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Genome-Wide Characterization of *HSP90* Gene Family in *Malus sieversii* and Their Potential Roles in Response to *Valsa mali* Infection

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Abstract: Heat shock protein 90 (*HSP90*) is highly conservative molecular chaperon produced by plants in response to adverse environmental stresses including fungal infection. In China, canker disease, caused by *Valsa mali*, is the main threat for *Malus sieversii*, an ancestor of the cultivated apple. In this study, a total of eight *HSP90* genes were identified from the *M. sieversii* genome and randomly distributed on eight chromosomes. According to gene structure and phylogenetic analysis, the *MsHSP90s* can be divided into five categories. The transcriptome analysis of *M. sieversii* under *V. mali* infection showed that the plant pathogen interaction pathway was identified as significantly enriched. RNA-seq data and qRT-PCR analysis demonstrated that the *MsHSP90-6a* gene was significantly repressed by *V. mali* infection. We further predicted cis-regulatory elements on the promotor region of *MsHSP90* genes and identified canonical SHE motifs. Our results improve our understanding of the *HSP90* gene family and provide a foundation for further studies of disease prevention in *M. sieversii*.

Keywords: canker; heat shock protein 90; *Malus sieversii*; fungal resistance

Citation: Haxim, Y.; Si, Y.; Liu, X.; Wen, X.; Kahar, G.; Ding, Y.; Li, X.; Zhang, D. Genome-Wide Characterization of *HSP90* Gene Family in *Malus sieversii* and Their Potential Roles in Response to *Valsa mali* Infection. *Forests* **2021**, *12*, 1232. <https://doi.org/10.3390/f12091232>

Academic Editor: Carol A. Loopstra

Received: 17 July 2021

Accepted: 6 September 2021

Published: 10 September 2021

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1. Introduction

Heat shock proteins (*HSPs*) are highly conserved protein families among the fungi, animals, and plants that respond to a wide variety of stress. *HSP90s* are key molecular chaperons that regulate proteostasis under both physiological and stress conditions in eukaryotic cells [1].

Studies on the biological functions of *HSP90s* in plants are relatively late than in fungi and mammalian model systems. Recently, a large number of *HSP90* genes have been identified in various plant species, including *Arabidopsis thaliana* (L.) Heynh. [2], *Oryza sativa* L. [3], *Glycine max* (L.) Merr. [4], *Cucumis sativus* L. [5], *Populus trichocarpa* Torr. & A.Gray ex Hook. [6], and *Nicotiana tabacum* L. [7]. The number of *HSP90* genes in the plant genome varies from plant to plant. For instance, seven, nine, and fifteen *HSP90* genes were identified in *Arabidopsis* [2], rice [3], and soybean [4], respectively.

Recent studies indicated that *HSP90s* mediate both abiotic and biotic stress resistances in plants [5,8–10], and the expression patterns of *HSP90* genes under different biotic and abiotic stress conditions have been widely analysed. *HSP90* genes have reported increased expression under heat stress [11] or salt stress [12] in *Arabidopsis* while being downregulated under cold stress [13] in wheat. It has been shown that heat shock factors (HSFs) are responsible for the activation of *HSP* genes in plants by binding to the heat shock response element (HSE), which is a specific DNA sequence (nGAAn) observed in

HSP gene promoters [14]. Under stress conditions, HSF binds to HSEs in the promoter of HSP90 genes and initiates transcription of HSP90 genes [15,16].

In recent years, it has become apparent that HSP90s mediate disease resistance in plants. Transcriptional response of HSP90 genes against various pathogens are varied in different host plants. Three HSP90 genes were upregulated in *Medicago truncatula* Gaertn. by *Acyrtosiphon pisum* (Harris, 1776) infestation [17]. Bacterial pathogen, *Ralstonia solani*, infection in *Nicotiana benthamiana* Domin induced HSP90 gene expression [18]; however, a viral pathogen, *Stem Grooving Virus*, infection in *Malus domestica* (Suckow) Borkh. suppressed the host HSP90 gene expression [18]. Recent studies showed that plant HSP90 genes also display different expression patterns during fungal infection, and it seems plants have evolved pathogen-specific immune response strategies. Stripe rust (*Puccinia striiformis*) and powdery mildew (*Blumeria graminis*) are important diseases of wheat (*Triticum aestivum* L.). The large-scale transcriptome comparison reveals that HSP90 genes were upregulated in response to *B. graminis* while repressed by infection of *P. striiformis* [19].

As the primary progenitor of all cultivated apple species, *Malus sieversii* (Ledeb.) M. Roem. is mainly distributed in central Asia, and featured higher genetic diversity and disease resistance providing genetic materials for the molecular breeding of cultivated apples [20,21]. Valsa canker disease (caused by *Valsa mali*), however, has been threatening the *M. sieversii* population [22,23]. Recent studies showed that HSP90s play an essential role in plant R (resistance) protein-mediated immune response. R proteins recognize pathogen virulence proteins, and subsequently trigger host immune response, hypersensitive response, or systemic acquired resistance [24]. However, the characteristic and functional identification of the HSP90 gene family in *M. sieversii* and transcriptional response upon the infection of *V. mali* has not yet been reported. Therefore, exploring the biological function of the MsHSP90 gene family provides new insight into the resistance mechanism of *M. sieversii* response to the infection of *V. mali*.

In this study, we identified eight HSP90 genes based on *M. sieversii* genome and transcriptome data. Subsequently, we characterized the gene structure and phylogenetic relationship of HSP90 gene family in *M. sieversii*. Our work extends our knowledge of the function of MsHSP90 in response to *V. mali* infection.

2. Materials and Methods

2.1. Plant Material and Pathogen Infection

Six-month-old *M. sieversii* seedlings were purchased from Nature and Wildlife Conservation Station of Xinyuan County, Xinjiang, China. These seedlings were planted in pots containing soil and vermiculite and grown at 24 °C in 16/8 h light/dark cycles. For the fungal pathogen *V. mali*, isolate EGI1 [22] was grown on the Potato Dextrose Agar (PDA) medium (Rishui BioTechnologies, China) plate at 25 °C for three days. The plant infection by *V. mali* was performed as described by Liu et al., 2020 [22]. Briefly, the mycelial plugs (diameter: 5 mm) were excised from the 3-days-cultured isolate EGI1 on PDA medium. The leaves were wounded by a fabric pattern wheel and then inoculated with these mycelial plugs. These inoculated leaves were placed in dishes tapped with parafilm and kept in darkness at 25 °C.

2.2. RNA Isolation and Real Time Quantitative PCR Analysis

Total RNA was isolated from the *M. sieversii* leaf samples collected at different time points (0 dpi, 1 dpi, 2 dpi, 5 dpi) after *V. mali* infection by using Plant RNA Kit (Omega, No. R6827, China) according to instructions. cDNA was synthesized with 1 µg of total RNA using PrimeScript RT reagent Kit with gDNA Eraser (Takara, No. RR047Q, China). To validate expression level of MsHSP90s, sequence specific primers (Table S1) were designed using DNAMAN software version 9.0 (Lynnon BioSoft) and synthesized by Sangon Biotech (Shanghai, China). Transcript level of target genes was detected by TB Green Premix Ex Taq II kit (Takara, No. RR820A, China) on the CFX96 Real-Time PCR Detection System (Bio-Rad, CFX96, USA). The thermal profile for qRT-PCR was as follows: preheating at

95 °C for 30 s; cycling stage: 95 °C for 5 s, 58 °C for 30 s, 40 cycles; melt curve analysis was produced to confirm the specificity of amplification. The relative transcription levels of *MsHSP90s* were analyzed with the $2^{-\Delta\Delta CT}$ method [25]. The *EF-1 α* (Elongation factor 1- α) gene was used as an internal reference gene [26]. Each sample contained three biological replicates and each biological replicate contained three technical replicates. Statistical analysis was performed by ANOVA test using SPSS v.18 software.

2.3. RNA-Seq Data Analysis

To analyse differentially expressed genes (DEG) of *M. sieversii* in response to *V. mali* infection, the PacBio and Illumina transcriptome sequencing data (NCBI BioProject: PRJNA687214) were used [27]. For the sampling of the PacBio and Illumina sequencing, barks of twigs in *M. sieversii* near the canker were separately harvested at the time points of 0, 1, 2, and 5 dpi, and each sample contained three biological replicates. The FPKM (fragments per kilobase of transcript per million fragments mapped) value was calculated for each gene. Differential expression analysis of three biological replicates per condition was performed using the DESeq R package (1.18.0). The resulting *p*-values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Transcripts with an adjusted *p*-value < 0.05 and log₂ fold change ≥ 2 were assigned as differentially expressed. The log₂ fold change values of the *MsHSP90* genes were used for bar plot generation.

2.4. Genome Wide Identification of the *MsHSP90* Gens and Gene Sequence Analysis

The amino acid sequences of *HSP90* from *P. trichocarpa*, *Vitis vinifera* L., *O. sativa*, and *M. domestica* were retrieved from the NCBI (<https://www.ncbi.nlm.nih.gov/>, accessed on 15 May 2021) databases, *Arabidopsis thaliana* genome data were obtained from TAIR (<http://www.arabidopsis.org/>, accessed on 15 May 2021) database. These sequences were used as queries for identifying homologous genes in the *M. sieversii* genome (NCBI genome ID: JAHTLV000000000, unpublished data). Briefly, the local protein BLAST database of *M. sieversii* was generated with the BLAST 2.2.24 program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 15 May 2021), and putative orthologous genes of query were searched using an all-against-all protein BLAST based on the reciprocal best similarity match.

The sequences were aligned by Clustal X2 (<http://www.clustal.org/>, accessed on 15 May 2021), and the phylogenetic tree was generated using MEGA-X software (<https://www.megasoftware.net/>, accessed on 15 May 2021) by the neighbor-joining (NJ) method with bootstrap value 1000.

The exon–intron organization of the *HSP90* genes were visualized by Gene Structure Display Server 2.0 (<http://gsds.cbi.pku.edu.cn/>, accessed on 15 May 2021) [28]. The coding sequences were aligned to their corresponding genomic sequences. The alignment was manually adjusted, if necessary.

The corrected transcripts were annotated based on KEGG database (<http://www.genome.jp/kegg>, accessed on 15 May 2021). For the KEGG enrichment or pathway analysis, the KOBAS3.0 [29] method was used, and visualized by Pathview package of R/Bioconductor.

All databases used in this study shall be subject to the version in May 2021.

2.5. Prediction of Transcription Factor Binding Sites of *MsHSP90* Genes Promoter

The promoter sequence (2.5 kb DNA sequence upstream of the start codon) of each *HSP90* gene was retrieved from the *M. sieversii* genome (NCBI genome ID: JAHTLV000000000, unpublished data). Non-redundancy and high-quality transcription factor binding motifs were derived from the promoters of each *MsHSP90* genes using the PlantRegMap.3.3 tools [30]. Transcription factor binding sites were scanned from promoters of each *MsHSP90* gene sequence, *Malsu dom estica* served as reference species, and the threshold *p*-value was adjusted to 1×10^{-4} .

For the prediction of HSEs from promoters of *MsHSP90s*, we searched for the occurrence of HSE sequences in consensus with [nGAAn/nTTCn] in the promoter regions

Further analysis showed that 73 differentially expressed genes across the time points were involved in plant-pathogen interaction pathways (Figure 1). Infection of *V. mali* triggered overexpression of the ETI (effector triggered immunity)-related core genes, such as resistance gene *RPM1* (resistance to *P. syringae* pv. *maculicola*) and *RPS2* (resistance to *P. syringae*) or *RIN4* (*RPM1*interacting protein 4). Interestingly, *HSP90* was downregulated while its interacting partners *RAR1* and *SGT1* remained constant.

3.2. Identification of HSP90 Gene Family in *M. sieversii*

To accurately identify the members of *HSP90* gene family in *M. sieversii*, we first confirmed the homology of the *HSP90* gene family in the *M. domestica* genome. A total of eight *MdHSP90* genes were identified, and were mapped onto different chromosomes (Table 1, Figure 2b). Furthermore, we used a local BLAST search against the *M. sieversii* genome. The *HSP90* proteins from *M. domestica*, *A. thaliana*, *P. trichocarpa*, *V. vinifera*, and *O. sativa* were served as a query sequence, and total of eight *MsHSP90* genes were identified (Table 1, Figure 2c). To further elucidate the evolutionary relationship of the *HSP90* gene family, the phylogenetic tree was constructed using *HSP90* genes from different plant species by the neighbor-joining method.

Figure 2. Bioinformatic analysis of *HSP90* genes. (a) Phylogenetic tree of *HSP90* proteins from different species. Protein sequences were aligned by ClustalW algorithm, and phylogenetic tree was generated by using the neighbor-joining method with 1000 bootstrap in MEGA X. Different shapes represent different species. Chromosomal locations of the 8 *MdHSP90* (b) and *MsHSP90* (c) genes. MG2C online tools were used (http://mg2c.iask.in/mg2c_v2.1/, accessed on 15 May 2021) to depict chromosomal location, and the gene was marked with short lines. (d) Gene structure of *MsHSP90* and *MdHSP90* genes. GSDS2.0 (<http://gsds.cbi.pku.edu.cn/>, accessed on 15 May 2021) online tools [28] were used to visualize exon-intron structures.

As shown in Figure 2a, the *HSP90* genes were classified into seven categories (*HSP90*-1~7), and the *MsHSP90* and *MdHSP90* genes were clustered into five different categories (Figure 2d). For the *MdHSP90* gene family, each of the *MdHSP90*-1, *MdHSP90*-2, and *MdHSP90*-6 genes has two homologues while *MdHSP90*-5 and *MdHSP90*-7 was clustered with single members. For the *MsHSP90* gene family, however, each of the *MsHSP90*-1, *MsHSP90*-2, and *MsHSP90*-7 genes has two members while *MsHSP90*-5 and *MsHSP90*-6 have single members. We performed exon-intron structure analysis for *MsHSP90* and *MdHSP90* genes. Our results showed a high degree of similarity in the same branch (Figure 2d).

Table 1. HSP90 genes in *M. domestica* and *M. sieversii*.

Gene Symbol	Length of CDS (bp)	Chromosomes	Genomic Coordinates	Strand	Number of Amino Acids in Protein
<i>MdHSP90-1a</i>	2112	7	NC_041795.1: 34410217-34414101	+	703
<i>MdHSP90-1b</i>	2112	1	NC_041789.1: 30385625-30388837	+	703
<i>MdHSP90-2a</i>	2100	11	NC_041799.1: 3210146-3213261	—	699
<i>MdHSP90-2b</i>	2100	3	NC_041791.1: 2915642-2918677	—	699
<i>MdHSP90-5</i>	2385	16	NC_041804.1: 3808810-3813939	—	794
<i>MdHSP90-6a</i>	2421	17	NC_041805.1: 9778825-9785023	—	806
<i>MdHSP90-6b</i>	2430	9	NC_041797.1: 9428843-9434534	—	809
<i>MdHSP90-7</i>	2457	8	NC_041796.1: 866442-871008	+	818
<i>MsHSP90-1a</i>	2490	9	Chr9:35058827-35061327	+	829
<i>MsHSP90-1b</i>	1512	13	Chr13:32200739-32203239	+	503
<i>MsHSP90-2a</i>	2100	5	Chr5:3353408-3350908	—	699
<i>MsHSP90-2b</i>	2085	8	Chr8:2754830-2752330	—	694
<i>MsHSP90-5</i>	2385	6	Chr6:3862971-3860471	—	794
<i>MsHSP90-6</i>	2415	10	Chr10:27515958-27518458	+	804
<i>MsHSP90-7a</i>	2457	1	Chr1:622597-625097	+	818
<i>MsHSP90-7b</i>	2457	15	Chr15:32199616-32197116	—	818

3.3. Expression of MsHSP90 Genes in Response to *V.mali*

To investigate how *MsHSP90* genes respond to a *V. mali* attack, we analysed *M. sieversii* transcriptome data under *V. mali* infection. A total of eight *HSP90* transcripts from *M. sieversii* were subjected to further differential expression analysis through comparisons of foldchange expression. *MsHSP90-6* was significantly downregulated across each time point, while *MsHSP90-5* did not respond to fungal infection. *MsHSP90-2a/b* decreased significantly in the early stage but slightly increased at the late stage of infection. To validate the expression pattern of each transcript, we quantified the transcription level of each *MsHSP90* gene by qRT-PCR at different time points after infection. Expression analysis revealed that relative expression of all *MsHSP90* genes was decreased by infection of *V. mali* (Figure 3, b). The qRT-PCR results of *MsHSP90* transcripts were consistent with the RNA-seq data (Figure 3, a).

Figure 3. Expression of *HSP90* genes in *M. sieversii* during *V. mali* infection. The normalized expression level (FPKM) from the RNA-seq data were shown in red line on right vertical axis and relative expression level from qPCR were shown in green bar on right vertical axis. Verification of the expression of *MdHSP90s* in *M. sieversii* under *V. mali* infection by qRT-PCR. *MsEF1-α* was used as an internal reference gene. The relative expression data were analyzed by one-way ANOVA method. “*” represented significantly different ($p < 0.05$, $n = 3$), and error bars indicate mean $\pm$ SE.

3.4. Prediction of Potential Cis-Elements on HSP90s

To explore potential transcriptional mechanism of *HSP90* genes, we searched for the occurrence of HSE sequences in the promoter regions (within 2500 bp upstream of the coding region) of 8 *MsHSP90* genes. Analysis of the promoter region showed that except *MsHSP90-6* other *MsHSP90* genes have at least single HSE motif (Table 2), strongly suggesting that *MsHSP90* genes are regulated by HSFs. No HSE motif was identified on the promoter region of *MsHSP90-6* inferred that *MsHSP90-6* gene may be targeted by other transcription factors rather than HSFs.

Table 2. The list of HSE motifs identified in the promoters of *MsHSP90* genes.

Gene Name	Start	End	p-Value	q-Value	Matched Sequence
<i>MsHSP90-1a</i>	40	49	7.01×10^{-5}	0.672	–GTTACGAAC–
<i>MsHSP90-1b</i>	2139	2148	1.19×10^{-5}	0.232	–GGAACGTTCC–
	2176	2185	2.63×10^{-4}	0.509	–AGAACATTCT–
	2435	2444	2.63×10^{-4}	0.509	–AGAAAATTTCG–
	2457	2466	2.63×10^{-4}	0.509	–CGAATTTTCT–
<i>MsHSP90-2a</i>	194	203	1.76×10^{-4}	0.509	–CGAAAATTCC–
	1754	1763	1.76×10^{-4}	0.509	–AGAACGTTCA–
	445	454	2.63×10^{-4}	0.509	–AGAAAATTTCG–
	1447	1456	2.89×10^{-4}	0.672	–TTTCATGAAA–
<i>MsHSP90-2b</i>	117	126	1.76×10^{-4}	0.509	–GGAATTTTTCG–
<i>MsHSP90-5</i>	1301	1310	2.89×10^{-4}	0.509	–TGAATTTTCT–
	2244	2253	1.76×10^{-4}	0.672	–GTTCCAGAAA–
	2290	2299	1.76×10^{-4}	0.672	–TTTCCAGAAAG–
<i>MsHSP90-7a</i>	1540	1549	2.63×10^{-4}	0.509	–TGAAC TTTC A–
<i>MsHSP90-7b</i>	1182	1191	1.76×10^{-4}	0.509	–GGAATTTTCG–
	2372	2381	1.76×10^{-4}	0.672	–TTTCTGGAAC–

To investigate whether other transcription factors besides SHF potentially target the *MsHSP90* genes, we scan transcription binding sites on the promoter regions of eight *MsHSP90* genes by plant regulatory data and analysis platform PlantRegMap.3.3 (<http://plantregmap.gao-lab.org/>, accessed on 15 May 2021) and a total of 430 binding sites of 145 transcription factors were identified. Binding sites of bZIP, BBR-BPC, ERF, Dof, and MIKC_MADS families were identified as top five abundant binding sites (Figure 4).

Figure 4. Prediction of potential transcription factors that binding sites of *MsHSP90* genes. The promoter regions (within 2500 bp upstream of the coding region) of 8 *MsHSP90* genes were obtained from the *M. sieversii* genome and analysed by PlantRegMap.3.3 tools (http://plantregmap.gao-lab.org/binding_site_prediction.php, accessed on 15 May 2021). Each dot represents the number of transcription factor binding sites in the *MsHSP90* gene promoter region. Threshold *p*-value $\leq 1 \times 10^{-5}$.

4. Discussion

HSP90 is a type of chaperone that is highly conserved throughout biology kingdoms, and widely involved in various biological processes. To date, the *HSP90* gene family has been identified in various plants, such as *A. thaliana* [2], rice [32], and *N. tabacum* [7]. The apple genome was successfully sequenced and accurate sequence information or annotations are available [33]. However, the genome-wide identification of the *HSP90* gene family and their expression patterns under fungal infection has not been conducted in apples, especially wild apples, *M. sieversii*.

M. sieversii is a wild species native to the mountains of central Asia and eastern China. Recent study showed that *M. sieversii* in Xinjiang, China is the most primitive and principal ancestor of the domesticated apple and is therefore a globally important genetic resource [20]. In Xinjiang, however, the natural habitat of *M. sieversii* has declined by expansion and development of agriculture and the population is shrinking by other natural disasters such as canker diseases caused by the necrotrophic pathogen *V. mali* [22]. Therefore, exploring the disease resistance gene resources in *M. sieversii* upon the infection of *V. mali* has great value in disease prevention.

To uncover the potential function of *HSP90* genes in *M. sieversii* under *V. mali* infection, in the first place we determined eight *HSP90* genes in *M. sieversii* (Table 1). The *MsHSP90* genes were mainly distributed on both ends of the chromosome (Figure 2c) which was similar to the distribution of *M. domestica* (Figure 2b), rice [32], and *Nicotiana tabacum* [7] *HSP90* genes. Different numbers of introns (2 to 19 introns) were identified in different *MsHSP90* gene sequences; *MsHSP90-6* has the maximum numbers of introns while *MsHSP90-2a/b* has the minimum number of introns, implying the diverse sensitivity of gene transcription regulation (Figure 2d). Subsequently, the eight transcripts were identified in *M. sieversii* full-length transcriptome data based on *MsHSP90* gene sequences. The *MsHSP90* genes shared extremely high similarity in the number of sequence compositions with *MdHSP90s*, suggesting that *M. domestica* maintained some of its ancestors' traits in the respect of *HSP90* molecular evolution, implying functional redundancy.

HSP90 plays a crucial role in host fungal defence strategies. To investigate the expression pattern of *HSP90* genes in response to fungal infection in plants, the published data of *HSP90* gene expression in response to *Penicillium expansum* [21], *Alternaria alternata* [34], and *Phytophthora infestans* [35] were collected and fold change expression data were obtained. The expression of *HSP90* genes were varied in different host species. The expression of *HSP90-7* can be host-specific or pathogen-specific. In *M. domestica*, for instance, the *HSP90-7* gene was repressed by infection of *P. expansum* while unchanged in the late phase of *A. alternata* infection (Figure S2). The same expression pattern of *HSP90-7* was observed in two different populus species in response to the same pathogen. It seems that different plants employ different *HSP90s* in response to different fungal pathogens and also implying that each *HSP90* genes was regulated by different transcription factors or has extremely distinct transcriptional regulatory elements.

Recent studies have demonstrated that *HSP90* proteins mediate signal transduction in plant–pathogen interaction pathways in plants. *HSP90* interacts with SGT1 (suppressor of the G-two allele of *skp1*) and RAR1 (required for *Mla12* resistance), and modulates the immune response by regulating R protein stability [36–39]. Hsp90-SGT1-RAR1 complex in *Arabidopsis* plays a key role in assisting in R protein-mediated disease resistance [37]. Despite the *HSP90*, RAR1, and SGT1 proteins interacting with each other, the diversity in transcriptional regulation of their encoding genes reflects their distinct functions. In *Arabidopsis*, for example, transcription level of *AtSGT1a* and *AtSGT1b* was elevated by *Peronospora parasitica* infection [40], but no significant change was observed at the protein level [41].

In this study, transcriptome data analysis indicated that the plant–pathogen interaction pathway in *M. sieversii* was enriched (Figure S1) and the *MsHSP90* gene was downregulated, while *SGT1* and *RAR1* were unchanged in response to *V. mali* infection (Figure 1). qRT-PCR analysis of *MsHSP90* gene expressions also demonstrated that all members of the *MsHSP90*

gene family were repressed by *V. mali* infection (Figure 3), implying that *V. mali* undermined the *M. sieversii* immune system by blocking *HSP90*-mediated signal transduction. In order to investigate how host plant *HSP90* gene expression is modulated in response to fungal infection, we collected the published expression data of *HSP90* genes from *A. thaliana*, *M. domestica*, and two populus species in response to different fungal pathogens. Only a single gene from *Populus deltoids*, *PaHSP90-4a*, was downregulated by *Marssonina brunnea* infection while *PaHSP90-3* was upregulated (Figure S2). In other cases, the *HSP90* gene expression showed fluctuation, indicating that it is not common that the pathogen targets host *HSP90* to interfere with the immune system so that it successfully evades host immune response such as hypersensitive response.

To understand how *MsHSP90* genes were transcriptionally regulated, we analysed the promoter of *MsHSP90* genes. We detected canonical HSE (nGAAnnTTCn) in the promoter of *MdHSP90* genes, implying that HSE regulatory elements are essential for *V. mali* response. We further identified various transcription binding elements (Figure 4) on the promoter region of *MsHSP90* genes suggesting that each *MsHSP90* gene expression may be regulated by different transcription factor. Our results imply the complexity of *MsHSP90* gene expression and versatility of *HSP90* in distinct biological pathways.

In this study, a total of eight *HSP90* genes in *M. sieversii* were identified and characterized by the bioinformatic analyses. A total of eight *HSP90* transcripts in *M. sieversii* were detected in transcriptome data under *V. mali* infection, and *MsHSP90* genes were repressed in response to *V. mali* infection. We anticipate that *V. mali* may facilitate infection by repressing *MsHSP90* gene expression so that it diminishes host immune response. Our study also indicated that *MsHSP90* may be regulated by various transcription factors in response to different stress. This study could not only provide a scientific foundation for the comprehensive understanding of the apple *HSP90* gene family, but could also be helpful in screening candidate genes for canker disease prevention.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/f12091232/s1>, Figure S1: KEGG pathway enrichment analysis of differentially expressed genes response to *V. mali* infection in *M. sieversii*. Figure S2. Expression profiles of *HSP90* genes in response to different plant fungal pathogens. Table S1: List of the qRT-PCR primers of the *MsHSP90* genes.

Author Contributions: The experimental design, data analyzation, manuscript organization were completed by Y.H., Y.S., X.L. (Xiaojie Liu), Y.D. and X.W. were assistant with RNA quantification. D.Z. and X.L. (Xiaoshuang Li) conceived the project, supervised the analysis, and critically revised the manuscript. G.K. was revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (2019QZKK0502030403), the Science and Technology Plan Project of Xinjiang Uygur Autonomous Region (2016B03040), the Youth Innovation Promotion Association, Chinese Academy of Sciences (No. 2018478) and the China Postdoctoral Science Foundation (2021M693380).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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