _Rlm9_. The QR was expressed in cotyledons in terms of both lower lesion scores (see
Table 1 of Hubbard and Peng [11]) and more restricted *Lm* colonization [12]. Plants were grown in Sunshine #3 soil-less mix (Sun Gro Horticulture Canada Ltd., Vancouver, BC) to which 12.5 g L⁻¹ Osmocote Plus 16-9-12 (N-P-K; Scotts Miracle-Gro Canada, Mississauga, ON) had been added. For all experiments, except those involving the time series that did not involve DAB staining to detect ROS, canola plants were grown in 72-well flats and placed in a growth chamber set to 22°C and 16°C during the 16 hours of light (approximately 280-575 μmol m⁻² s⁻¹) and 8 hours of darkness, respectively. Plants intended for the time series microscopic examination were grown either as described above or in the greenhouse in 10 cm square pots, exposed to a mix of natural and fluorescent (430W Philips high pressure sodium lamps) light, and inoculated with water, 12CC09 or 12CC09-GFP. Isolate 12CC09 was included as a control to determine if fluorescence observed could be attributed to GFP.

Plants were divided into *Lm*-inoculated and mock-inoculated. Within each inoculation treatment, plants were split between cultivars. The RNA-Seq and time-series microscopy experiments were repeated three times, as were the experiments that involved staining for hydrogen peroxide (ROS) with DAB. The protease inhibitor experiments were carried out five times.

For RNA-seq experiments, within each replicate, there were six seedlings per treatment (Westar or 74-44 BL, mock or 12CC09-GFP inoculated), divided at random into two blocks of three plants. At 7 days post inoculation (dpi), cotyledon samples were taken for RNA extraction and subsequent RNA-seq, from three of these seedlings. The other three seedlings were maintained until 14 dpi and rated for infection severity on the 0-9 scale [21, 22].

**RNA extraction, library preparation and sequencing**

Samples, measuring 5-10mm × 5-10mm, were collected from the area adjacent to and containing the lesion on each lobe of the cotyledons at 7 dpi (Fig. 1A). Samples were flash frozen in liquid nitrogen and stored at -80°C to await RNA extraction. Samples from one lobe (lobe 1, 2 or 3) were pooled from three replicates, each containing three seedlings, for a total of nine samples per RNA extraction. RNA was only extracted from one of the inoculated lobes.

Cotyledon tissue was ground in liquid nitrogen by vortexing in 50mL Nalgene Oak Ridge tubes
containing two metal balls. RNA was extracted from 40-50 mg of the ground and frozen tissue using
the QIAGEN RNeasy Plant mini kit on a QIAcube with a DNase I on-column digestion. The
concentration and integrity of the resulting RNA was assessed via Nanodrop and Experion (Bio-Rad
Canada, Mississauga, ON) automated electrophoresis, respectively.

Sequencing libraries were prepared using a Illumina® TruSeq™ RNA Sample Preparation Kit, pooled
and sequenced on the Illumina HiSeq 2500 at McGill University and Genome Quebec Innovation
Center (740 ave Dr Penfield, suite 7104, Montreal, QC), using one lane of V4 PE 125bp.

RNA-seq data analysis

Adapter sequences were removed with Trimmomatic (version 0.32) [23]. Subsequently, reads were
aligned to the B. napus and Lm reference genomes (downloaded from Genoscope
http://www.genoscope.cns.fr/brassicanapus/data/ and the Joint Genome Institute, Genome Portal
http://genome.jgi.doe.gov/, respectively) via STAR (version 2.4.2a) [24]. Next, gene models were
defined using the GenomicFeatures package in R, and the reads were counted using the R package
GenomicAlignments [25]. Differential expression analysis was conducted in R (version 3.3.1 or 3.3.2)
using the DESeq2 package [26]. Genes were considered differentially expressed if they had a log base
2 (log₂) fold change in expression above 2 or below -2 and an adjusted p-value under 0.05.

Differentially expressed genes (DEGs) were scored based on expression (basemean), adjusted p-value
(padj) and log₂ fold change in expression (basemean):

Venn diagrams (Fig. 3) were used to identify DEGs that were unique to each combination of
contrastting treatments: inoculated Westar versus inoculated 74-44 BL, mock inoculated Westar
versus mock inoculated 74-44 BL, mock versus Lm inoculated Westar and mock versus Lm inoculated
74-44 BL. DEGs were also subdivided into those with higher expression in the former of the two
treatments being contrasted (positive, Fig. 3A) and those upregulated in the latter of the two
contrastted treatments (negative, Fig. 3B).

Enrichment analysis based on gene ontology (GO) terms was performed by using the Blast2Go-pro
suite [27]. All B. napus genes were searched against the non-redundant protein database from
National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) using BLASTX algorithm
with an E-value threshold of $10^{-5}$. All BLAST hits were then mapped to the GO database to retrieve GO terms that associated with each hit. Subsequently, all *B. napus* genes were searched against the InterPro database (http://www.ebi.ac.uk/interpro/) and annotated by merging the Blast2GO and InterPro results. GO terms that were significantly enriched in DEGs were identified by comparing with the whole genome background with a false discovery rate (FDR) $\leq 0.05$.

**Time series infection in cotyledons examined with microscopy**

Both fluorescent and bright-field images were collected with a Zeiss Stereo-Lumar epifluorescence microscope, equipped with a NeoLumar S 0.8× objective and an Axiocam 512 camera. Light was provided by a KL-2500 LCD bulb and a HBO100 mercury lamp for bright field and fluorescent microscopy, respectively.

Two separate sets of time series microscopy experiments were carried out. In the first set of experiments, cotyledons were detached from the plant for imaging at 3, 7, 10 and 14 dpi. In the second set of experiments, imaging was done at 3, 5, 7, 9 and 11 dpi. The second set of experiments also included colorimetric staining for hydrogen peroxide. For both sets of experiments, different plants were used at each time point. Because the lesion area and the area colonized by *Lm* hyphae at 3 dpi were frequently zero, or close to zero, data from this time point is not presented.

For each inoculation site, parameters were measured with the aid of ZEN 2 pro and/or ZEN 2.3 lite (blue edition, © Carl Zeiss Microscopy GmbH, 2011) software. The software automatically takes the image magnification into account. Bright field images of the top surface of each cotyledon were used to measure the area of the lesion (mm$^2$), the distance from the inoculation point to the most distant edge of the lesion (mm) and the area stained hydrogen peroxide (mm$^2$). The area colonized by hyphae (mm$^2$) and the distance from the edge of inoculation wound to the furthest hyphal tip (mm) (first set of time series experiments only) were quantified using fluorescent images. The Zen Active Contour and/or Polygon Contour and Length tools were used to collect area and distance data, respectively.

**Colorimetric detection of hydrogen peroxide**

The area staining for the reactive oxygen species (ROS) hydrogen peroxide in *B. napus* cotyledons
infected by *Lm* isolate 12CC09-GFP, was measured at 7 dpi. Lesion area and area colonized by GFP-tagged *Lm* hyphae was measured as described above for the time series infection experiment.

Subsequently, detached cotyledons were placed in a solution of DAB at room temperature. After 40 (first two experiments) or 90 (third experiment) min, the cotyledons were vacuum infiltrated with DAB for approximately 2 to 3 hours and then boiled in 95% ethanol for approximately 10 to 20 min at 70°C to remove the chlorophyll, making the DAB staining more visible. The cotyledons were stored in 95% ethanol prior to measurement of the area stained brown for hydrogen peroxide under a dissecting microscope.

Assessment of genomic DNA degradation as a marker of programmed cell death

Samples of canola cotyledons were collected as described for RNA extraction. The samples were freeze dried, and ground to a fine powder in 2mL tubes with one 3 mm tungsten carbide bead per tube in a TissueLyser (Qiagen) for 5 min at 25 hertz at room temperature. Genomic DNA was extracted using the QIAGEN DNeasy Plant mini kit according to the manufacturer’s instructions.

Extracted DNA was diluted to 50 ng/μl. The integrity of the resulting DNA was assessed using Experion DNA 12K analysis kit (Bio-Rad Canada) on an Experion automated electrophoresis system according to the manufacturer’s instructions.

Statistical analysis

Statistical analyses were done using SAS (version 9.3). Data were assessed for homogeneity of variance and normality, respectively, using Bartlett’s Test and Shapiro-Wilk Test. Data from mock-inoculated plants, which consisted exclusively of zeros, were excluded from statistical analysis.

For ratings of infection severity in parallel with RNA-seq, when only the inoculated plants are considered, a randomized complete block design (RCBD) was used, with a total of 9 replicates (3 per experiment) and 3 subsamples (plants) per replicate (Fig. 1B). This data was pooled from all plants in a given experiment and \( y = \log_{10}(x+10) \) transformed. Means were compared via a t-test using proc GLM. For the time series experiments based on microscopic examinations that did not include colorimetric hydrogen peroxide detection (Fig. 1E), data was subjected to a \( y = \log_{10}(x+10) \) transformation prior to statistical analysis. Each parameter (lesion area, area colonized by GFP-tagged...
Results
Infection symptoms and *Lm* hyphal growth in cotyledons
Among the seedlings inoculated and grown in parallel with those used for RNA-Seq, Westar showed higher infection ratings than 74-44 BL at 14 dpi (Fig. 1B). In separate experiments, however, the appearance and size of lesions, as well as the distance from the inoculation wound to lesion edge, were similar between the two cultivars at 7 dpi, while the area colonized by *Lm* hyphae and the distance from the inoculation site to the most distal hyphal tips were greater in Westar (Fig. 1C and E). By 10 and 14 dpi, all of the measurements had become greater in Westar than 74-44 BL (Fig. 1D and E).

RNA-sequencing
For each treatment, 14.1-17.8 million paired-end reads were produced per library. When reads were considered singly, an average of 33.5 ± 2.0, 21.3 ± 0.06, 33.0 ± 2.0 and 28.2 ± 1.8 million reads were mapped to the *B. napus* genome from mock-inoculated Westar, *Lm*-inoculated Westar, mock-inoculated 74-44 BL and *Lm*-inoculated 74-44 BL, respectively. In comparison, an average of 10.1 ±
1.2, 5.8 ± 0.6, 0.07 ± 0.002 and 0.07 ± 0.010 reads were mapped to the *L. maculans* genome from *Lm*-inoculated Westar, inoculated 74-44 BL, mock-inoculated Westar and mock-inoculated 74-44 BL, respectively. A higher percentage of reads mapped to the *L. maculans* genome in inoculated Westar as compared to inoculated 74-44 BL (Fig. 2A). Principle component analysis (PCA) indicated that the treatments grouped tightly together in terms of their alignment to the *B. napus* genome (Fig. 2B).

**Gene expression in *L. maculans***

When the criteria of adjusted p-value ≤ 0.05 and log$_2$ fold change ≥ 2 in expression were applied, there were only 16 differentially expressed *Lm* genes between inoculated Westar and 74-44 BL. Three DEGs were more highly expressed in inoculated 74-44 BL as compared to inoculated Westar (Table 1) while thirteen DEGs showed the reverse trend (Table 2).

The *Lm* DEGs were all expressed at low levels. When all *Lm* genes were considered, there were eight genes with basemean expression values over 10,000 (ranging from 14,346 to 40,534). Three genes had expression values between 1,000 and 9,999, 85 between 100 and 999 and 152 between 50 and 99. There were a total of 12,119 genes with non-zero expression values. The most highly expressed DEG had a basemean of 40.

The three DEGs upregulated in *Lm* inoculated 74-44 BL had sequence similarities to genes encoding a short chain dehydrogenase/reductase, a pyoverdine biosynthesis and a hypothetical protein, respectively. Pyoverdine is a siderophore biosynthesized by *Pseudomonads* [28]. Zwiers et al. [29] found a gene encoding an ABC-transporter with a pyoverdine biosynthesis motif in the fungus *Mycosphaerella graminicola*; ABC-transporters can play a role in virulence of fungal pathogens towards host plants [30, 31]. It is unclear what role, if any, these upregulated genes played during the infection of 74-44BL by *Lm*.

**Gene expression in *B. napus***

**Genes upregulated in inoculated 74-44 BL**

There were 908 DEGs upregulated in inoculated 74-44 BL, relative to inoculated Westar, but not differentially expressed between any other pairs of treatments. Two DEGs showed basemean expression levels over 10,000, six had basemeans between 5000 and 9999, and 65 had basemeans
between 4,999 and 1,000.

Five DEGs with similarities to peptidases were among those with the highest scores. Indeed, the three DEGs with the highest scores were all putative peptidases. The DEG with the highest score is BnaA01g17570D, which has InterPro domains suggesting it is a cysteine peptidase belonging to family C1, sub-family C1A, papain family. Another DEG with a high score is BnaA09g52180D, a putative cysteine peptidase. The legumain peptidase C13 (BnaA01g04000D), also known as a vacuole processing enzyme (VPE), and BnaC02g00130D which has similarity to a protease involved in the degradation of Rubisco, were also upregulated. Additionally, numerous chlorophyll A-B binding proteins showed very high basemeans and were more highly expressed in inoculated 74-44 BL than inoculated Westar.

An ATPase of AAA-type, with protein BLAST similarity to RuBisCO activase, was also differentially expressed. The protein BLAST results also indicate that this DEG is potentially involved in endoplasmic reticulum (ER) to Golgi membrane budding.

Glycoside hydrolases, including a beta-galactosidase (BnaA04g04110D) and an alpha-1,6-glucosidases, pullulanase-type (BnaA10g25820D) are differentially expressed, with very small adjusted p-values. A putative lactate/malate dehydrogenase (BnaC02g00740D) is also differentially expressed, albeit with a less significant adjusted p-value and higher basemean expression than BnaA04g04110D or BnaA10g25820D. The DEG BnaA03g11710D, with a thiazole biosynthetic enzyme InterPro domain, also has protein sequence similarity to a ribulose-1,5-biphosphate synthetase. Table 3 and Fig. 3 summarize the putative functions of DEGs that are more highly expressed in inoculated 74-44 BL as compared to inoculated Westar.

GO term enrichment analysis of these 908 DEGs was consistent with the results presented in Table 3 and Fig. 3 in which many of GO terms with the lowest FDR were related to photosynthesis and light responses. Furthermore, three GO terms were linked to hydrogen peroxide. While none of the enriched GO terms suggested peptidase activities, the GO term with the second lowest FDR was associated with cysteine biosynthesis (Table 5). This is consistent with the putative cysteine peptidase activity of BnaA01g17570D (Table 3).
**Genes upregulated in inoculated Westar**

A total of 640 DEGs were more highly expressed in inoculated Westar as compared to inoculated 74-44 BL, but not differentially expressed when any other pair of treatments were compared. The expressions of these DEGs ranged from a basemean of 3,410 to 1.25, with only 11 DEGs showing basemeans over 1,000. Twenty eight DEGs had basemeans between 500 and 999, while 73 had basemeans between 100 and 499. The remaining 527 DEGs had basemeans under 100. The DEG with the highest score, BnaC09g20030D, showed similarity to a Bax inhibitor-1.

BnaCnnng58090D, a DEG with a basemean of 2,354, is similar to a development/cell death domain (DCD). BnaC08g42820D is a DEG similar to a heat shock protein 70. BnaA04g06220D and BnaA09g26960D have similarities to Sec23/Sec24 and Sec61/SecY, respectively. Sec23 and sec24 are part of the coat protein II (COPII) complex, involved in ER to Golgi vesicle transport [32]. Five DEGs, BnaA08g26550D, BnaA06g05280D, BnaC06g24690D, BnaA07g09950D and BnaCnnng06680D appeared similar to small GTPases. These DEGs have basemeans ranging from 972 to 3,100. Table 4 and Fig. 3 summarize these DEGs.

GO terms related to the ER, ER stress, vesicle transport and the cellular endomembrane system were enriched. None of the enriched GO terms, however, were associated with PCD. One enriched GO term was related to response to hydrogen peroxide (Table 6). BnaCnnng58090D is not associated with any GO terms.

**Hydrogen peroxide in cotyledons**

RNA-seq results suggested that ROS, such as hydrogen peroxide, may play a role in the QR to *Lm* carried by 74-44 BL. To validate this finding, DAB staining was used to quantify the area of ROS production surrounding the infection site.

**Hydrogen peroxide at seven days post inoculation**

The size of visible lesion, area of hyphal colonization and area with ROS detection in cotyledons varied, depending on the cultivar and parameter measured. In inoculated Westar, the area colonized by hyphae (as visualized by GFP fluorescence) and area staining positive for hydrogen peroxide were both larger than the area of necrotic lesions, while the former two parameters were not different from
each other (Fig. 4A). In contrast, the lesion size and area colonized by GFP-tagged *Lm* hyphae did not differ in 74-44 BL, whereas the area with ROS staining was bigger than that of former two. As with the results in Fig. 1, the lesion size did not differ between Westar and 74-44 BL at 7 dpi, while the area colonized by *Lm* hyphae was substantially greater in Westar. The area with ROS staining did not differ between the cultivars at 7 dpi either (Fig. 4).

**Hydrogen peroxide time series experiment**

When examined over time post inoculation, most of the parameters measured tended to increase over time. Westar and 74-44 BL responded differently to the *Lm* infection. In Westar, the lesions were consistently smaller than either the area colonized by *Lm* hyphae or the area with ROS staining (Tukey adjusted p ≤ 0.05) (Fig. 5). In 74-44 BL, however, the area stained for hydrogen peroxide was larger than that occupied by fungal hyphae or visible lesions (Tukey adjusted p ≤ 0.05), and the area of pathogen colonization was either smaller than (11 dpi) or not different (5, 7 and 9 dpi) from the size of lesion (Tukey adjusted p ≤ 0.05; Fig. 5).

**Genomic DNA degradation as an indicator of programmed cell death**

Because the RNA-seq results suggested that PCD could play a role in QR to *Lm* in 74-44 BL, we examined degradation of genomic DNA as a proxy for PCD. No difference in genomic DNA degradation was apparent between any of the treatments by either agarose gel electrophoresis or Experion 12K (Fig. 6).

**Impact of protease inhibitors on *Lm* infection of cotyledons**

Results from the RNA-seq experiments led us to hypothesize that proteases could contribute to 74-44 BL QR to *Lm*. We attempted to test this hypothesis by treating cotyledons with several protease inhibitors. The direct application of protease inhibitors to surface of Westar or 74-44 BL cotyledons did not have a significant impact on either the lesion size or the area colonized by *Lm* hyphae within a given cultivar. However, the latter was consistently greater in Westar than in 74-44 BL cotyledons, regardless of the protease inhibitor used (Fig. 7).

**Discussion**

It is generally thought that QR to *Lm* is not expressed in canola cotyledons [2]. In this study, however, we found that the infection severity on *Lm*-inoculated cotyledons differed quantitatively between
Westar (susceptible) and 74-44 BL (with QR). In addition to larger lesions, the area of *Lm* hyphal colonization was greater in Westar than in 74-44 BL; often the hyphal growth extended beyond the borders of visible lesions in Westar, while this was not the case for 74-44 BL. Huang et al. [20, 33] also measured QR to *Lm* in young *B. napus* plants and found, in some cases, restricted *Lm* growth correlated with reduced blackleg in more mature plants [33]. Huang et al. [33] also found partial overlap in quantitative trait loci (QTL) contributing to *Lm* resistance at both plant developmental stages. We used RNA-seq to explore DEGs between inoculated Westar and 74-44 BL as a first step to understanding the molecular mechanisms of this cotyledon-stage QR.

Many of the highest scoring DEGs, upregulated in inoculated Westar, against inoculated 74-44 BL, relate to the control of PCD, endomembrane vesicle trafficking between the ER and Golgi, as well as molecular chaperones, cation transporters, protein glycosylases and degradation enzymes (Table 4). The GO terms enriched in these DEGs also suggest a role in endomembrane vesicle transport (Table 6). The gene BnaCnng58090D, with sequence similarity to a DCD domain, was upregulated in inoculated Westar (Table 4). DCD domains can stimulate a hypersensitive response, considered a form of PCD in plants [34]. Other upregulated genes with putative roles in endomembrane transport to/from the ER are potentially related to ER stress, which can trigger DCD-mediated PCD [35]. BnaC09g20030D, which is similar to a Bax inhibitor-1, was also upregulated. Bax inhibitor-1 inhibits PCD [36]. Hence, it seems reasonable to hypothesize that the cotyledon infection triggers the expression of BnaCnng58090D in susceptible plants, but that the hypersensitive response that a DCD would otherwise promote may be prevented by the activation of BnaC09g20030D, the Bax inhibitor-1. However, the lack of differences observed in genomic DNA degradation argues against the above hypothesis. Consistently, the GO enrichment analysis did not uncover any GO terms related to PCD (Tables 5 and 6). Fragmentation of genomic DNA can be associated with plant PCD, including PCD that mimics apoptosis in animal cells, and is involved in normal plant developmental processes. For example Hoeberichts et al. [37] found PCD-linked DNA breakdown during petal senescence. In another example, Abdelmigid and Morsi [38] found DNA fragmentation in plant cells dying as a results of exposure to toxins. However, the results of Ruberti et al. [39] showed the complex and
incompletely understood role of Bax inhibitor-1 in plant PCD. Therefore our results showing no differences in the degradation of genomic DNA as a proxy for PCD cannot be clearly interpreted at this point.

PCD in general and Bax inhibitor-1 in particular, play a role in plant resistance to other pathogens. For example, Babaeizad et al. [40] found that overexpression of Bax inhibitor-1 in barley led to increased susceptibility to the biotrophic fungal pathogen *Blumeria graminis* f.sp. *hordei*, the causal agent of powdery mildew in barley. This finding is consistent with the upregulation of Bax inhibitor-1 in Westar in the current study, which corresponded to greater biotrophic growth of *Lm* hyphae asymptptomatically beyond the borders of necrotic lesions. Consistent with the idea that increased PCD can lead to resistance to biotrophic infection and susceptibility to necrotrophic colonization, Scotton et al. [41] observed that constitutive overexpression of Bax inhibitor-1 resulted in elevated resistance to the necrotrophic pathogens *Sclerotinia sclerotiorum*, *Sclerotium rolfsii* and *Botrytis cinerea*.

When 74-44 BL was inoculated, numerous peptidases were more highly expressed than in inoculated Westar. Specifically, putative papain cysteine peptidases (BnaA01g17570D and BnaA09g52180D) likely found in the plant vacuole [42, 43], as well as putative legumain peptidase C13 (BnaA01g04000D), also known as a vacuole processing enzyme (VPE), were upregulated. VPEs are, as suggested by the name, located in plant vacuoles [44, 45], as shown in Fig. 8. In addition, BnaC02g00130D, which has similarity to a protease involved in the degradation of RuBisCO, is also upregulated in inoculated 74-44 BL. These genes may also be involved in PCD [reviewed by Zamyatnin [46]]. During PCD, the plant cell vacuole ruptures, releasing proteases, which then degrade cellular components [47]. Protease-mediated PCD is essential for plant hypersensitive responses [reviewed by Sueldo and van der Hoorn [48]] which limit the spread of pathogens during the biotrophic phase of infection.

The protease inhibitor experiments were intended to test the hypothesis that at least some of the differentially expressed peptidases are involved in limiting the latent growth of *Lm* hyphae - hyphal growth beyond the edge of the visible lesion - in 74-44 BL. Possibly, this could occur through a role of peptidases in PCD. The lack of differences between the treatments were not understood. However,
because the protease inhibitors were applied to the surface of inoculated cotyledons, it is possible that they inhibited fungal proteases, which may be, to some extent, required by *Lm* for infection. Another possibility is that the applied protease inhibitors were unable to penetrate the cotyledon cuticles and thus failed to interact with plant proteases. It is also possible that proteases do not make a significant contribution to QR to *Lm* in 74-44 BL; this is supported by the lack of protease- or peptidase-related GO terms in the enriched GO terms (Table 5).

Chlorophyll A-B binding proteins, which are a source of ROS [49], were also upregulated in inoculated 74-44 BL, relative to Westar. ROS, including hydrogen peroxide, can act as pro-PCD signals [reviewed by Galvez-Valdivieso and Mullineaux [50]]. The conjecture that the upregulated chlorophyll A-B binding proteins are involved in triggering PCD is supported by our findings that, in inoculated 74-44 BL, the area that stained for hydrogen peroxide was similar to that in Westar, at 5 and 7 dpi, despite the area colonized by *Lm* hyphae being smaller in 74-44 BL than in Westar (Fig. 4 and 5). This may indicate that hydrogen peroxide is produced in 74-44 BL beyond the hyphal front, contributing to the restriction to the hyphal growth. In contrast, *Lm* hyphae grew beyond the zone of hydrogen peroxide production in Westar at 5 and 7 dpi; it appears that ROS production was not able to catch up with the *Lm* hyphal growth. At 9 and 11 dpi, however, the area occupied by hyphae and ROS were not different. Potentially the more rapid production of hydrogen peroxide in 74-44 BL, relative to *Lm* hyphal spreading, is able to prevent the biotrophic growth seen in Westar. Other DEGs that are related to the photosynthetic process include BnaA03g11710D, the putative thiazole biosynthetic enzyme and/or ribulose-1,5-bisphosphate synthetase. Thiazole is a precursor of vitamin B1, or thiamine, which can activate plant defenses [51]. Thus, it is reasonable to speculate that increased vitamin B1 biosynthesis may also contribute to the *Lm* resistance displayed by 74-44 BL. The fact that Ahn et al. [52] and Boubakri et al. [53] found a relationship between vitamin B1-induced disease resistance and hydrogen peroxide suggests a potential link between overregulation of BnaA03g11710D and increased hydrogen peroxide production, relative to the area colonized by *Lm* hyphae in 74-44 BL (Fig. 4 and 5). Additionally, three GO terms linked to hydrogen peroxide were identified by the GO enrichment analysis of the DEGs upregulated in *Lm* inoculated 74-44 BL, as
compared to Westar (Table 5); these are consistent with the suggested roles of hydrogen peroxide in the QR response of 74-44 BL to \( Lm \).

One of the DEGs upregulated in \( Lm \)-inoculated Westar relative to inoculated 74-44 BL (but not differentially expressed between any other pair of treatments) is a heat shock protein 70 which could be linked to the upregulation of Bax inhibitor-1. Qi et al. [54] noted that overexpression of a heat shock protein 70 inhibited PCD induced by hydrogen peroxide; this finding is consistent with our observation that hydrogen peroxide was produced in a larger area, relative to \( Lm \) hyphal colonization, in 74-44 BL cotyledons (Fig. 4 and 5).

The DEGs involved in endomembrane trafficking, such as small GTPases, sec23/sec24, sec61 and WD40 repeats, could be involved in ER stress and the unfolded protein response (UPR). Both of these processes can, if not resolved, lead to PCD [reviewed by Williams et al. [55]]. As shown in Fig. 8, vesicle trafficking between the Golgi and ER, and vice versa, is linked to traffic to the vacuole, where VPE-mediated PCD takes place. A VPE is one of the proteases upregulated in \( Lm \)-inoculated 74-44 BL.

Furthermore, Bax inhibitor-1 is ER localized [56], suggesting that it could also be linked to the ER to/from Golgi vesicle traffic. The UPR occurs in the ER and is, as the name implies, linked to improperly folded proteins. If stress to the ER is severe enough, the UPR can induce PCD [reviewed by Cui et al. [57]]. Hence DEGs with potential roles in protein folding that are more highly expressed in Westar than 74-44 BL (both inoculated), such as the putative peptidyl-prolyl cis-trans isomerase BnaC03g44640D, could also be linked to PCD. The fact that differences were not observed in genomic DNA degradation could suggest that the UPR might be only a signaling mechanism related to QR, and not necessarily PCD. A role for the UPR in canola resistance to \( Lm \) is consistent with the findings of Arrano-Salinas et al. [58] documenting a link between the UPR and plant immunity. Indeed, the UPR could also be related to ROS production [59]. Further research into the potential roles of the UPR, endomembrane dynamics and ROS production in plant defenses are merited for a better understanding of QR to blackleg of canola.

**Conclusion**

QR observed in 74-44 BL cotyledons involves restricting tissue colonization by \( Lm \). This is likely due,
at least in part, to the production of ROS beyond the pathogen hyphal growth. ROS may be linked to signal transduction and endomembrane vesicle trafficking. The ability of QR to reduce the growth of \textit{Lm} hyphae in cotyledons can be significant because it may limit the pathogen movement from infected leaves into the stem, where the most damaging form of the disease takes place. Further research is needed to clarify the molecular and cellular mechanisms involved. Such work, in conjunction with exploration of putative modes of action of QR in other canola cultivars and life stages, could help facilitate judicious use of QR-carrier canola for improved management of blackleg in Canada and around the world.

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

The work was conducted at the Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada. MH and GP conceived the study and designed the experiments. MH carried out the majority of the lab, growth chamber, greenhouse and microscopy work. She also conducted the bioinformatics and statistical analysis and wrote the paper. CZ transformed \textit{Lm} with GFP and carried out the analysis of genomic DNA fragmentation and GO enrichment analysis. GP and CZ provided input into the data interpretation, composition of the manuscript and editing the manuscript.

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References
1. Fitt BDL, Brun H, Barbetti MJ, Rimmer SR: **World-wide importance of Phoma Stem Canker (Leptosphaeria maculans and L. biglobosa) on Oilseed Rape (Brassica napus)**. *European Journal of Plant Pathology* 2006, **114**(1):3-15.

2. Delourme R, Chèvre AM, Brun H, Rouxel T, Balesdent MH, Dias JS, Salisbury P, Renard M, Rimmer SR: **Major gene and polygenic resistance to Leptosphaeria maculans in Oilseed Rape (Brassica napus)**. *European Journal of Plant Pathology* 2006, **114**(1):41-52.

3. Larkan NJ, Lydiate DJ, Parkin IA, Nelson MN, Epp DJ, Cowling WA, Rimmer SR, Borhan MH: **The Brassica napus blackleg resistance gene LepR3 encodes a receptor-like protein triggered by the Leptosphaeria maculans effector AVRLM1**. *New Phytol* 2013, **197**(2):595-605.

4. Yu F, Lydiate DJ, Rimmer SR: **Identification of two novel genes for blackleg resistance in Brassica napus**. *Theor Appl Genet* 2005, **110**(5):969-979.

5. Yu F, Gugel RK, Kutcher HR, Peng G, Rimmer SR: **Identification and mapping of a novel blackleg resistance locus LepR4 in the progenies from Brassica napus x B. rapa subsp. sylvestris**. *Theor Appl Genet* 2013, **126**(2):307-315.

6. Raman R, Taylor B, Lindbeck K, Coombes N, Barbulescu D, Salisbury P, Raman H: **Molecular mapping and validation of Rlm1 gene for resistance to Leptosphaeria maculans in canola (Brassica napus L.).** *Crop and Pasture Science* 2012, **63**(10):1007.

7. Larkan NJ, Lydiate DJ, Yu F, Rimmer SR, Borhan MH: **Co-localisation of the blackleg resistance genes Rlm2 and LepR3 on Brassica napus chromosome A10**. *BMC Plant Biol* 2014, **14**:387.

8. Parlange F, Daverdin G, Fudal I, Kuhn ML, Balesdent MH, Blaise F, Grezes-Besset B, Rouxel T: **Leptosphaeria maculans avirulence gene AvrLm4-7 confers a dual
recognition specificity by the Rlm4 and Rlm7 resistance genes of oilseed rape, and circumvents Rlm4-mediated recognition through a single amino acid change. *Mol Microbiol* 2009, **71**(4):851-863.

9. Kumar V, Paillard S, Fopa-Fomeju B, Falentin C, Deniot G, Baron C, Vallee P, Manzanares-Dauleux MJ, Delourme R: *Multi-year linkage and association mapping confirm the high number of genomic regions involved in oilseed rape quantitative resistance to blackleg*. *Theor Appl Genet* 2018, **131**(8):1627-1643.

10. Raman H, Raman R, Diffey S, Qiu Y, McVittie B, Barbulescu DM, Salisbury PA, Marcroft S, Delourme R: *Stable quantitative resistance loci to Blackleg disease in canola (Brassica napus L.) over continents*. *Frontiers in plant science* 2018, **9**:1622.

11. Hubbard M, Peng G: *Quantitative resistance against an isolate of Leptosphaeria maculans* (blackleg) in selected Canadian canola cultivars remains effective under increased temperatures. *Plant Pathology* 2018, **67**:1329–1338.

12. Soomro WM: *Characterizing Avr genes of Leptosphaeria maculans and resistance responses among commercial canola cultivars in western Canada* Saskatoon, Saskatchewan, Canada: University of Saskatchewan; 2016.

13. Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ: *Shades of gray: the world of quantitative disease resistance*. *Trends Plant Sci* 2009, **14**(1):21-29.

14. Haddadi P, Ma L, Wang H, Borhan MH: *Genome-wide transcriptome analyses provides insights into the lifestyle transition and effector repertoire of Leptosphaeria maculans during colonization of Brassica napus seedlings*. *Mol Plant Pathol* 2015.
15. Sonah H, Zhang X, Deshmukh RK, Borhan MH, Fernando WG, Belanger RR: 

**Comparative transcriptomic analysis of virulence factors in Leptosphaeria maculans during compatible and incompatible interactions with canola.** 
*Frontiers in plant science* 2016, 7:1784.

16. Becker MG, Zhang X, Walker PL, Wan JC, Millar JL, Khan D, Granger MJ, Cavers JD, Chan AC, Fernando DWG et al: **Transcriptome analysis of the Brassica napus-Leptosphaeria maculans pathosystem identifies receptor, signaling and structural genes underlying plant resistance.** *Plant J* 2017, 90(3):573-586.

17. Hao Y, Wang T, Wang K, Wang X, Fu Y, Huang L, Kang Z: **Transcriptome analysis provides insights into the mechanisms underlying wheat plant resistance to Stripe Rust at the adult plant stage.** *PLoS One* 2016, 11(3):e0150717.

18. Joshi RK, Megha S, Rahman MH, Basu U, Kav NN: **A global study of transcriptome dynamics in canola (Brassica napus L.) responsive to Sclerotinia sclerotiorum infection using RNA-Seq.** *Gene* 2016, 590(1):57-67.

19. Larkan NJ, Raman H, Lydiate DJ, Robinson SJ, Yu F, Barbulescu DM, Raman R, Luckett DJ, Burton W, Wratten N et al: **Multi-environment QTL studies suggest a role for cysteine-rich protein kinase genes in quantitative resistance to blackleg disease in Brassica napus.** *BMC Plant Biol* 2016, 16(1):183.

20. Huang YJ, Qi A, King GJ, Fitt BD: **Assessing quantitative resistance against Leptosphaeria maculans (phoma stem canker) in Brassica napus (oilseed rape) in young plants.** *PLoS One* 2014, 9(1):e84924.

21. Kutcher HR, Balesdent MH, Rimmer SR, Rouxel T, Chèvre AM, Delourme R, Brun H: **Frequency of avirulence genes in Leptosphaeria maculans in western Canada.** *Canadian Journal of Plant Pathology* 2010, 32(1):77-85.

22. Koch E, Badawy HMA, Hoppe HH: **Differences between aggressive and non-**
aggressive single spore lines of *Leptosphaeria maculans* in cultural characteristics and phytotoxin production. *Journal of Phytopathology* 1989, **124**:52-62.

23. Bolger AM, Lohse M, Usadel B: *Trimmomatic: a flexible trimer for Illumina sequence data*. *Bioinformatics* 2014, **30**(15):2114-2120.

24. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR: *STAR: ultrafast universal RNA-seq aligner*. *Bioinformatics* 2013, **29**(1):15-21.

25. Lawrence M, Huber W, Pages H, Aboyoun P, Carlson M, Gentleman R, Morgan MT, Carey VJ: *Software for computing and annotating genomic ranges*. *PLoS Comput Biol* 2013, **9**(8):e1003118.

26. Love MI, Huber W, Anders S: *Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2*. *Genome biology* 2014, **15**(12):550.

27. Conesa A, Gotz S: *Blast2GO: A comprehensive suite for functional analysis in plant genomics*. *Int J Plant Genomics* 2008, **2008**:619832.

28. Wendenbaum S, Demange P, Dell A, Meyer JM, Abdallah MA: *The structure of pyoverdine Pa, the siderophore of Pseudomonas aeruginosa*. *Tetrahedron Letters* 1983, **24**(44):4877-4880.

29. Zwiers LH, Roohparvar R, de Waard MA: *MgAtr7, a new type of ABC transporter from Mycosphaerella graminicola involved in iron homeostasis*. *Fungal genetics and biology : FG & B* 2007, **44**(9):853-863.

30. Kim Y, Park SY, Kim D, Choi J, Lee YH, Lee JH, Choi W: *Genome-scale analysis of ABC transporter genes and characterization of the ABCC type transporter genes in Magnaporthe oryzae*. *Genomics* 2013, **101**(6):354-361.

31. Yin Y, Wang Z, Cheng D, Chen X, Chen Y, Ma Z: *The ATP-binding protein FgArb1 is*
essential for penetration, infectious and normal growth of *Fusarium graminearum*. *New Phytol* 2018, **219**(4):1447-1466.

32. Yorimitsu T, Sato K, Takeuchi M: **Molecular mechanisms of Sar/Arf GTPases in vesicular trafficking in yeast and plants**. *Frontiers in plant science* 2014, **5**:411.

33. Huang YJ, Paillard S, Kumar V, King GJ, Fitt BDL, Delourme R: **Oilseed rape (Brassica napus) resistance to growth of Leptosphaeria maculans in leaves of young plants contributes to quantitative resistance in stems of adult plants**. *PLoS One* 2019, **14**(9):e0222540.

34. Tenhaken R, Doerks T, Bork P: **DCD - a novel plant specific domain in proteins involved in development and programmed cell death**. *BMC bioinformatics* 2005, **6**:169.

35. Reis PA, Carpinetti PA, Freitas PP, Santos EG, Camargos LF, Oliveira IH, Silva JC, Carvalho HH, Dal-Bianco M, Soares-Ramos JR et al: **Functional and regulatory conservation of the soybean ER stress-induced DCD/NRP-mediated cell death signaling in plants**. *BMC Plant Biol* 2016, **16**(1):156.

36. Kawai-Yamada M, Ohori Y, Uchimiya H: **Dissection of Arabidopsis Bax inhibitor-1 suppressing Bax-, hydrogen peroxide-, and salicylic acid-induced cell death**. *Plant Cell* 2004, **16**(1):21-32.

37. Hoeberichts FA, de Jong AJ, Woltering EJ: **Apoptotic-like cell death marks the early stages of gypsophila (Gypsophila paniculata) petal senescence**. *Postharvest Biology and Technology* 2005, **35**(3):229-236.

38. Abdelmigid HM, Morsi MM: **Cytotoxic and molecular impacts of allelopathic effects of leaf residues of Eucalyptus globulus on soybean (Glycine max)**. *J Genet Eng Biotechnol* 2017, **15**(2):297-302.

39. Ruberti C, Lai Y, Brandizzi F: **Recovery from temporary endoplasmic reticulum**
stress in plants relies on the tissue-specific and largely independent roles of
bZIP28 and bZIP60, as well as an antagonizing function of BAX-Inhibitor 1
upon the pro-adaptive signaling mediated by bZIP28. *Plant J* 2018, 93(1):155-
165.

40. Babaeizad V, Imani J, Kogel KH, Eichmann R, Huckelhoven R: **Over-expression of
the cell death regulator BAX inhibitor-1 in barley confers reduced or
enhanced susceptibility to distinct fungal pathogens.** *Theor Appl Genet* 2009,
118(3):455-463.

41. Scotton DC, Azevedo MD, Sestari I, Da Silva JS, Souza LA, Peres LE, Leal GA, Jr.,
Figueira A: **Expression of the *Theobroma cacao* Bax-inhibitor-1 gene in tomato
reduces infection by the hemibiotrophic pathogen *Moniliophthora perniciosa*.**
*Mol Plant Pathol* 2016.

42. Diaz-Mendoza M, Velasco-Arroyo B, Gonzalez-Melendi P, Martinez M, Diaz I: **C1A
cysteine protease-cystatin interactions in leaf senescence.** *J Exp Bot* 2014,
65(14):3825-3833.

43. Okamoto T, Shimada T, Hara-Nishimura I, Nishimura M, Minamikawa T: **C-terminal
KDEL sequence of a KDEL-tailed cysteine proteinase (sulfhydryl-
endopeptidase) is involved in formation of KDEL vesicle and in efficient
vacuolar transport of sulfhydryl-endopeptidase.** *Plant Physiology* 2003,
132(4):1892-1900.

44. Hara-Nishimura I, Inoue K, Nishimura M: **A unique vacuolar processing enzyme
responsible for conversion of several proprotein precursors into the mature
forms.** *FEBS J* 1991, 294:89-93.

45. Hara-Nishimura I, Nishimura M: **Proglobulin processing enzyme in vacuoles
isolated from developing pumpkin cotyledons.** *Plant Physiol* 1987, 85:440-445.
46. Zamyatnin AA, Jr.: **Plant proteases involved in regulated cell death.** *Biochemistry (Mosc)* 2015, **80**(13):1701-1715.

47. Zheng Y, Zhang H, Deng X, Liu J, Chen H: **The relationship between vacuolation and initiation of PCD in rice (Oryza sativa) aleurone cells.** *Sci Rep* 2017, **7**:41245.

48. Sueldo DJ, van der Hoorn RAL: **Plant life needs cell death, but does plant cell death need Cys proteases?** *FEBS J* 2017, **284**(10):1577-1585.

49. Xu YH, Liu R, Yan L, Liu ZQ, Jiang SC, Shen YY, Wang XF, Zhang DP: **Light-harvesting chlorophyll a/b-binding proteins are required for stomatal response to abscisic acid in Arabidopsis.** *J Exp Bot* 2012, **63**(3):1095-1106.

50. Galvez-Valdivieso G, Mullineaux PM: **The role of reactive oxygen species in signalling from chloroplasts to the nucleus.** *Physiol Plant* 2010, **138**(4):430-439.

51. Ahn IP, Kim S, Lee YH: **Vitamin B1 functions as an activator of plant disease resistance.** *Plant Physiol* 2005, **138**(3):1505-1515.

52. Ahn IP, Kim S, Lee YH, Suh SC: **Vitamin B1-induced priming is dependent on hydrogen peroxide and the NPR1 gene in Arabidopsis.** *Plant Physiol* 2007, **143**(2):838-848.

53. Boubakri H, Wahab MA, Chong J, Bertsch C, Mliki A, Soustre-Gacounolle I: **Thiamine induced resistance to Plasmopara viticola in grapevine and elicited host-defense responses, including HR like-cell death.** *Plant Physiol Biochem* 2012, **57**:120-133.

54. Qi Y, Wang H, Zou Y, Liu C, Liu Y, Wang Y, Zhang W: **Over-expression of mitochondrial heat shock protein 70 suppresses programmed cell death in rice.** *FEBS Lett* 2011, **585**(1):231-239.

55. Williams B, Verchot J, Dickman MB: **When supply does not meet demand-ER**
stress and plant programmed cell death. *Frontiers in plant science* 2014, 5:211.

56. Ishikawa T, Watanabe N, Nagano M, Kawai-Yamada M, Lam E: **Bax inhibitor-1: a highly conserved endoplasmic reticulum-resident cell death suppressor.** *Cell Death Differ* 2011, 18(8):1271-1278.

57. Cui J, Chen B, Wang H, Han Y, Chen X, Zhang W: **Glucosidase II beta-subunit, a novel substrate for caspase-3-like activity in rice, plays as a molecular switch between autophagy and programmed cell death.** *Sci Rep* 2016, 6:31764.

58. Arrano-Salinas P, Dominguez-Figueroa J, Herrera-Vasquez A, Zavala D, Medina J, Vicente-Carbajosa J, Meneses C, Canessa P, Moreno AA, Blanco-Herrera F: **WRKY7, -11 and -17 transcription factors are modulators of the bZIP28 branch of the unfolded protein response during PAMP-triggered immunity in Arabidopsis thaliana.** *Plant Sci* 2018, 277:242-250.

59. Ozgur R, Uzilday B, Iwata Y, Koizumi N, Turkan I: **Interplay between the unfolded protein response and reactive oxygen species: a dynamic duo.** *J Exp Bot* 2018, 69(14):3333-3345.

Tables
Table 1 Differentially expressed genes (DEGs) in *Leptosphaeria maculans* that are more highly expressed in *L. maculans* inoculated 74-44 BL than in inoculated Westar.

| Locus tag     | Expression | log2 Fold Change | Adjusted p-value | Score   | InterPro IDs                                                                 |
|---------------|------------|------------------|------------------|---------|-----------------------------------------------------------------------------|
| LEMA_P093320.1 | 40.32      | -2.10            | 8.28E-04         | -2.61E+02 | similar to short chain dehydrogenase/reductase family oxidoreductase       |
|               |            |                  |                  |         | IPR0021                                                                      |
| LEMA_P012380.1 | 12.05      | -2.04            | 6.76E-04         | -7.81E+01 | hypothetical protein                                                       |
|               |            |                  |                  |         | IPR0007                                                                      |
| LEMA_P055320.1 | 3.96       | -2.13            | 1.54E-04         | -3.22E+01 | hypothetical protein                                                       |
|               |            |                  |                  |         | non                                                                         |

Table 2 Differentially expressed genes (DEGs) in *Leptosphaeria maculans* that are more highly expressed in *L. maculans* inoculated Westar than in inoculated 74-44 BL.
| Locus tag          | Expression | log2 Fold Change | Adjusted p-value | Score     | Product                                          | InterPro IDs                      |
|-------------------|------------|-----------------|------------------|-----------|--------------------------------------------------|-----------------------------------|
| LEMA_P111660.1    | 30.84      | 2.11            | 3.36E-03         | 1.61E+02  | predicted protein                                |不代表                    |
| LEMA_P098600.1    | 7.34       | 2.24            | 1.16E-09         | 1.47E+02  | similar to 1,3,8-naphthalenetol reductase        | IPR0, IPR0, IPR0              |
| LEMA_P123340.1    | 16.62      | 2.63            | 2.79E-03         | 1.12E+02  | similar to amine oxidase                         | IPR0                          |
| LEMA_P081810.1    | 12.41      | 2.68            | 1.21E-02         | 6.36E+01  | similar to nonribosomal peptide synthase GliP    | IPR0                          |
| LEMA_P072910.1    | 6.66       | 2.02            | 1.96E-03         | 3.65E+01  | hypothetical protein                             | IPR0, IPR0                   |
| LEMA_P100110.1    | 2.64       | 3.30            | 6.76E-04         | 2.97E+01  | hypothetical protein                             | IPR0                          |
| LEMA_P103080.1    | 7.17       | 2.63            | 3.49E-02         | 2.75E+01  | hypothetical protein                             | IPR0                          |
| LEMA_P032040.1    | 3.21       | 2.39            | 3.32E-04         | 2.67E+01  | hypothetical protein                             | IPR0                          |
| LEMA_P081730.1    | 4.72       | 2.31            | 4.36E-02         | 1.49E+01  | similar to Methyltransferase type 11             | IPR0                          |
| LEMA_P081800.1    | 3.09       | 3.13            | 4.36E-02         | 1.32E+01  | similar to cytochrome P450                        | IPR0                          |
| LEMA_P081720.1    | 1.93       | 2.90            | 3.55E-02         | 8.11E+00  | similar to aflatoxin B1 aldehyde reductase member 2 | IPR0                          |
| LEMA_P013060.1    | 0.81       | 2.13            | 5.36E-03         | 3.93E+00  | hypothetical protein                             |不代表                    |
| LEMA_P026690.1    | 0.71       | 2.73            | 4.47E-02         | 2.60E+00  | similar to FAD binding domain protein            |不代表                    |

**Table 3** Differentially expressed genes (DEGs) in *Brassica napus* that are more highly expressed in *Leptosphaeria maculans* inoculated cotyledons of 74-44 BL (74-44 Lm) relative to inoculated Westar (Westar Lm) (score ≤ -2 x 10⁴).
| Accession      | Description                             | E-value | Identity | Coordinates |
|---------------|-----------------------------------------|---------|----------|--------------|
| BnaA01g04000D | 5722.37 -3.01 3.54E-11 -1.80E+05        |         |          |              |
| BnaA09g52180D | 7220.10 -2.14 2.34E-08 -1.18E+05        |         |          |              |
| BnaA07g20510D | 2050.02 -4.29 9.36E-12 -9.70E+04        |         |          |              |
| BnaC04g10780D | 13062.01 -2.30 6.65E-03 -6.53E+04        |         |          |              |
| BnaC02g00130D | 2502.42 -2.67 3.01E-09 -5.69E+04        |         |          |              |
| BnaA04g04110D | 521.69 -2.56 3.25E-32 -4.21E+04         |         |          |              |
| BnaA09g13710D | 5954.02 -2.48 1.41E-03 -4.21E+04        |         |          |              |
| BnaA05g29390D | 8516.26 -2.06 4.03E-03 -4.21E+04        |         |          |              |
| BnaA10g25820D | 518.97 -2.95 1.64E-27 -4.09E+04         |         |          |              |
| Accession     | Description                          | Value   | Significance | Fold/Domain                                      |
|---------------|--------------------------------------|---------|--------------|-------------------------------------------------|
| BnaA02g00670D| Immunoglobulin-like fold             | 2921.18 | -2.37        | 1.89E-06 -3.96E+04                              |
| BnaA03g44930D| Immunoglobulin E-set                 | 603.62  | -2.32        | 1.79E-28 -3.89E+04                              |
| BnaA10g26060D| Glycoside hydrolase, family 13       | 4072.11 | -2.42        | 2.31E-04 -3.58E+04                              |
| BnaA03g11710D| Glutamyl synthase, alpha subunit     | 2828.36 | -2.03        | 8.30E-07 -3.49E+04                              |
| BnaAnng14750D| Glutamylation-type TIM barrel        | 2232.72 | -2.28        | 2.74E-07 -3.34E+04                              |
| BnaA09g01080D| Kelch repeat type 1                  | 5335.01 | -2.04        | 1.26E-03 -3.16E+04                              |
| BnaAnng00160D| Kelch-type beta propeller            | 3182.66 | -2.73        | 3.39E-04 -3.02E+04                              |
| BnaC04g30870D| Ribulose bisphosphate carboxylase    | 4634.79 | -2.49        | 2.70E-03 -2.97E+04                              |
| BnaC08g49610D| Ribulose bisphosphate carboxylylase  | 1395.47 | -2.92        | 5.17E-08 -2.97E+04                              |
| BnaA09g19870D| Ribulose bisphosphate carboxylase    | 4432.54 | -2.24        | 1.04E-03 -2.96E+04                              |
| BnaAnng10080D| Photosystem II PsbO, manganese-stabilising | 2261.06 | -2.06        | 9.55E-07 -2.80E+04                              |
| BnaA09g19870D| Photosystem II PsbP, oxygen evolving complex | 2261.06 | -2.06        | 9.55E-07 -2.80E+04                              |
| Gene Symbol     | Log2 Fold Change | Fold Change | Adjusted p-value | Gene Symbol     | Log2 Fold Change | Fold Change | Adjusted p-value |
|-----------------|------------------|-------------|------------------|-----------------|------------------|-------------|------------------|
| BnaC03g59520D   | -2.66            | 3.90E-03    | -2.17E+04        | IPR016123       | Mog1/P          |            |
|                 |                  |             |                  | IPR001344       | Chloro             |
|                 |                  |             |                  | IPR022796       | Chloro             |
|                 |                  |             |                  | IPR023329       | Chloro             |
| BnaCnnng24140D  | -2.20            | 7.27E-03    | -2.17E+04        | IPR001344       | Chloro             |
|                 |                  |             |                  | IPR022796       | Chloro             |
|                 |                  |             |                  | IPR023329       | Chloro             |
| BnaA05g30550D   | -2.66            | 1.03E-31    | -2.14E+04        | IPR001344       | Chloro             |
|                 |                  |             |                  | IPR011545       | DNA/RN             |
|                 |                  |             |                  | IPR014014       | Helicas             |
|                 |                  |             |                  | IPR014001       | Helicas             |
| BnaA08g17660D   | -3.79            | 3.06E-05    | -2.05E+04        | IPR001344       | Chloro             |
|                 |                  |             |                  | IPR022796       | Chloro             |
|                 |                  |             |                  | IPR023329       | Chloro             |
| BnaC05g04590D   | -2.21            | 4.83E-04    | -2.02E+04        | IPR002683       | Photosystem         |
|                 |                  |             |                  | IPR016123       | Mog1/P             |
|                 |                  |             |                  | IPR019050       | Lke-Sn              |
|                 |                  |             |                  | IPR025609       | FDF doi            |
|                 |                  |             |                  | IPR025761       | Lsm14              |
|                 |                  |             |                  | IPR025762       | FF                 |
|                 |                  |             |                  | IPR025768       | DFDF doi           |
| BnaC07g11970D   | -2.29            | 1.82E-25    | -2.01E+04        | IPR010920       | Like-Sn             |
|                 |                  |             |                  | IPR00719        | Protein             |
|                 |                  |             |                  | IPR01245        | Serine-             |
|                 |                  |             |                  | IPR002290       | Serine/             |
|                 |                  |             |                  | IPR008271       | Serine/             |
|                 |                  |             |                  | IPR009856       | Light re            |
|                 |                  |             |                  | IPR011009       | Protein             |
|                 |                  |             |                  | IPR013320       | Concans             |
|                 |                  |             |                  | IPR017441       | Protein             |
| BnaA09g52250D   | -2.65            | 4.40E-07    | -2.01E+04        | IPR020635       | Tyrosin             |
|                 |                  |             |                  | IPR001478       | PDZ do              |
|                 |                  |             |                  | IPR004447       | C-termi             |
| BnaC08g28700D   | -2.83            | 2.03E-08    | -2.00E+04        | IPR005151       | Interph             |

Table 4 Differentially expressed genes (DEGs) that are more highly expressed in *Leptosphaeria maculans* inoculated Westar than in inoculated 74-44 BL (scores < 2 x 10^4).
| Gene name     | Expression | Score     | InterPro ID | Description                                |
|--------------|------------|-----------|-------------|--------------------------------------------|
| BnaC09g20030 | 3410.28    | 1.15E-28  | IPR006213   | Bax inhibitor 1, conserved site            |
|              | D          | 2.20      | IPR006214   |                                            |
| BnaA04g06220 | 1236.46    | 6.75E-33  | IPR006895   | Bax inhibitor 1-related                    |
|              | D          | 2.82      | IPR006896   |                                            |
|              |            | 1.12E+0   | IPR007123   |                                            |
| BnaCnng58090 | 2353.53    | 2.71E-16  | IPR013989   | Zinc finger, Sec23/Sec24-type              |
|              | D          | 2.39      | IPR013990   |                                            |
| BnaC08g42820 | 767.75     | 7.76E-26  | IPR018181   | Gelsolin domain                           |
|              | D          | 4.53      | IPR018181   |                                            |
| BnaAnng22050 | 1884.66    | 5.24E-17  | IPR025753   | Development/cell death domain.             |
|              | D          | 2.62      | IPR025753   |                                            |
| BnaA08g26550 | 971.89     | 1.70E-31  | IPR024156   | Small GTP-binding protein domain           |
|              | D          | 2.67      | IPR024156   |                                            |
| BnaA06g05280 | 1986.35    | 2.64E-15  | IPR003593   | AAA+ ATPase domain                         |
|              | D          | 2.35      | IPR003593   |                                            |
| BnaA06g31440 | 866.61     | 1.64E-21  | IPR001179   | Peptidyl-prolyl cis-trans isomerase domain |
|              | D          | 3.55      | IPR001179   |                                            |
| BnaC08g18340 | 954.57     | 1.15E-20  | IPR002208   | SecY/SEC61-alpha family                    |
|              | D          | 3.24      | IPR002208   |                                            |
| BnaC06g24690 | 3099.53    | 1.99E-09  | IPR003579   | Small GTPase superfamily, Rab type         |
|              | D          | 2.28      | IPR003579   |                                            |
| BnaA09g26960 | 2590.93    | 6.08E-11  | IPR001179   | SecY/SEC61-alpha family                    |
|              | D          | 2.07      | IPR001179   |                                            |
| BnaC03g44640 | 850.32     | 3.08E-30  | IPR001179   |                                            |
|              | D          | 2.14      | IPR001179   |                                            |
| Accession  | Score | q-value | E-value | Protein Name                      | Domain Information                        | IPR Numbers          |
|------------|-------|---------|---------|----------------------------------|-------------------------------------------|----------------------|
| BnaA05g20940 | 604.35 | 6.18 | 6.22E-15 | 5.31E+04 | Peptidyl-prolyl cis-trans isomerase, FKBP-type | IPR004314, IPR025521 |
| BnaA07g09950  | 983.34 | 2.13 | 4.51E-26 | 5.30E+04 | Domain of unknown function DUF239 | IPR004314, IPR025521 |
| BnaA05g16820  | 220.73 | 5.24 | 3.74E-45 | 5.14E+04 | Domain of unknown function DUF4409 | IPR004314, IPR025521 |
| BnaC03g41930  | 847.71 | 2.16 | 6.09E-25 | 4.42E+04 | Small GTPase superfamily, Rab type | IPR004314, IPR025521 |
| BnaCnng06680  | 1100.64 | 2.28 | 1.06E-15 | 3.75E+04 | Small GTPase superfamily, SAR1-type | IPR004314, IPR025521 |
| BnaC03g39420  | 417.07 | 2.37 | 2.23E-37 | 3.63E+04 | Small GTPase superfamily, ARF/SAR type | IPR004314, IPR025521 |
| BnaA07g34510  | 449.54 | 3.01 | 5.05E-27 | 3.56E+04 | Small GTPase superfamily, ARF type | IPR004314, IPR025521 |
| BnaA09g05230  | 746.34 | 2.25 | 3.04E-19 | 3.10E+04 | Small GTP-binding protein domain | IPR004314, IPR025521 |
| BnaA03g00370  | 346.01 | 3.02 | 3.74E-30 | 3.07E+04 | Small GTPase superfamily, SAR1-type | IPR004314, IPR025521 |
| BnaC03g07130  | 999.98 | 2.79 | 1.08E-11 | 3.06E+04 | Small GTPase superfamily, ARF/SAR type | IPR004314, IPR025521 |
| BnaA01g07540  | 610.05 | 2.38 | 1.84E-18 | 2.58E+04 | Small GTP-binding protein domain | IPR004314, IPR025521 |
| BnaC08g25990  | 545.89 | 2.91 | 8.40E-16 | 2.40E+04 | Small GTPase superfamily, ARF type | IPR004314, IPR025521 |
| BnaA09g32460  | 744.70 | 2.54 | 3.00E-13 | 2.37E+04 | Small GTP-binding protein domain | IPR004314, IPR025521 |
Table 5 Gene Ontology (GO) term enrichment of differentially expressed genes (DEGs) in *Brassica napus* that are more highly expressed in *Leptosphaeria maculans* inoculated 74-44 (74-44 Lm) than in inoculated Westar (Westar Lm).

| GO ID    | GO Name                                                                 | GO Category          | FDR     | P-Value   | % T  |
|----------|-------------------------------------------------------------------------|----------------------|---------|-----------|------|
| GO:0006364 | rRNA processing                                                          | BIOL. PROC.          | 2.02E-68 | 4.68E-71  | 11.4 |
| GO:0019344 | cysteine biosynthetic process                                            | BIOL. PROC.          | 8.58E-57 | 3.68E-59  | 9.5  |
| GO:0009773 | photosynthetic electron transport in photosystem I                      | BIOL. PROC.          | 1.78E-54 | 9.06E-57  | 5.5  |
| GO:0009637 | response to blue light                                                  | BIOL. PROC.          | 5.32E-42 | 3.63E-44  | 6.3  |
| GO:0010218 | response to far red light                                               | BIOL. PROC.          | 4.06E-41 | 2.91E-43  | 5.7  |
| GO:0009773 | photosynthetic electron transport in photosystem I                      | BIOL. PROC.          | 1.78E-54 | 9.06E-57  | 5.5  |
| GO:0019344 | cysteine biosynthetic process                                            | BIOL. PROC.          | 8.58E-57 | 3.68E-59  | 9.5  |
| GO:0009773 | photosynthetic electron transport in photosystem I                      | BIOL. PROC.          | 1.78E-54 | 9.06E-57  | 5.5  |
| GO:0009637 | response to blue light                                                  | BIOL. PROC.          | 5.32E-42 | 3.63E-44  | 6.3  |
| GO:0010218 | response to far red light                                               | BIOL. PROC.          | 4.06E-41 | 2.91E-43  | 5.7  |
| GO:0006364 | rRNA processing                                                          | BIOL. PROC.          | 2.02E-68 | 4.68E-71  | 11.4 |
| GO:0019344 | cysteine biosynthetic process                                            | BIOL. PROC.          | 8.58E-57 | 3.68E-59  | 9.5  |
| GO:0009773 | photosynthetic electron transport in photosystem I                      | BIOL. PROC.          | 1.78E-54 | 9.06E-57  | 5.5  |
| GO:0009637 | response to blue light                                                  | BIOL. PROC.          | 5.32E-42 | 3.63E-44  | 6.3  |
| GO:0010218 | response to far red light                                               | BIOL. PROC.          | 4.06E-41 | 2.91E-43  | 5.7  |
| GO:0009773 | photosynthetic electron transport in photosystem I                      | BIOL. PROC.          | 1.78E-54 | 9.06E-57  | 5.5  |
| GO:0009637 | response to blue light                                                  | BIOL. PROC.          | 5.32E-42 | 3.63E-44  | 6.3  |
| GO:0010218 | response to far red light                                               | BIOL. PROC.          | 4.06E-41 | 2.91E-43  | 5.7  |
| GO:0006364 | rRNA processing                                                          | BIOL. PROC.          | 2.02E-68 | 4.68E-71  | 11.4 |
| GO:0019344 | cysteine biosynthetic process                                            | BIOL. PROC.          | 8.58E-57 | 3.68E-59  | 9.5  |
| GO:0009773 | photosynthetic electron transport in photosystem I                      | BIOL. PROC.          | 1.78E-54 | 9.06E-57  | 5.5  |
| GO:0009637 | response to blue light                                                  | BIOL. PROC.          | 5.32E-42 | 3.63E-44  | 6.3  |
| GO:0010218 | response to far red light                                               | BIOL. PROC.          | 4.06E-41 | 2.91E-43  | 5.7  |
| GO:0006364 | rRNA processing                                                          | BIOL. PROC.          | 2.02E-68 | 4.68E-71  | 11.4 |
| GO:0019344 | cysteine biosynthetic process                                            | BIOL. PROC.          | 8.58E-57 | 3.68E-59  | 9.5  |
| GO:0009773 | photosynthetic electron transport in photosystem I                      | BIOL. PROC.          | 1.78E-54 | 9.06E-57  | 5.5  |
| GO:0009637 | response to blue light                                                  | BIOL. PROC.          | 5.32E-42 | 3.63E-44  | 6.3  |
| GO:0010218 | response to far red light                                               | BIOL. PROC.          | 4.06E-41 | 2.91E-43  | 5.7  |
| GO:0006364 | rRNA processing                                                          | BIOL. PROC.          | 2.02E-68 | 4.68E-71  | 11.4 |
| GO:0019344 | cysteine biosynthetic process                                            | BIOL. PROC.          | 8.58E-57 | 3.68E-59  | 9.5  |
| GO:0009773 | photosynthetic electron transport in photosystem I                      | BIOL. PROC.          | 1.78E-54 | 9.06E-57  | 5.5  |
| GO:0009637 | response to blue light                                                  | BIOL. PROC.          | 5.32E-42 | 3.63E-44  | 6.3  |
| GO:0010218 | response to far red light                                               | BIOL. PROC.          | 4.06E-41 | 2.91E-43  | 5.7  |
| GO:0019761 | glucosinolate biosynthetic process | BIOL. PROC. | 11 | 5.61E-11 | 13 | 1.6E-12 | 3.4% |
| GO:0009768 | photosynthesis, light harvesting in photosystem I | BIOL. PROC. | 11 | 5.82E-11 | 12 | 1.67E-12 | 0.8% |
| GO:0009965 | leaf morphogenesis | BIOL. PROC. | 11 | 8.63E-12 | 12 | 2.5E-12 | 3.7% |
| GO:0010310 | regulation of hydrogen peroxide metabolic process | BIOL. PROC. | 11 | 8.38E-12 | 12 | 2.1E-12 | 3.4% |
| GO:0009595 | detection of biotic stimulus | BIOL. PROC. | 10 | 1.07E-10 | 11 | 3.13E-11 | 2.7% |
| GO:0009409 | response to cold | BIOL. PROC. | 10 | 1.20E-10 | 12 | 3.54E-11 | 6.7% |
| GO:0042742 | defense response to bacterium | BIOL. PROC. | 10 | 4.60E-10 | 11 | 1.38E-11 | 5.6% |
| GO:0045893 | positive regulation of transcription, DNA-templated | BIOL. PROC. | 10 | 7.56E-10 | 11 | 2.31E-11 | 5.2% |
| GO:0030093 | chloroplast photosystem I | CELL. COMP. | 09 | 1.35E-09 | 11 | 4.25E-10 | 0.6% |
| GO:0010598 | NAD(P)H dehydrogenase complex (plastoquinone) | CELL. COMP. | 09 | 1.92E-09 | 11 | 6.12E-10 | 0.9% |
| GO:0000165 | MAPK cascade | BIOL. PROC. | 09 | 3.02E-09 | 11 | 9.72E-10 | 3.8% |
| GO:0016117 | carotenoid biosynthetic process | BIOL. PROC. | 09 | 4.30E-09 | 10 | 1.40E-09 | 2.5% |
| GO:0009862 | systemic acquired resistance, salicylic acid mediated signaling pathway | BIOL. PROC. | 09 | 4.89E-09 | 10 | 1.60E-10 | 3.7% |
| GO:0006636 | unsaturated fatty acid biosynthetic process | BIOL. PROC. | 09 | 7.19E-09 | 10 | 2.38E-10 | 2.0% |
| GO:0042793 | plastid transcription | BIOL. PROC. | 08 | 1.36E-08 | 10 | 4.59E-09 | 1.7% |
| GO:0019843 | rRNA binding | MOL. FUNCT. | 08 | 2.36E-08 | 10 | 8.12E-09 | 2.0% |
| GO:0031348 | negative regulation of defense response | BIOL. PROC. | 08 | 3.18E-08 | 09 | 1.10E-09 | 3.9% |
| GO:0004565 | beta-galactosidase activity | MOL. FUNCT. | 08 | 3.28E-08 | 09 | 1.14E-09 | 1.2% |
| GO:0006697 | salicylic acid biosynthetic process | BIOL. PROC. | 08 | 6.73E-08 | 09 | 2.41E-09 | 3.1% |
| GO:0010319 | stromule | CELL. COMP. | 08 | 7.34E-08 | 09 | 2.65E-09 | 1.5% |
| GO:0003959 | NADPH dehydrogenase activity | MOL. FUNCT. | 07 | 1.63E-08 | 09 | 6.08E-10 | 0.7% |
| GO:0009867 | jasmonic acid mediated signaling pathway | BIOL. PROC. | 07 | 3.78E-08 | 08 | 1.45E-09 | 3.7% |
| GO:0019898 | extrinsic component of membrane | CELL. COMP. | 07 | 6.14E-08 | 08 | 2.41E-09 | 2.0% |
| GO:0009780 | photosynthetic NADP+ reduction | BIOL. PROC. | 06 | 2.39E-08 | 08 | 9.58E-10 | 0.4% |
| GO:0006655 | phosphatidyglycerol biosynthetic process | BIOL. PROC. | 06 | 3.26E-08 | 07 | 1.31E-09 | 1.5% |
| GO:0010196 | nonphotochemical quenching | BIOL. PROC. | 06 | 4.07E-08 | 07 | 1.65E-09 | 0.6% |
| GO:0031969 | chloroplast membrane | CELL. COMP. | 06 | 4.09E-08 | 07 | 1.66E-09 | 2.6% |
| GO:0016226 | iron-sulfur cluster assembly | BIOL. PROC. | 06 | 9.42E-08 | 07 | 3.95E-09 | 1.7% |
| GO:0010363 | regulation of plant-type hypersensitive response | BIOL. PROC. | 05 | 1.77E-08 | 07 | 7.54E-10 | 3.9% |
| GO:0006612 | protein targeting to membrane | BIOL. PROC. | 05 | 2.48E-08 | 06 | 1.07E-09 | 4.1% |
| GO:0042550 | photosystem I stabilization | BIOL. PROC. | 05 | 3.00E-08 | 06 | 1.30E-09 | 0.4% |
| GO:0047100 | glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) activity | MOL. FUNCT. | 05 | 3.00E-08 | 06 | 1.30E-09 | 0.4% |
| GO:0042549 | photosystem II stabilization | BIOL. PROC. | 05 | 3.09E-08 | 06 | 1.34E-09 | 0.5% |
| GO:0005762 | mitochondrial large ribosomal subunit | CELL. COMP. | 05 | 3.21E-08 | 06 | 1.41E-09 | 0.8% |
| GO:0042335 | cuticle development | BIOL. PROC. | 05 | 5.20E-08 | 06 | 2.31E-09 | 1.2% |
| GO:0032544 | plastid translation | BIOL. PROC. | 05 | 5.35E-08 | 06 | 2.38E-09 | 0.6% |
| GO:0018316 | peptide cross-linking via L-cystine | BIOL. PROC. | 05 | 6.38E-08 | 06 | 2.87E-09 | 0.3% |
| GO:0009783 | photosystem II antenna complex | CELL. COMP. | 05 | 6.67E-08 | 06 | 3.03E-09 | 0.4% |
| Annotation                                                      | MOL. FUNCT. | BIOL. PROC. | CELL. COMP. | MOL. FUNCT. | BIOL. PROC. | CELL. COMP. | MOL. FUNCT. | BIOL. PROC. | CELL. COMP. | MOL. FUNCT. | BIOL. PROC. |
|----------------------------------------------------------------|------------|-------------|-------------|------------|-------------|-------------|------------|-------------|-------------|------------|-------------|
| GO:0030246 carbohydrate binding                               | 7.67E-05   | 3.51E-06    |             |            |             |             |            |             |             |            |             |
| GO:0003735 structural constituent of ribosome                 | 8.24E-05   | 3.80E-06    |             |            |             |             |            |             |             |            |             |
| GO:0009106 lipoate metabolic process                           | 1.11E-04   | 5.19E-06    |             |            |             |             |            |             |             |            |             |
| GO:0016984 ribulose-bisphosphate carboxylase activity          | 1.22E-04   | 5.76E-06    |             |            |             |             |            |             |             |            |             |
| GO:0000038 very long-chain fatty acid metabolic process        | 1.37E-04   | 6.58E-06    |             |            |             |             |            |             |             |            |             |
| GO:0006546 glycine catabolic process                           | 1.42E-04   | 6.86E-06    |             |            |             |             |            |             |             |            |             |
| GO:009782 photosystem I antenna complex                        | 1.48E-04   | 7.14E-06    |             |            |             |             |            |             |             |            |             |
| GO:009108 coenzyme biosynthetic process                        | 1.59E-04   | 7.69E-06    |             |            |             |             |            |             |             |            |             |
| GO:0071454 cellular response to anoxia                         | 2.85E-04   | 1.42E-06    |             |            |             |             |            |             |             |            |             |
| GO:0050661 NADP binding                                       | 3.70E-04   | 1.85E-06    |             |            |             |             |            |             |             |            |             |
| GO:009505 plant-type cell wall                                 | 5.17E-04   | 2.62E-06    |             |            |             |             |            |             |             |            |             |
| GO:0006005 L-fucose biosynthetic process                       | 7.55E-04   | 3.92E-06    |             |            |             |             |            |             |             |            |             |
| GO:0050832 defense response to fungus                          | 7.64E-04   | 3.97E-06    |             |            |             |             |            |             |             |            |             |
| GO:0042744 hydrogen peroxide catabolic process                 | 8.28E-04   | 4.34E-06    |             |            |             |             |            |             |             |            |             |
| GO:005528 FK506 binding                                       | 9.23E-04   | 4.89E-06    |             |            |             |             |            |             |             |            |             |
| GO:005247 voltage-gated chloride channel activity              | 9.41E-04   | 5.01E-06    |             |            |             |             |            |             |             |            |             |
| GO:009533 chloroplast stromal thylakoid                        | 1.11E-03   | 5.97E-06    |             |            |             |             |            |             |             |            |             |
| GO:0090042 tubulin deacetylation                               | 1.45E-03   | 8.07E-06    |             |            |             |             |            |             |             |            |             |
| GO:0051721 protein phosphatase 2A binding                      | 1.45E-03   | 8.07E-06    |             |            |             |             |            |             |             |            |             |
| GO:0042903 tubulin deacetylase activity                        | 1.45E-03   | 8.07E-06    |             |            |             |             |            |             |             |            |             |
| GO:0043014 alpha-tubulin binding                               | 1.45E-03   | 8.07E-06    |             |            |             |             |            |             |             |            |             |
| GO:0034707 chloride channel complex                            | 1.49E-03   | 8.27E-06    |             |            |             |             |            |             |             |            |             |
| GO:0010242 oxygen evolving activity                            | 1.49E-03   | 8.28E-06    |             |            |             |             |            |             |             |            |             |
| GO:0043086 negative regulation of catalytic activity           | 1.58E-03   | 8.82E-06    |             |            |             |             |            |             |             |            |             |
| GO:0031012 extracellular matrix                                | 1.73E-03   | 9.75E-06    |             |            |             |             |            |             |             |            |             |
| GO:0006833 water transport                                     | 1.80E-03   | 1.03E-05    |             |            |             |             |            |             |             |            |             |
| GO:0000413 protein peptidyl-prolyl isomerization               | 1.91E-03   | 1.09E-05    |             |            |             |             |            |             |             |            |             |
| GO:0003755 peptidyl-prolyl cis-trans isomerase activity        | 1.91E-03   | 1.09E-05    |             |            |             |             |            |             |             |            |             |
| GO:0009508 plastid chromosome                                  | 2.02E-03   | 1.16E-05    |             |            |             |             |            |             |             |            |             |
| GO:1902476 chloride transmembrane transport                    | 2.22E-03   | 1.29E-05    |             |            |             |             |            |             |             |            |             |
| GO:0080153 negative regulation of reductive pentose-phosphate cycle | 2.55E-03   | 1.50E-05    |             |            |             |             |            |             |             |            |             |
| GO:0050577 GDP-L-fucose synthase activity                      | 2.55E-03   | 1.50E-05    |             |            |             |             |            |             |             |            |             |
| GO:0015996 chlorophyll catabolic process                       | 2.57E-03   | 1.51E-05    |             |            |             |             |            |             |             |            |             |
| GO:0009737 response to abscisic acid                           | 2.75E-03   | 1.62E-05    |             |            |             |             |            |             |             |            |             |
| GO:0050821 protein stabilization                              | 2.84E-03   | 1.68E-05    |             |            |             |             |            |             |             |            |             |
| GO:0045036 protein targeting to chloroplast                    | 3.14E-03   | 1.88E-05    |             |            |             |             |            |             |             |            |             |
| GO:0004040 amidase activity                                    | 3.22E-03   | 1.93E-05    |             |            |             |             |            |             |             |            |             |
| GO:0045454 cell redox homeostasis                              | 3.37E-03   | 2.03E-05    |             |            |             |             |            |             |             |            |             |
| GO:0042631 | cellular response to water deprivation | BIOL. PROC. | 3.49E-03 | 2.12E-04 | 1.2|
| GO:0005509 | calcium ion binding | MOL. FUNCT. | 3.49E-03 | 2.11E-04 | 2.5|
| GO:0043481 | anthocyanin accumulation in tissues in response to UV light | BIOL. PROC. | 3.83E-03 | 2.35E-04 | 1.5|
| GO:0010189 | vitamin E biosynthetic process | BIOL. PROC. | 3.89E-03 | 2.11E-04 | 0.4|
| GO:0043864 | indoleacetamide hydrolase activity | MOL. FUNCT. | 3.89E-03 | 2.11E-04 | 0.2|
| GO:0031679 | NADH dehydrogenase (plastoquinone) activity | MOL. FUNCT. | 3.89E-03 | 2.11E-04 | 0.2|
| GO:0004857 | enzyme inhibitor activity | MOL. FUNCT. | 4.22E-03 | 2.66E-04 | 1.9|
| GO:0015250 | water channel activity | MOL. FUNCT. | 4.65E-03 | 2.96E-04 | 0.7|
| GO:0010019 | chloroplast-nucleus signaling pathway | BIOL. PROC. | 4.74E-03 | 3.04E-04 | 0.3|
| GO:0016151 | nickel cation binding | MOL. FUNCT. | 4.74E-03 | 3.04E-04 | 0.3|
| GO:0016709 | oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen | MOL. FUNCT. | 5.04E-03 | 3.23E-04 | 1.1|
| GO:0045735 | nutrient reservoir activity | MOL. FUNCT. | 5.72E-03 | 3.69E-04 | 0.8|
| GO:0000311 | plastid large ribosomal subunit | CELL. COMP. | 5.79E-03 | 3.75E-04 | 0.4|
| GO:0030091 | protein repair | BIOL. PROC. | 5.83E-03 | 3.79E-04 | 0.5|
| GO:0009269 | response to desiccation | BIOL. PROC. | 6.09E-03 | 3.97E-04 | 0.7|
| GO:0097339 | glycolate transmembrane transport | BIOL. PROC. | 7.20E-03 | 4.78E-04 | 0.2|
| GO:1901975 | glycerate transmembrane transport | BIOL. PROC. | 7.20E-03 | 4.78E-04 | 0.2|
| GO:0043879 | glycolate transmembrane transporter activity | MOL. FUNCT. | 7.20E-03 | 4.78E-04 | 0.2|
| GO:0008531 | riboflavin kinase activity | MOL. FUNCT. | 7.20E-03 | 4.78E-04 | 0.2|
| GO:0009671 | nitrate:proton symporter activity | MOL. FUNCT. | 7.20E-03 | 4.78E-04 | 0.2|
| GO:0010301 | xanthoxin dehydrogenase activity | MOL. FUNCT. | 7.20E-03 | 4.78E-04 | 0.2|
| GO:0045550 | geranylgeranyl reductase activity | MOL. FUNCT. | 7.20E-03 | 4.78E-04 | 0.2|
| GO:1901974 | glycerate transmembrane transporter activity | MOL. FUNCT. | 7.20E-03 | 4.78E-04 | 0.2|
| GO:0009772 | photosynthetic electron transport in photosystem II | BIOL. PROC. | 7.55E-03 | 5.04E-04 | 0.4|
| GO:0009695 | jasmonic acid biosynthetic process | BIOL. PROC. | 7.55E-03 | 5.04E-04 | 0.4|
| GO:0010118 | stomatal movement | BIOL. PROC. | 1.12E-02 | 7.61E-04 | 0.6|
| GO:0008465 | glycerate dehydrogenase activity | MOL. FUNCT. | 1.15E-02 | 7.92E-04 | 0.2|
| GO:0071277 | cellular response to calcium ion | BIOL. PROC. | 1.15E-02 | 7.92E-04 | 0.2|
| GO:0050278 | sedoheptulose-bisphosphatase activity | MOL. FUNCT. | 1.15E-02 | 7.92E-04 | 0.2|
| GO:0008974 | phosphoribulokinase activity | MOL. FUNCT. | 1.15E-02 | 7.92E-04 | 0.2|
| GO:0008465 | glycerate dehydrogenase activity | MOL. FUNCT. | 1.15E-02 | 7.92E-04 | 0.2|
GO:0046406  magnesium protoporphyrin IX methyltransferase activity
GO:0052689  carboxylic ester hydrolase activity
GO:0055070  copper ion homeostasis
GO:0042351  'de novo' GDP-L-fucose biosynthetic process
GO:0009051  pentose-phosphate shunt, oxidative branch
GO:0051537  2 iron, 2 sulfur cluster binding
GO:0031408  oxylipin biosynthetic process
GO:0016628  oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor
GO:0004345  glucose-6-phosphate dehydrogenase activity
GO:0050162  oxalate oxidase activity
GO:0009740  gibberellic acid mediated signaling pathway
GO:0006048  UDP-N-acetylglucosamine biosynthetic process
GO:0008106  alcohol dehydrogenase (NAD+) activity
GO:0045490  pectin catabolic process
GO:0019747  regulation of isoprenoid metabolic process
GO:0017148  negative regulation of translation
GO:0009344  nitrite reductase complex [NAD(P)H]
GO:0031409  pigment binding
GO:0009496  plastoquinol--plastocyanin reductase activity
GO:0045156  electron transporter, transferring electrons within the cyclic electron transport pathway of photosynthesis activity
GO:0010304  PSII associated light-harvesting complex II catabolic process
GO:0042132  fructose 1,6-bisphosphate 1-phosphatase activity
GO:1902066  regulation of cell wall pectin metabolic process
GO:0009769  photosynthesis, light harvesting in photosystem II
GO:0048487  beta-tubulin binding
GO:0008124  4-alpha-hydroxytetrahydrobiopterin dehydratase activity
GO:0009073  aromatic amino acid family biosynthetic process
GO:0046688  response to copper ion
GO:0051287  NAD binding
GO:0016122  xanthophyll metabolic process
GO:0004332  fructose-bisphosphate aldolase activity
GO:0009279  cell outer membrane
GO:0045252  oxoglutarate dehydrogenase complex
GO:0004149  dihydrolipoyllysine-residue succinyltransferase activity
| GO:0051920 | peroxiredoxin activity | MOL. FUNCT. | 2.89E-02 | 2.22E-03 | 0.3% |
| GO:0030145 | manganese ion binding | MOL. FUNCT. | 2.92E-02 | 2.24E-03 | 0.6% |
| GO:0016575 | histone deacetylation | BIOL. PROC. | 2.93E-02 | 2.25E-03 | 0.4% |
| GO:0009750 | response to fructose | BIOL. PROC. | 3.14E-02 | 2.44E-03 | 1.5% |
| GO:0048359 | mucilage metabolic process involved in seed coat development | BIOL. PROC. | 3.26E-02 | 2.54E-03 | 0.4% |
| GO:0006066 | alcohol metabolic process | BIOL. PROC. | 3.45E-02 | 2.70E-03 | 1.8% |
| GO:0010270 | photosystem II oxygen evolving complex assembly | BIOL. PROC. | 3.54E-02 | 2.79E-03 | 0.2% |
| GO:0004560 | alpha-L-fucosidase activity | MOL. FUNCT. | 3.54E-02 | 2.79E-03 | 0.2% |
| GO:0080093 | regulation of photorespiration | BIOL. PROC. | 4.32E-02 | 3.46E-03 | 0.2% |
| GO:0031998 | regulation of fatty acid beta-oxidation | BIOL. PROC. | 4.32E-02 | 3.46E-03 | 0.2% |
| GO:0010617 | circadian regulation of calcium ion oscillation | BIOL. PROC. | 4.32E-02 | 3.46E-03 | 0.2% |
| GO:0010258 | NADH dehydrogenase complex (plastoquinone) assembly | BIOL. PROC. | 4.32E-02 | 3.46E-03 | 0.2% |
| GO:0009517 | PSII associated light-harvesting complex II | CELL. COMP. | 4.32E-02 | 3.46E-03 | 0.2% |
| GO:0004615 | phosphomannomutase activity | MOL. FUNCT. | 4.32E-02 | 3.46E-03 | 0.2% |
| GO:0006662 | glycerol ether metabolic process | BIOL. PROC. | 4.39E-02 | 3.53E-03 | 0.5% |

Table 6 GO term enrichment of DEGs in *Brassica napus* that are more highly expressed in *Leptosphaeria maculans* inoculated Westar (*Westar Lm*) than in inoculated 74-44 (*74-44 BL Lm*).
| GO ID       | GO Name                                         | GO Category      | FDR     | P-Value   | % Test |
|------------|-------------------------------------------------|------------------|---------|-----------|--------|
| GO:0042542 | response to hydrogen peroxide                   | BIOL. PROC.      | 2.31E-08| 1.11E-11  | 4.06%  |
| GO:0006457 | protein folding                                 | BIOL. PROC.      | 2.31E-08| 1.27E-11  | 5.31%  |
| GO:0034976 | response to endoplasmic reticulum stress        | BIOL. PROC.      | 3.77E-08| 2.91E-11  | 5.78%  |
| GO:0009644 | response to high light intensity                | BIOL. PROC.      | 4.19E-07| 6.00E-10  | 5.91%  |
| GO:006984  | ER-nucleus signaling pathway                    | BIOL. PROC.      | 5.21E-04| 3.16E-06  | 0.94%  |
| GO:006888  | endoplasmic reticulum to Golgi vesicle-mediated transport | BIOL. PROC.      | 9.53E-04| 6.98E-06  | 2.19%  |
| GO:006499  | N-terminal protein myristoylation               | BIOL. PROC.      | 9.53E-04| 7.14E-06  | 2.19%  |
| GO:0034605 | cellular response to heat                       | BIOL. PROC.      | 4.59E-03| 4.29E-05  | 0.94%  |
| GO:006094  | gluconeogenesis                                 | BIOL. PROC.      | 5.10E-03| 4.94E-05  | 2.66%  |
| GO:1901617 | organic hydroxy compound biosynthetic process   | BIOL. PROC.      | 5.50E-03| 5.51E-05  | 5.00%  |
| GO:0010498 | proteasomal protein catabolic process           | BIOL. PROC.      | 1.84E-02| 2.34E-04  | 2.97%  |
| GO:009862  | systemic acquired resistance, salicylic acid mediated signaling pathway | BIOL. PROC.      | 2.81E-02| 3.83E-04  | 2.66%  |
| GO:001561  | fatty acid alpha-oxidation                      | BIOL. PROC.      | 3.83E-02| 5.91E-04  | 0.31%  |
| GO:007105  | nitrogen compound transport                     | BIOL. PROC.      | 4.23E-02| 6.58E-04  | 11.72% |
| GO:005788  | endoplasmic reticulum lumen                     | CELL. COMP.      | 4.92E-10| 1.08E-13  | 2.34%  |
| GO:009505  | plant-type cell wall                            | CELL. COMP.      | 2.13E-05| 5.40E-08  | 3.91%  |
| GO:005886  | plasma membrane                                 | CELL. COMP.      | 4.49E-05| 1.24E-07  | 20.00% |
| GO:003126  | COPI vesicle coat                               | CELL. COMP.      | 6.20E-05| 1.80E-07  | 1.09%  |
| GO:000327  | lytic vacuole within protein storage vacuole    | CELL. COMP.      | 8.68E-05| 3.25E-07  | 0.63%  |
| GO:002266  | cytosolic ribosome                              | CELL. COMP.      | 9.32E-03| 9.96E-05  | 3.13%  |
| GO:005774  | vacuolar membrane                               | CELL. COMP.      | 2.16E-02| 2.79E-04  | 5.00%  |
| GO:009506  | plasmodesma                                     | CELL. COMP.      | 2.83E-02| 3.95E-04  | 7.03%  |
| GO:003756  | protein disulfide isomerase activity            | MOL. FUNCT.      | 1.82E-04| 8.43E-07  | 1.09%  |
| GO:005198  | structural molecule activity                    | MOL. FUNCT.      | 1.20E-03| 9.40E-07  | 5.00%  |
| GO:005524  | ATP binding                                     | MOL. FUNCT.      | 2.30E-03| 2.05E-05  | 14.53% |
| GO:004674  | protein serine/threonine kinase activity        | MOL. FUNCT.      | 1.28E-02| 1.48E-04  | 6.09%  |
| GO:004842  | ubiquitin-protein transferase activity          | MOL. FUNCT.      | 1.58E-02| 1.91E-04  | 3.28%  |
| GO:005509  | calcium ion binding                             | MOL. FUNCT.      | 1.80E-02| 2.26E-04  | 2.81%  |
| GO:0051670 | inulinase activity                              | MOL. FUNCT.      | 2.83E-02| 3.96E-04  | 0.31%  |
| GO:005261  | glucan endo-1,3-beta-glucanase activity, C-3 substituted reducing group | MOL. FUNCT.      | 4.85E-02| 8.24E-04  | 0.31%  |
| GO:005262  | glucan endo-1,4-beta-glucanase activity, C-3 substituted reducing group | MOL. FUNCT.      | 4.85E-02| 8.24E-04  | 0.31%  |
| GO:0051699 | fructan beta-fructosidase activity              | MOL. FUNCT.      | 4.85E-02| 8.24E-04  | 0.31%  |
Figures

Approximate size and location of cotyledon samples taken for RNA-Seq analysis (A).
Infection severity (0-9 scale), at 14 days after inoculation, in cotyledons of Westar and 74-
44 (B), grown in the same flats as those used for RNA-Seq analysis. Lesions and GFP expressing fungal hyphal growth in Westar and 74-44 cotyledons at 7 (C) and 14 (D) days post inoculation with the L. maculans isolate 12CC09-GFP. The lesion size (area), area colonization by the fluorescent hyphae, distance from the inoculation wound to the furthest edge of the lesion or distance from the wound to the furthest hyphal tip (E). Bars or data points with the same letter at a given time point in the same panel are not different (t-test, \( p \leq 0.05 \)).
Figure 2

Percent of reads mapped to the L. maculans genome (A) and principle component analysis (PCA) plot, produced by DESeq2 (B).
Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/) of upregulated (A) and downregulated (B) DEGs between L. maculans inoculated Westar (WLm) and 74-44 (7Lm), mock inoculated Westar (WM) and 74-44 (7M), and between mock and inoculated Westar or mock and inoculated 74-44. DEGs are upregulated in the underlined treatment.
Area of visible lesions, hyphal colonization and positive 3,3-diaminobenzidine (DAB) staining for hydrogen peroxide, a reactive oxygen species (ROS), in Westar and 74-44 cotyledons at 7 days post inoculation with the L. maculans isolate 12CC09-GFP. Bars with the same letter of the same case are not significantly different (A). Capital letters indicate comparisons between cultivars for a given parameter (Wilcoxon two-sample test, p ≤ 0.05). Lower case letters denote comparisons between parameters, within a cultivar (Tukey adjusted p ≤0.05).

Panel B shows the appearance of representative DAB-stained cotyledons.
Figure 5

Time series of the area of visible lesions, hyphal colonization and positive 3,3-diaminobenzidine (DAB) staining for hydrogen peroxide, a reactive oxygen species (ROS), in Westar and 74-44 cotyledons. Values with the same letter, at the same time point, are not significantly different (Tukey adjusted $p \leq 0.05$).
Genomic DNA from mock-inoculated Westar (Westar-mock), Westar inoculated with L. maculans (Westar-Lm), mock-inoculated 74-44 BL (74-44-mock) or 74-44 BL inoculated with L. maculans (74-44-Lm), separated on an Experion 12K chip in order to assay genomic DNA degradation as a marker of programmed cell death.
Impact of protease inhibitors on lesion size (A) and area colonized by GFP-tagged L. maculans hyphae (B) in Westar and 74-44 cotyledons at 7 days post inoculation. Bars with the same letter, within a given panel, are not significantly different (Tukey adjusted $p \leq 0.05$).
Figure 8

A proposed model on how some of the most highly-expressed DEGs (differentially expressed genes) may interact, potentially resulting in programmed cell death. ER: Endoplasmic reticulum.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
Supple Fig 1.pdf