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ORIGINAL ARTICLE

Temporal expression profiles of defense-related genes involved in *Lactuca sativa- Sclerotinia sclerotiorum* interactions

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Abstract

Sclerotinia sclerotiorum, a destructive fungal pathogen with an extensive host range infecting more than 400 plant species, causes lettuce drop on the leafy green lettuce that potentially have an enormous economic impact on lettuce cultivation worldwide. To gain insights into how lettuce regulates its defense pathways, gene expression profiles of five defense-related genes (*LsPRB1*, *LsSOD*, *LsERF1*, *LsLTC1*, and *LsHPL1*) triggered following infection of susceptible Mazandaran line 1 (ML1) and tolerant Jahrom (Jah) lettuce accessions by the *S. sclerotiorum* were compared by the real-time quantitative RT-PCR (RT-qPCR) approach. In the current study, we observed temporal and quantitative gene expression fluctuations between two examined accessions of *L. sativa* in response to *S. sclerotiorum* attack. All genes, except *LsHPL1*, were up-regulated earlier (24 hours after inoculation) in the Jah accession compared with the susceptible one. This data implies strong defense responses established in the tolerant accession to arrest the fungal growth, but it resulted in restricting lesion development rather than in preventing infection. This research contributes to a better understanding of the kinetics of lettuce reactions induced following *S. sclerotiorum* infection and may be employed to develop effective strategies to manage lettuce drop.

Keywords Lettuce drop · *Sclerotinia sclerotiorum* · RT-qPCR technique · Gene expression · Defense responses

Introduction

In all-natural environments, plants are invaded by various pathogens, exploiting the biosynthetic capabilities of the host plant. To efficiently defend themselves against these threats, plants have evolved elaborate mechanisms to recognize and respond to pathogen attacks (Chisholm et al. 2006). The plant immunity system consists of two interconnected tiers of receptors governing recognition of microbes and inducing appropriate defense responses based on the lifestyle of the invading pathogen to restrict its growth. The first line is achieved by the recognition of invariant molecular patterns that are commonly known as pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) located on the surface of the plant cell, mediating PAMP-triggered immunity (PTI), resulting in preventing further colonization of the host (Dodds and Rathjen 2010; Jones and Dangl 2006). In turn, fungal pathogens secrete effector proteins to suppress PTI, culminating in host susceptibility (Lo Presti et al. 2015). Co-evolutionary interactions between plants and pathogens resulted in the development of resistance proteins encoded by resistance (*R*) genes in plants recognizing these effector proteins leading to the effector-triggered immunity (ETI), the second line of defense that is often accompanied by local cell death at the attempted site of infection (van der Burgh and Joosten 2019). The triggered defense reactions include reinforcement of plant cell walls, biosynthesis of phytoalexins, inducing phytohormone signaling pathways, accumulation of reactive oxygen species (ROS), and synthesizing pathogenesis-related (PR) proteins (Tsuda and Katagiri 2010). Many of these reactions undergo temporally transcriptional reprogramming of the corresponding defense genes mainly, depending on types of interactions (compatibility/incompatibility) (Dixon et al. 1994).

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Sclerotinia sclerotiorum (Lib.) de Bary is a notorious fungal pathogen with an extensive host range infecting more than 400 plant species (Boland and Hall 1994; Purdy 1979). A number of these host plants are agronomically important crops, including *Brassica napus* (canola/oilseed rape) and *Lactuca sativa* (garden lettuce) (Clarkson et al. 2007; Zhao et al. 2007). This fungus causes lettuce drop on the leafy green lettuce, consuming as a fresh vegetable worldwide. This disease can occur all over the world and causes remarkable yield losses of up to 60% in individual fields (Hao and Subbarao 2005).

S. sclerotiorum is considered as a typical necrotrophic fungal pathogen, infecting host plant species through secreting cell wall degrading enzyme, producing toxin, and inducing host cell death (Kabbage et al. 2015). The life cycle of *S. sclerotiorum* is initiated by the germination of sclerotia, which is a resistant structure surviving in the infested soils for several years to either produce apothecia or mycelium (Ben-Yephet et al. 1993). Subsequently, apothecia release abundant ascospores, landing on host tissues, and germinating under favorable conditions. Under cool and moist environmental conditions, this fungus quickly colonizes the infected host tissue, leading to disease symptoms developments such as water-soaked areas followed by the formation of a white and cotton-like mycelium culminating in stunting and wilting of the host (Phillips 1987; Willetts and Wong 1980).

Lettuce drop management is a challenging task since *S. sclerotiorum* persists in the soil for up to 10 years through the generation of resistant sclerotia (Ben-Yephet et al. 1993) and the production of abundant airborne ascospores, playing pivotal roles in establishing the infection cycle of this damaging fungus. More importantly, the necrotrophic nature of this fungus in such a way to kill the host cell through a variety of strategies is a pivotal factor to make more difficult its control (Subbarao 1998).

Current measures to manage lettuce drop are mainly achieved through fungicide applications, including dicloran (Botran), iprodione (Rovral), and vinclozolin (Ronilan), providing a modest level of control against this disease in most conditions (Matheron and Matejka 1989; Matheron and Porchas 2004; Subbarao 1998). Nevertheless, the iprodione and vinclozolin were unable to manage lettuce drop under intensive lettuce production. This issue is associated with the rapid degradation of these fungicides in the soils, followed by repeated field applications. (Martin et al. 1991). Furthermore, fungicide-resistant strains and cross-resistant to different types of fungicides have been reported for the mentioned fungicides (Davet and Martin 1993; Hubbard et al. 1997). Therefore, the most reasonable and practical measure to manage lettuce drop is an integrated pest management (IPM) approach (Hayes et al. 2010; Subbarao 1998). Host resistance is the most environmentally friendly and safe element to incorporate into an IPM strategy for managing this destructive disease. Extensive screening efforts have been conducted to identify resistant lettuce genotypes with complete resistance against *S. sclerotiorum* complying with the gene-for-gene model, but these were unsuccessful (Hayes et al. 2010). On the other hand, partial resistance towards lettuce drop was observed in some cultivars, accession forms, and wild relatives, but the mechanisms of resistance were often attributed to plant architecture and growth rather than a physiological mechanism, operating to inhibit the fungal colonization (Hayes et al. 2010; Lebeda et al. 2014; Whipps et al. 2002). Recently, the genetic basis of lettuce resistance against lettuce drop was investigated in cv. Eruption, exhibiting a high level of resistance toward this disease (Hayes et al. 2010) and two genes governing partial resistance against lettuce drop explaining up to 41% of the phenotypic variations were genetically mapped on linkage groups (1 and 5) (Mamo et al. 2019).

The rarity of central genes playing major roles in lettuce defense in response to *S. sclerotiorum* infection has been an essential constraint to employ genetic resistance to combat with lettuce drop. Despite the mapping of two quantitative trait loci (QTLs), the molecular basis of responses happening in the host plant following pathogen attack, and the kinetics of plant defense in *S. sclerotiorum*-lettuce pathosystem has never been described. However, the expression profiles of defense-related genes in response to *S. sclerotiorum* in other host species such as *Brassica napus* and *Glycine max* were investigated to determine the gene differentially expressed between resistant and susceptible genotypes (Seifbarghi et al. 2017; Westrick et al. 2019).

The genes included in this study were chosen to represent the key defense-related genes providing resistance against biotrophic and necrotrophic fungal plant pathogens. *PRB1* is among the most abundantly produced proteins in plants upon pathogen attack, and its expression has long been employed as a molecular marker for salicylic acid-mediated disease resistance (Breen et al. 2017). *SOD* is a superoxide dismutase, which is a critical antioxidant enzyme in scavenging the reactive oxygen species produced during oxidative burst events in a plant following biotic and abiotic stresses (Alscher et al. 2002). *ERF1* is a transcription factor controlling the expression of pathogen response genes, and integrating signals from ethylene and jasmonate network in plant defense that eventually, inhibits disease development (Lorenzo et al. 2003). *LTC1* encodes a sesquiterpene synthase involved in defense response against a fungal pathogen (Yadav et al. 2019). *HPL1* encodes a hydroperoxide lyase playing central roles in mediating plant-specific defense responses (Tong et al. 2012).

In the current study, we aimed to compare the gene expression profiles of the defense-related genes induced following infection of susceptible (ML1), and tolerant (Jah) lettuce accessions. We employed the RT-qPCR approach to provide novel insights into how this plant finely regulates its defense response to protect itself against this invading fungal pathogen.

Materials and methods

Sampling and fungal isolation

Lettuce plants showing water-soaked lesions covered by numerous sclerotia were collected from Varamin County, Tehran Province, Iran, and transferred to the laboratory to perform fungal isolation. To this aim, sampled tissues were washed to eliminate the saprophytic contaminations and then were surface-sterilized through ethanol 70% for 30 s, and rinsed twice by sterile water. Subsequently, the dried samples were cut into around 5 cm segments and placed on the potato dextrose agar (PDA), followed by an incubation period of 5–7 days at 25 °C. Fungal isolate purification was performed via transferring a single hyphal tip grown on 2% water agar (WA) to a new PDA plate. To molecularly identify the isolated *Sclerotinia*, total genomic DNA was extracted based on the previously described protocol (Sambrook and Russell 2006). The PCR amplification was performed to target ITS-rDNA region in 20 µl reaction volume containing 0.2 unit/µl *Taq* DNA polymerase (Ampliqon, Denmark); 1.5 mM $MgCl_2$; 0.2 µM of ITS-1 primer (5'-CGTAGGTGAACCTGCGG-3') and ITS-4 primer (5'-TCCTCCGCTTATTGATATGC-3') and 10 ng genomic DNA of each isolate. PCR products were sequenced at Bioneer company (South Korea), and the obtained data were edited by the BioEdit V. 7.2.5 tool (Hall 2004). Eventually, the edited sequences were blasted in the GenBank nucleotide database (Johnson et al. 2008) to confirm the fungal species of the isolated *Sclerotinia.*

Plant materials and infection assay

Seeds of Iranian lettuce accessions were collected from all over Iran and used in this study to identify susceptible and tolerant accessions. Additionally, we included *L. serriola*, the wild progenitor of cultivated lettuce (Johnson et al. 2000), to compare the defense reactions of this accession to the modern lettuce (Table 1). We considered *L. serriola* as a wild progenitor (WP) accession here. Lettuce plants used in the infection assay were grown in the glasshouse with a minimum temperature of 15 °C in modular trays and then, re-cultivated into 9 cm plastic pots with watering from below. In all assays, plants were inoculated with *S. sclerotiorum* mycelia plugs, when they were 4–6 week-old with 5–7 fully expanded leaves. Once the seedlings were around 6-week-old, the inoculum, the PDA plugs excised from actively growing edges of fungal colonies, were positioned in wounds made on the leaves by a needle and sealed with a parafilm. The inoculated plants were subsequently covered with a plastic bag and maintained in a plastic cage in the dark for 48 h at 23–25 °C. The inoculated seedlings (one plant containing two leaves per each biological replicate) were then transferred to the greenhouse and disease development was monitored by five days after inoculation. We evaluated the disease development of each accession by calculating the percentage of the necrotic area, extending to the exterior border, where we placed the mycelial plugs (Table 1). To aim this, we cut the inoculated leaves and stuck on the white paper to take pictures of all harvested leaves. We calculated the necrotic areas by visual assessments and confirmed it by the Image J tool for some

Table 1 Lettuce accessions and cultivars used in the infection assay. Experiments were repeated *in triplo* and the percentage of disease development was assessed through calculating the necrotic areas formed on the inoculated leaves

No.	Accession name	Percentage of disease development		
		Rep 1	Rep 2	Rep 3
1	Abtavil	28	24	22
2	Borazjan	27	29	33
3	siah Dezful	30	20	20
4	Piche Ahvaz	15	15	20
5	Shadeghan	20	30	30
6	Ghom	15	20	25
7	Karaj	15	20	29
8	sefed Neyshabur	28	25	29
9	siah Neyshabur	22	28	29
10	Gorgan	28	32	27
11	Varsh	10	15	14
12	Babolye 1	28	25	30
13	Piche Babolye 3	11	16	14
14	Mazandaran Line 1	50	47	40
15	Mazandaran Line 2	20	22	20
16	Mazandaran Line 3	30	30	32
17	Mazandaran Line 4	33	34	30
18	Mazandaran Line 5	20	23	17
19	Mazandaran Line 6	20	18	20
20	Mazandaran Line 7	30	31	33
21	Mazandaran Line 8	28	29	26
22	Mazandaran Line 9	31	28	39
23	Mazandaran Line 10	30	33	37
24	Mazandaran Line 11	24	22	31
25	Mazandaran Line 12	30	32	31
26	Mazandaran Line 13	20	22	28
27	Mazandaran Line 14	10	15	19
28	Mazandaran Line 15	35	33	37
29	Mazandaran Line 16	10	10	20
30	Mazandaran Line 17	17	20	15
31	Mazandaran Line 18	20	10	15
32	Varamin 1	30	31	37
33	Varamin 2	21	19	32
34	Varamin 3	30	35	33
35	Shiraz	20	10	30
36	Kazeroon	30	20	30
37	Hamedan	30	10	20
38	Nahavand	26	32	37
39	Pars Abad	17	20	18
40	Ardebil	19	17	15
41	Jahrom	8	11	9
42	Fasa	15	10	17
43	*Lactuca serriola* (WP)	37	30	33
44	Commercial AL	29	25	30
45	Commercial BL	32	28	29
46	Commercial RL	15	14	19

cases to be sure of what we have calculated. Experiments were repeated *in triplo.*

Selectin of candidate defense-related genes and primer design

A total of five defense-related genes (*LsPRB1*, *LsSOD*, *LsERF1*, *LsLTC1*, and *LsHPL1*) were selected based on the previous studies, describing these genes as an important key player in plant-microbe interactions to mediate defense response in host species. As the fully sequenced genomes or even the coding sequences of candidate genes in Iranian lettuce accessions are unavailable, we blasted the NCBI database with the stored sequence of *L. sativa* for each gene to identify the homologous of the candidate genes in the closely-related species. Afterward, we used Vector NTI (Lu and Moriyama 2004) to run multiple alignments for the retrieved sequence to determine the most conserved region of each gene in *L. sativa* compared with that of closely-related species. In the next step, we employed the conserved region to design the required primers. The Primer3Plus tool (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) was employed to design primers for qPCR assay (Table 2). For efficient PCR design amplicon size (< 200 bp), melting temperature (60 °C) and product size of less than 200 bp were considered. The genome of *L. sativus* available at NCBI was browsed, and transcript sequence of interesting genes was retrieved and downloaded in fasta format. Subsequently, the downloaded sequences were uploaded in Primer3Plus to design required primers. The presence of any primer dimers and non-specific amplification was identified post-PCR through the analysis of melting curve data.

RNA isolation and qRT-PCR

In planta expression analyses of candidate genes were conducted through real-time quantitative RT-PCR (qPCR) method. Lettuce accessions were inoculated with *S. sclerotiorum* as previously described, and leaf samples were collected in three biological replicates (one plant having two leaves per each biological replicate), flash frozen and ground in liquid nitrogen using a mortar and pestle. Total RNA was extracted either from ground leaves using the Total RNA Extraction Kit (Vivantis, Taiwan), and subsequently, DNA contamination was removed using the DNase I (Fermentas, USA). First-strand cDNA was synthesized from approximately two μg of total RNA primed with oligo(dT) using the ExcelRT™ Reverse Transcriptase (SMOBIO, Taiwan) according to the manufacturer's instructions. One μl of the resulting cDNA was used in a 25 μl PCR reaction using a RealQ Plus Master Mix Green (Ampliqon, Denmark) and run and analyzed using an Applied Biosystems StepOne system. We applied the constitutively expressed *L. sativa* actin gene as a reference gene to normalize the relative expression of each gene as this is the most frequently used gene in the qPCR technique (Borowski et al. 2014). Subsequently, the comparative C (t) method described previously (Schmittgen and Livak 2008) was employed to calculate the relative expression for each gene.

Results

Phenotypic evaluation of lettuce genotypes

As we aimed to evaluate the gene expression pattern of lettuce genes, playing at an early stage of the infection process in

Table 2 Primers used in this study

No	Code	Functional predicted	NCBI ID	Primer sequence (5'–3')
1	*PRB1*	Pathogenesis related protein-1	XM_023883232.1	F:ATGGGACAGTCGTGTGGCTAGTTT R:TGTTCACAGCATCTACACCG GTCA
2	*ERF1*	Ethylene-responsive element binding protein1 homolog	XM_023900790.1	F:TCGCCGGTGATGTCCAGTTATCAA R:TGTTTCCCTCTCTGCTGGTTCACA
3	*HPL1*	Fatty acid hydroperoxide lyase	XM_023907774.1	F:CGTTAGGATCCGCCGACCGC R:TCCTTCCTTGCCCGCCCGTA
4	*LTC1*	Sesquiterpene synthase	AF489964	F:AACGAGGGATGCCTTAAGCC R:CCCGGAAAAGTAAACCCATCG
5	*SOD*	Superoxide dismutase	AJ310450.1	F: CTTCCAGCCTTCAACAACGC R: ATTAGGCCTCCAAACGAGCC
6	*Actin*	*Lactuca sativa* putative actin 7	XM_023905463	F: AACTGGAATGGTGAAGGCTGG R: TTGTAGAAAGTGTGATGCCA

response to *S. sclerotiorum* attack, we scored all inoculated plants at 96 hours after inoculation (hai) to determine the contrasting accessions showing the highest susceptibility and tolerance. The data are shown as a percentage of disease development formed on each leaf (Table 1). Based on the obtained data, we selected accessions named Mazandaran Line 1 (ML1) and Jahrom (Jah) as susceptible and tolerant ones, respectively, to use in the subsequent expression analysis (Fig. 1). The percentage of lesion formation for ML1 and Jah was calculated 45.6% and 9.3%, respectively, while that of WP was 33.3%.

Expression profiling of five defense-related genes

Expression profiles of five defense-related genes (*LsPRB1*, *LsSOD*, *LsERF1*, *LsLTC1*, and *LsHPL1*) implicated in defense response were investigated by qRT-PCR in two contrastingly responding lettuce accessions along with a wild progenitor (WP) of cultivated lettuce. The expression profiling of selected genes was monitored at various time courses, including 24, 48, 72, and 96 hai. *LsPRB1* was specifically up-regulated in the tolerant Jah accession compared with that of susceptible one, reaching a peak at 24 hai, while this gene was lowly expressed in the WP accession. *LsSOD* transcript accumulated both highly and rapidly in the Jah accession compared with that of susceptible ML1 accession at 24 hai, whereas this gene was slightly expressed in the WP accession in all tested time courses. *LsERF1* has a bimodal expression pattern reaching a peak at 24 and 72 hai in the Jah accession compared with that of susceptible ML1 accession, while *LsLTC1* followed the same expression trend, but it was highly expressed at 24 and 96 hai. Interestingly, the highest transcript accumulation of *LsHPLI* was in the WP accession at 48 hai, which was in contrast with other observed expression patterns (Fig. 2).

Discussion

We employed the qPCR technique to monitor the expression profiles of five defense-related genes in lettuce-*S. sclerotiorum* interactions to gain insight into how lettuce finely regulates its defense pathway to defend itself efficiently. To achieve this goal, we collected 42 Iranian lettuce accessions along with three commercial cultivars and *L. serriola* (the WP accession) to identify genetic resources, which are tolerant or resistant toward lettuce drop. Subsequently, all accessions were infected with S. *sclerotiorum* isolate collected from Varamin County, Tehran Province, Iran, where lettuce plant is widely grown. Our infection assay resulted in identifying a susceptible ML1 accession and the tolerant Jah accession. These two selected accessions were subsequently inoculated with *S. sclerotiorum* isolate, and the infected leaves were collected 24, 48, 72, and 96 hai to evaluate the gene expression patterns of interesting genes. In this study, we observed temporal and quantitative gene expression fluctuations between two examined accessions of *L. sativa* in response to *S. sclerotiorum* attack. All genes, except *LsHPL1*, were up-regulated earlier (24 hai) in the Jah accession

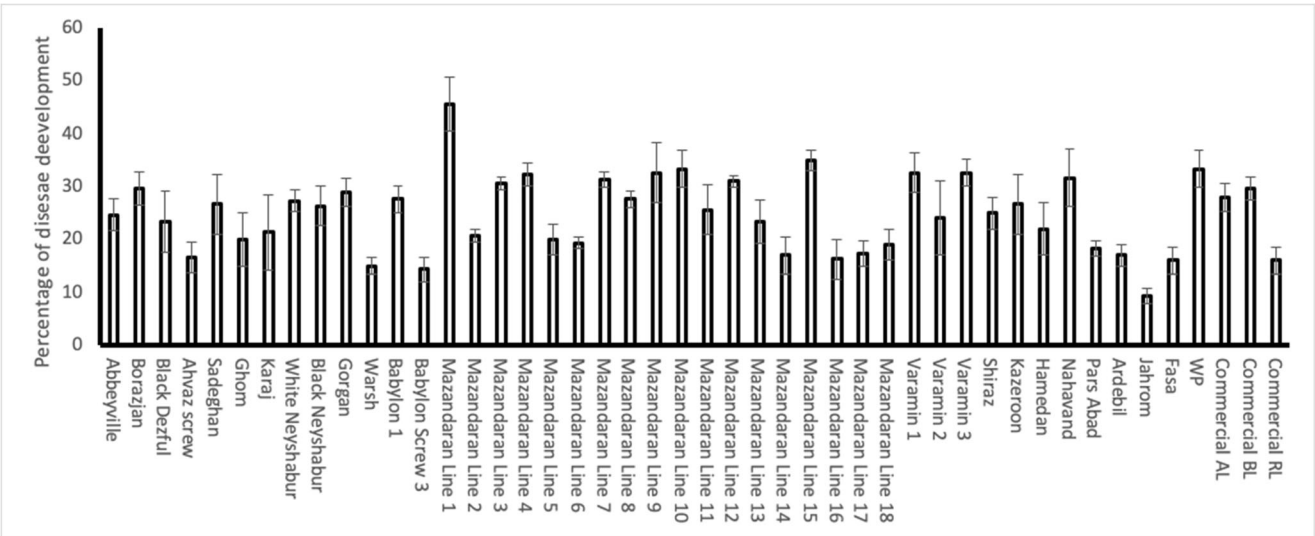

Fig. 1 The percentage of disease development of 46 lettuce accessions and cultivars in response to *Sclerotinia sclerotiorum* infection. 4–6 week-old plants were inoculated by placing a PDA plugs excised from actively growing edges of fungal colonies on the wounded leaf, and sealed with a parafilm. The disease development of each accession was calculated as the percentage of the necrotic area extending to the exterior border where the mycelial plug was placed. Experiments were repeated *in triplo* and the evaluation of the inoculated leaves were conducted at 96 hpi. The Y-axis represents the percentage of disease development, while the X-axis shows the name of examined accessions

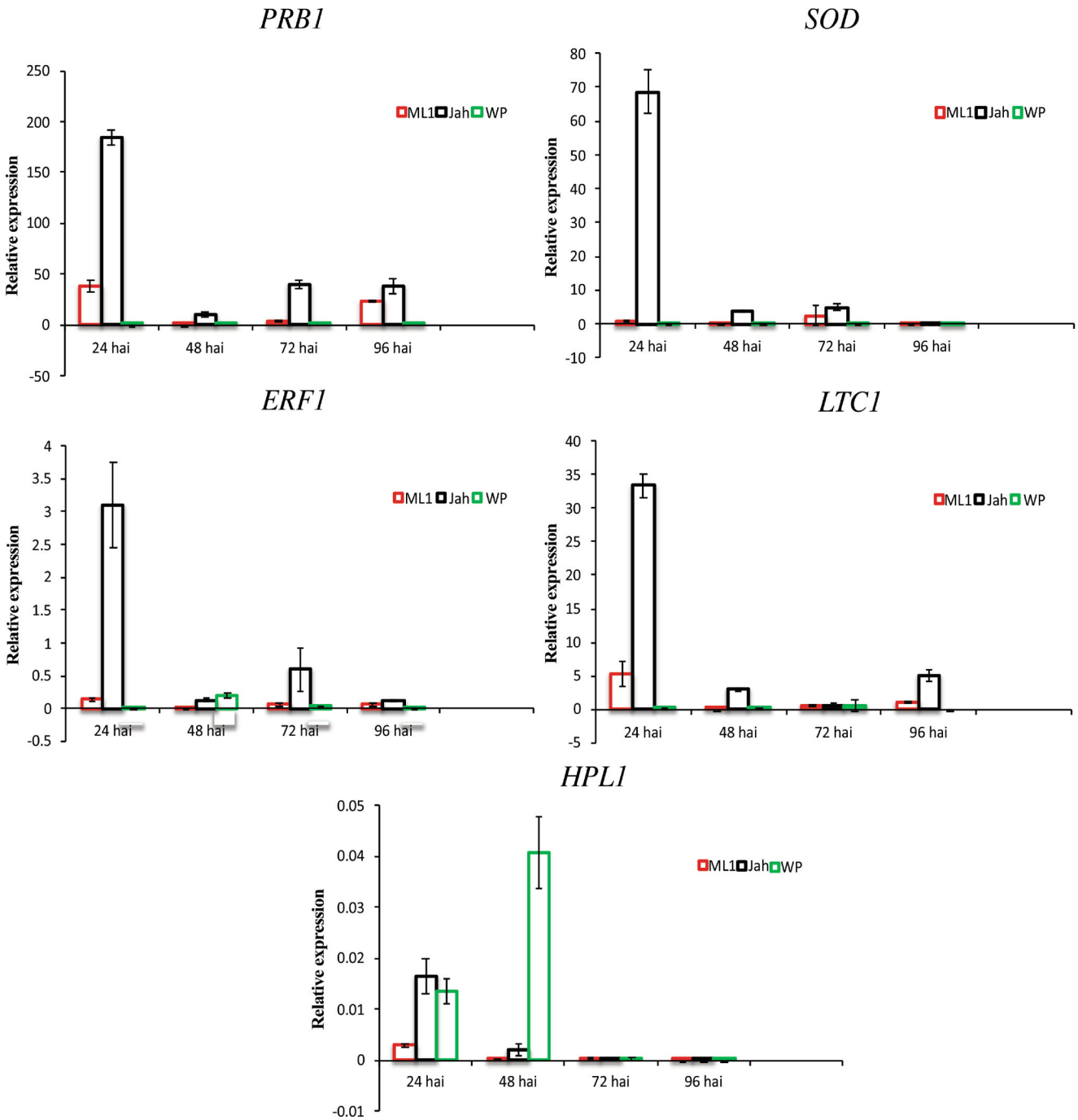

Fig. 2 *In planta* expression levels of five defense-related genes triggered in lettuce upon infection by *Sclerotinia sclerotiorum*. *In planta* expression profiles were evaluated during a time course (24–96 hours after inoculation) experiment, using the susceptible lettuce accession Mazandaran line 1 (ML1) and the tolerant accession Jahrom (Jah). The relative expression of tested genes was normalized with the constitutively expressed *Lactuca sativa* actin gene. Error bars represent the standard deviation of the mean from three biological replicates

compared with the susceptible one. This data implies probably strong defense responses established in the tolerant Jah accession to arrest the fungal growth, but this resulted in restricting lesion development rather than in preventing infection as observed phenotypically in the infection assay.

The fluctuation of gene expression profiles of defense-related genes is probably a pivotal element, functioning central roles in *L. sativa* interactions. Our expression analyses revealed that all examined genes in two contrastingly interactions were differentially expressed. Based on this data, we speculate that the temporal and quantitative differences in

gene expression might play a role in restricting disease development.

Phytohormones, including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are the central regulator of plant defense response, and they are elevated in plant tissues upon pathogen attack. SA signaling networks provide plant resistance to biotrophic pathogens, whereas JA- and ET- signaling pathways are involved in rendering resistance towards necrotrophs (Glazebrook 2005).

LsPRB1 transcripts were induced significantly at all tested time courses, but the highest peak was observed at 24 hai in the tolerant Jah accession compared with that of susceptible one. This stage is coincident with the early stage of the pathogenesis process, and the potential short biotrophic phase proposed to exist in the life cycle of *S. sclerotiorum*. Traditionally, this fungus is considered as a typical necrotrophic pathogen, but recently a short biotrophic stage is proposed in the life cycle of this fungus for the successful establishment of plant colonization (Kabbage et al. 2015). It is documented that salicylic acid-mediated defense resistance is triggered in host plants to combat biotrophs (Glazebrook 2005), and the *LsPRB1* expression is employed as a molecular marker for the triggering of the salicylic acid pathway (Breen et al. 2017). Our data agreed with a previously published study where it was shown that the expression level of *LsPRB1* was significantly induced during the response of *L. sativa* infected with the necrotrophic phytopathogen *Botrytis cinerea* and in the same study, it was observed that the *LsPRB1* transcript was highly triggered in the *L. sativa* infected with the biotrophic phytopathogen *Bremia lactucae* (fold changes of 32 and 35, respectively) (De Cremer et al. 2013). Another study revealed that the expression level of *LsPRB1* was highly activated in the model plant *Arabidopsis thaliana* in response to infection with *S. sclerotiorum* (Girard et al. 2017).

Here, the transcript abundance of gene-encoding the antioxidant enzyme superoxide dismutase (*SOD*) was significantly accumulated at 24 hai in the tolerant Jah accession compared with that of the susceptible ML1 one. This data is a reasonable indication revealing that this enzyme plays an essential role in detoxifying the generated free radical upon *S. sclerotiorum* invasion. *LsSOD* is known to function as the first line of defense against the elevated reactive oxygen species, a phenomenon considered as oxidative burst, generated in a plant in response to biotic and abiotic stresses (Alscher et al. 2002). To our knowledge, it is the first evidence evaluating the expression profiles of *LsSOD* in lettuce accessions to the fungal infection. Nevertheless, there are several lines of evidence demonstrated that lettuce exposed to abiotic stresses such as salinity and heavy metals enhanced the activity as well as the gene expression level of *LsSOD* to combat with the oxidative stress (Kolahi et al. 2020; Mahmoudi et al. 2012; Ruiz-Lozano et al. 2001). More interestingly, it was shown that lettuce plants treated with blue light suppressed remarkedly the development of gray mold caused by *B. cinerea.* This finding explained through this fact that an increase in the activity of antioxidant enzymes such as *LsSOD* was observed (Ruiz-Lozano et al. 2001).

Our results showed that *LsERF1* was significantly induced at 24 and 48 hai in the tolerant Jah accession compared with that of susceptible one, but the highest expression level was observed at 24 hai, indicating its expression may is a crucial factor in mediating resistance toward *S. sclerotiorum*. *LsERF1* is a transcriptional regulator and downstream of the ethylene pathway integrating this network with the jasmonic pathway to confer resistance against necrotrophic fungal pathogens. Based on our data, no transcriptome analysis or even gene expression data are available investigating the expression profile of *LsERF1* in lettuce in response to *S. sclerotiorum* attack. Microarray analysis conducted to investigate transcriptional changes in canola following infection with *S. sclerotiorum* confirmed an increase in transcript abundance of various ERFs such as ethylene responsive element binding factor 4 (*ERF4*) at 24 hai (Yang et al. 2007). Comparative transcriptome analysis of lettuce and Arabidopsis plants infected by *B. cinerea* demonstrated that *LsERF1* expression level at 18 and 24 hai was induced, suggesting a potential role of this transcription factor in conferring resistance against this necrotrophic phytopathogen (De Cremer et al. 2013; Mulema and Denby 2012). Moreover, overexpression of *LsERF1* in Arabidopsis provided enhanced resistance towards several necrotrophic fungi such as *B. cinerea* and *Plectosphaerella cucumerina* (Berrocal-Lobo et al. 2002). Northern analysis to monitor the expression level of *LsERF1* in Arabidopsis proved that this transcriptional regulator was induced following infection by the soil-borne pathogen *F. oxysporum* f. sp. *conglutinans*, and more interestingly, the enhanced resistance against this soil-borne plant pathogen mediated by *LsERF1* overexpression (Berrocal-Lobo and Molina 2004).

Our expression analysis revealed that *LsLTC1* was remarkably triggered at 24 and 96 hai in the tolerant Jah accession compared with that of the ML1, suggesting it probably plays a role in conferring defense response toward attack by *S. sclerotiorum*. *LsLTC1* encodes a sesquiterpene synthase that is associated with establishing the plant defense response following fungal attack. Overall, the terpenoids include the largest class of natural products, protecting either indirectly or directly plants against biotic and abiotic stresses, and also they extensively employed in the industrial sector as flavors (Singh and Sharma 2015; Yadav et al. 2019). For example, the terpene synthase *TPS10* plays a central role in the indirect protection of maize towards pests by attracting the natural enemies of herbivores (Schnee et al. 2006). Additionally, *MtTPS10*, encoding a putative sesquiterpene synthase, was up-regulated strongly at 2 hai in the seedlings and roots of *Medicago truncatula* inoculated by the oomycetes

Aphanomyces euteiches, the causal agent of root rot disease in legumes (Yadav et al. 2019).

Interestingly, the transcript abundance of *LsHPL1* encoding a hydroperoxide lyases was specifically accumulated in the WP accession compared with that of other tested accessions. This result suggests that *LsHPL1* probably acts as either resistance or susceptibility factor, inducing highly upon infection. Several reports demonstrated that *LsHPL1* plays a diverse role in mediating plant defense responses through the release of the green leaf volatiles (GLVs) and the regulation of genes implicated in the jasmonic acid (JA) pathway (Xin et al. 2014). Constitutive expression of *CsiHPL1*, a chloroplast-localized tea gene in tomato plant, resulted in developing a transgenic line with enhanced resistance to the necrotrophic fungal pathogen *Alternaria alternata* f. sp. *lycopersici* (AAL), causing the *Alternaria* stem canker of tomato (Tong et al. 2012; Xin et al. 2014).

To sum up, we found genes up-regulated differentially in the susceptible and tolerant accessions in the *L. sativa-S. sclerotiorum* interactions that probably play instrumental roles in restricting fungal colonization. We can consider them as potential candidates to be included in the subsequent functional analysis to prove their exact roles. Following completing their functional analysis, novel technology such as CRISPR-Cas9 could be applied to genetically manipulate them in the susceptible backgrounds in the future experiments (Ran et al. 2013). This genetic editing will be culminated in developing either tolerant or resistant plants not considered anymore as genetically modified (GM) plants, and they can tolerate the high virulence spectrum exhibited by the notorious fungal pathogen *S. sclerotiorum*. Finally, we propose to fully sequence the genomes of the Iranian lettuce accession, providing valuable genomic resources and enabling the researcher to precisely compare them at the molecular level. Additionally, the de novo transcriptome sequencing approach is highly recommended in the subsequent studies to compare these accessions at the transcriptome level.

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Data availability Data are available.

Compliance with ethical standards

Conflicts 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.

Code availability Not applicable.

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