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ORIGINAL PAPER

Genome-Wide Identification and Expression Analysis of the *CrRLK1L* Gene Family in Apple (*Malus domestica*)

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Abstract
It has been reported that members of the *Catharanthus roseus* receptor-like kinase1-like kinase (*CrRLK1L*) gene family detect cell wall integrity, cell-to-cell communication, and biotic and abiotic stress. We performed a comprehensive study including the genome-wide identification, characterization, and gene expression analysis of *CrRLK1Ls* in apple (*Malus domestica*). Sixty-seven *M. domestica CrRLK1Ls* (*MdCrRLK1Ls*) were identified based on their domain structure. Molecular weight and pI ranged from 52.36–141 kDa and 5.05–8.9, respectively. They were distributed across 16 of the 18 chromosomes and classified into five phylogenetic branches. Exon-intron structural analysis indicated a wide range of exon numbers. Collinearity analysis showed that both segmental-and tandem-duplication contributed to the expansion of this family. *Cis*-elements in the *MdCrRLK1L* promoter region responded mainly to light, circadian rhythm, phytohormones, and biotic or abiotic stress. Many members exhibited tissue-specific expression patterns and differentially expressed under biotic stresses, which may contribute to the different functional roles of *MdCrRLK1Ls* under physiological stress and/or pathological conditions. This study provides new insights into the *CrRLK1Ls* in *Malus* spp.

Keywords *CrRLK1L* · *Malus domestica* · Bioinformatic analysis · Expression pattern

Introduction

The first receptor-like kinase (RLK) identified in plants resembled animal receptor tyrosine kinases (Walker and Zhang 1990). In the following decades, other RLKs were identified in various plant species (Shiu et al. 2004; Lehti-Shiu et al. 2009). More than 600 and 1100 RLKs have been identified in *Arabidopsis thaliana* and rice (*Oryza sativa* L.), respectively (Shiu et al. 2004). A typical RLK contains a signal sequence, a transmembrane region, and a C-terminal domain with eukaryotic protein kinase signatures (Lehti-Shiu et al. 2012). RLKs may also vary greatly in their domain organization and extracellular domain sequence identity (Lehti-Shiu et al. 2012). Plant RLKs are transmembrane proteins that

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can detect many extracellular and endogenous signals through their extracellular domains. They can propagate these signals via their intracellular kinase domains (Walker 1994). Therefore, RLKs play pivotal roles in plant growth, development, and biotic and abiotic stress responses (Stracke et al. 2002; Wang et al. 2008).

Catharanthus roseus receptor-like kinase1-like kinases (*CrRLK1Ls*) are members of a distinct RLK subfamily first discovered in *C. roseus* (Schulze-Muth et al. 1996). At present, 17, 16, and over 40 *CrRLK1Ls* have been identified in *Arabidopsis thaliana*, rice, and cotton (*Gossypium* spp.), respectively (Boisson-Dernier et al. 2011; Han et al. 2011; Nguyen et al. 2015; Niu et al. 2016). *CrRLK1Ls* also have a putative extracellular carbohydrate-binding malectin-like domain (Boisson-Dernier et al. 2011), the presence of which suggests that this gene family participates in cell wall surveillance. They may coordinate cell growth, cell-to-cell communication, and cell wall remodeling during both vegetative and reproductive development (Lindner et al. 2012). Some *CrRLK1Ls* have been reported to play key roles in cell elongation, coordination of cell wall integrity, and cell-to-cell communication between male and female gametophytes (Lindner et al. 2012). Recently, it was shown that a *CrRLK1L* helps powdery mildew penetrate host cells (Kessler et al. 2010). Further investigation confirmed that *CrRLK1Ls* were activated by a secreted peptide known as rapid alkalinization factor (RALF), which suppresses primary root cell elongation in *Arabidopsis thaliana* (Haruta et al. 2014). Many different RALFs have since been identified in plants. Some of them mediate the expression of *CrRLK1Ls* and their co-receptor brassinosteroid insensitive 1-associated kinase 1 (BAK1), which itself regulates plant immune responses (Stegmann et al. 2017). Chen et al. (2016) showed that this protein interacts with guanine nucleotide exchange factors (GEFs). GEFs regulate RhoGTPases (RAC/ROP), which influence certain stress-induced responses, such as the regulation of reactive oxygen species (ROS) production (Greeff et al. 2012). The rapid accumulation of ROS has been proposed as a key step in biotic and abiotic stress responses (Fujita et al. 2006). Therefore, this *CrRLK1L* may be involved in stress response regulation through GEFs. Several *CrRLK1Ls* are regulated in response to drought and cold stress (Nguyen et al. 2015; Wang et al. 2008).

Apple (*Malus domestica* Borkh; family *Rosaceae*) is an important fruit crop and is beneficial to the economic development and human health (Gallai et al. 2009; Kellerhals 2009). Biotic stress (such as *Valsa* canker, fire blight, and replant disease) and abiotic stress (for example, frost, drought, and salinity) severely impede apple tree growth (Byers and Marini 1994; Reed and Mazzola 2015; Rodrigo 2000; Wang et al. 2016; Wu et al. 2017). The completed genome sequence for the diploid "Golden Delicious" apple variety allowed us to perform a comprehensive survey of the *CrRLK1L* family members (Velasco et al. 2010). Although *CrRLK1Ls* have been investigated in several other plant species, those involved in stress responses in apple remain poorly understood.

Using a high-quality whole-genome sequence from a reference strain, we performed the first genome-wide identification and analyses of phylogenetic relationships, *cis*-elements, and expression patterns of all *CrRLK1Ls* in *M. domestica*. Our results identified candidate *CrRLK1L* genes for functional analysis. These data will be invaluable in breeding programs implementing genetic engineering approaches to improve the adaptation of apple trees to biotic and abiotic stresses.

Methods

Database Search and Identification of *CrRLK1L* Genes in *Malus domestica*

The *M. domestica* v. 3 genomic sequence was obtained from the Genome Database of *Rosaceae* species (GDR) (https://www.rosaceae.org/species/malus/all). The *Arabidopsis thaliana* protein database was obtained from the *Arabidopsis* information resource (TAIR: http://www.arabidopsis.org). The *CrRLK1L* malectin-like (PF12819) and Pkinase (PF00069) domain databases were downloaded from Pfam (http://pfam.janelia.org/; Finn et al. 2014) and used as the query to scan the apple database. All *CrRLK1L* protein sequences were confirmed by the SMART online database (E-value ≤ 1E-5; http://smart.embl-heidelberg.de/; Schultz et al. 1998).

Bioinformatics Analysis of *CrRLK1L* Genes

The physicochemical characteristics of the *MdCrRLK1Ls* were identified using the online ExPASyProtParam tool (http://web.expasy.org/protparam/). Multiple sequence alignments of *MdCrRLK1Ls* were carried out using ClustalX v. 2.0 (Larkin et al. 2007). A phylogenetic tree was constructed using MEGA v. 5.12 (www.megasoftware.net; Tamura et al. 2011) adopting the neighbor-joining method, bootstrap-replicated 1000 times. The Gene Structure Display Server (http://gsds.cbi.pku.edu.cn, Hu et al. 2014) and SMART (http://smart.embl-heidelberg.de/; Schultz et al. 1998) were used to show the exons/introns and domains. The duplication pattern for each *MdCrRLK1L* was analyzed as follows: (a) local BLAST was performed to search for potential homologous gene pairs (E-value < 1E-5); (b) chromosomal distribution and gene duplication data were plotted using MCScanX (Wang et al. 2012). Genes separated by five or fewer loci within 100 kb were considered tandem duplicates (Hu et al. 2010). For *cis*-acting element analysis, genomic DNA sequences in the promoter region (− 1500 to − 1 bp) were downloaded from GDR then scanned for in the Plant

CARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, Lescot et al. 2002).

Organ-Specific Expression Patterns of *MdCrRLK1Ls*

High throughput RNA-sequencing data (GSE42873; Celton et al. 2014) from apple (*Malus* × *domestica* Borkh) tissues (flowers, fruit, leaves, roots, stems, seedlings, and seeds) were downloaded from NCBI GEO datasets (National Center for Biotechnology Information [NCBI]). Expression data for the identified genes were extracted using gene ID. A heat map and a clustering tree were constructed using MeV 4.9.0 (open source genomic analysis software; www.tm4.org).

Expression Pattern of *MdCrRLK1Ls*

MdCrRLK1L expression data were retrieved from the GEO datasetGSE42873 (tissue specific; Celton et al. 2014) and the Sequence Read Archive (SRA) database SRP034726 (apple response to *Valsa* canker; Yin et al. 2016), downloaded from the NCBI website. Other expression data in response to apple replant disease and apple scab were obtained from supplementary data of previously published studies (Gusberti et al. 2013; Shin et al. 2016). For all data, gene IDs and names were consistent with those in the GDR database. *MdCrRLK1L* expression data were extracted by gene ID using Microsoft *Excel* software.

Plant Materials and Treatment

For the tissue-specific expression analyses, roots and leaves from a 3-year-old Malling 26 (M. 26, *M. domestica*) and *M. baccata* were collected. To investigate the gene expression of apple in response to *Valsa* canker, 2-year-old branches were pruned from a 12-year-old "Tian Wang No. 1" Delicious apple trees on May 15, 2016. The branches were inoculated with *Valsa mali* and cultured for 10 d in darkness at 28 °C. A pure conidial culture of *V. mali* isolate GS-03 was kindly provided by the College of Plant Protection at Gansu Agricultural University, Lanzhou, China. GS-03 culture and incubation were carried out as described by Li et al. (2015). Unincubated and incubated phloem tissues were collected and stored in liquid nitrogen at − 80 °C.

Quantitative Real-Time PCR (RT-qPCR) Assay

Total RNA was extracted with a plant RNAout kit (Tiandz Inc., Beijing, China) and reverse-transcribed to produce cDNA using a PrimeScript™ RT Reagent kit with gDNA Eraser (Takara Bio Inc., Kusatsu, Shiga, Japan). The coding sequence (CDS) of each gene was retrieved from GDR and further confirmed using the NCBI website. Primers were designed using Primer v. 3 (http://primer3.ut.ee/). Sequences of primers are listed in Supplementary Table 1. The RT-qPCR assay was conducted on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with SYBR® Premix Ex Taq™ II (Tli RNase H Plus, Takara Bio Inc., Kusatsu, Shiga, Japan). Before the RT-qPCR assay, dissociation curves were constructed to verify the specificity of each primer pair. The relative expression levels of each gene were calculated by a comparative $2^{-\Delta\Delta CT}$method and normalized by the average expression level of two actin genes (Giorno et al. 2012; Bassett et al. 2014). The assay was run in triplicate.

Results

Identification of *MdCrRLK1L* Members

After a preliminary screen, 74 *MdCrRLK1L* candidates were retrieved from the apple protein database. The protein sequences ranged from 465 to 1257 amino acids (aa). Each had one or two *N*-terminal malectin (PF11721) or malectin-like (PF12819) domains, a transmembrane region, and a C-terminal Pkinase (PF00069) or Pkinase_Tyr (PF07714) domain. Normally, most Pkinase domain consisting of 250–300 aa is presenting RLK sequences (Hanks and Hunter 1995; Yamaguchi et al. 2001). During subsequent sequence analyses, certain candidates were excluded from the family because their domain structures differed from the others. The genes MDP0000228029, MDP0000297560, and MDP0000319483 had two Pkinase domains (one N-terminal and one C-terminal), and MDP0000322681 had two transmembrane regions. MDP0000234306, MDP0000176966, and MDP0000199384 had a C-terminal Pkinase or a Pkinase_Tyr domain shorter than 150 aa. Sixty-seven *MdCrRLK1Ls* were identified using Pfam and SMART (Table 1). One or two extra N- or C-terminal domains such as PDB, LRR, or Seipin (PF06775) were found in several *MdCrRLK1Ls*. The average molecular weight and pI of the MdCrRLK1Ls were 95.53 kDa and 6.5, respectively. The molecular weights ranged from 52.36–141 kDa and the pI ranged from 5.05–8.9.

An analysis with CELLO v .2.5(subcellular localization predictor; http://cello.life.nctu.edu.tw/) indicated that 64 MdCrRLK1Ls were localized to the plasma membrane (PM). The other three were predicted to be expressed on the chloroplast membrane (Chlo)/nuclear envelope (Nucl) (MDP0000228140), plasma membrane (PM)/Chlo (MDP0000199141), or PM/Nucl (MDP0000721825). However, the subcellular localization predictions for some of the proteins varied when different software was used. The pSORT prediction software (http://www.genscript.com/psort.html) found that 32, 23, and 6 proteins may localize to the PM, Chlo, and Nucl, respectively. A few proteins were localized to the vacuole (Vacu; MDP0000306337, MDP0000632093, and MDP0000199141), the cytosol (Cyto; MDP0000212691 and MDP0000281046) or the extracellular region (Extr; MDP0000413728).

Table 1 List of MdCrRLK1L genes in the *Malus domestica* genome

Gene ID in GDR	Amino acids	Mol. mass (kDa)	pI	Localization CELLO	Localization WoLF PSORT	Orthologsin NCBI
MDP0000007813	831	91.72	5.42	PM	PM	LOC103421664
MDP0000122720	620	68.27	5.72	PM	PM	–
MDP0000151537	816	89.81	5.99	PM	PM	LOC103454427
MDP0000158407	1028	113.56	6.19	PM	PM	–
MDP0000158644	825	91.03	7.52	PM	Chlo	–
MDP0000161440	835	92.98	7.04	PM	Chlo	LOC103404640
MDP0000161461	962	107.99	5.28	PM	PM	LOC103431864
MDP0000161886	776	86.11	8.33	PM	Chlo	–
MDP0000163412	942	105.37	6.98	PM	Chlo	LOC103435116
MDP0000163811	841	92.85	5.77	PM	PM	LOC103453535
MDP0000167949	878	98.73	5.88	PM	PM	LOC103443759
MDP0000171121	858	95.49	5.50	PM	PM	–
MDP0000186775	887	97.52	7.98	PM	Chlo	LOC103455543
MDP0000195070	1257	141.00	6.14	PM	PM	–
MDP0000195775	818	90.36	6.03	PM	Chlo	LOC103435951
MDP0000196032	934	103.50	6.47	PM	PM	–
MDP0000196035	1070	118.55	5.45	PM	PM	–
MDP0000197297	779	87.27	6.82	PM	Chlo	–
MDP0000199141	1066	118.97	8.68	PM, Chlo	Vacu	LOC103434654
MDP0000199305	869	96.78	5.50	PM	PM	–
MDP0000207688	1039	115.18	5.80	PM	Nucl	LOC103431967
MDP0000207770	1115	124.01	5.46	PM	PM	–
MDP0000212352	645	72.28	8.32	PM	Chlo	–
MDP0000212691	488	53.50	6.08	PM	Cyto	LOC103435243
MDP0000212717	905	100.84	5.52	PM	Chlo	–
MDP0000228140	1211	130.96	5.05	Chlo, Nucl	PM	–
MDP0000229405	838	92.68	5.69	PM	PM	LOC103435779
MDP0000232699	958	105.67	6.80	PM	PM	–
MDP0000247000	1176	129.86	7.34	PM	Nucl	LOC103433772
MDP0000249114	891	97.04	5.93	PM	Chlo	LOC103433772
MDP0000263999	922	102.98	8.67	PM	Chlo	LOC103443978
MDP0000267728	570	63.56	6.72	PM	Chlo	LOC103435246
MDP0000269684	523	57.10	8.59	PM	Chlo	–
MDP0000270938	956	107.14	6.80	PM	PM	–
MDP0000276599	465	52.36	5.63	PM	Chlo	–
MDP0000278907	858	94.14	5.75	PM	Chlo	LOC103438142
MDP0000281046	713	78.82	6.11	PM	Cyto	–
MDP0000292097	1197	133.39	6.64	PM	PM	–
MDP0000297735	811	89.89	6.24	PM	PM	LOC103404857
MDP0000304817	739	82.91	7.78	PM	PM	–
MDP0000306337	865	95.23	6.47	PM	Vacu	–
MDP0000307776	834	91.53	5.76	PM	Nucl	–
MDP0000310638	688	77.40	6.34	PM	PM	–
MDP0000314005	883	96.08	5.79	PM	PM	LOC103427681
MDP0000320718	1008	112.82	5.82	PM	PM	LOC103433332
MDP0000390677	678	74.10	5.93	PM	Nucl	LOC103427709
MDP0000413728	834	92.93	5.87	PM	Extr	LOC103442845
MDP0000420368	860	94.58	6.24	PM	PM	LOC103452778
MDP0000427444	896	99.50	7.00	PM	Nucl	LOC103428345

Table 1 (continued)

Gene ID in GDR	Amino acids	Mol. mass (kDa)	pI	Localization CELLO	Localization WoLF PSORT	Orthologsin NCBI
MDP0000445374	891	97.04	5.93	PM	Chlo	LOC103433772
MDP0000450994	846	94.98	6.47	PM	Chlo	LOC103411223
MDP0000451269	806	89.54	6.20	PM	Nucl	LOC103404640
MDP0000465335	892	98.84	7.57	PM	PM	LOC103404646
MDP0000465341	892	98.82	6.82	PM	PM	LOC103404644
MDP0000493959	842	93.89	8.18	PM	PM	LOC103442845
MDP0000593517	854	94.17	5.98	PM	PM	LOC103446108
MDP0000613486	891	97.04	5.93	PM	Chlo	LOC103433772
MDP0000632093	866	95.75	7.58	PM	Vacu	–
MDP0000642889	852	–	–	PM	Chlo	LOC103451923
MDP0000721825	579	64.50	7.20	PM, Nucl	Chlo	LOC103417120
MDP0000742436	973	107.39	8.90	PM	Chlo	LOC103439799
MDP0000852480	820	90.52	7.88	PM	Chlo	LOC103443100
MDP0000870778	905	100.66	6.00	PM	PM	LOC103442434
MDP0000891518	930	104.58	6.93	PM	PM	LOC103452074
MDP0000931970	770	84.15	5.05	PM	Chlo	LOC103427681
MDP0000944724	854	–	–	PM	PM	LOC103435626
MDP0000945548	832	91.37	5.31	PM	PM	LOC103447770

Phylogenetic Analysis of *MdCrRLK1L* Family from Apple, *Arabidopsis thaliana*, and Rice

To investigate the evolutionary relationship among the *CrRLK1L* genes of apple, *Arabidopsis thaliana,* and rice, rooted phylogenetic trees were generated using 67 *CrRLK1L* protein sequences from apple, 17 from *A. thaliana*, and 16 from rice. As shown in Fig. 1, the *CrRLK1L* phylogram was divided into five sub-clusters. The apple *MdCrRLK1L* family included 23 members in cluster I, 11 in cluster II, 21 in cluster IV, and 10 in cluster V. Four *Arabidopsis CrRLK1L* members belonged to cluster III, 3 to cluster IV, and 10 to cluster V. Groups I and II contained only apple *CrRLK1L* proteins and accounted for 34 members. Thus, the *CrRLK1L* proteins showed a species-specific evolutionary classification.

To gain further insight into the evolution of the *CrRLK1L* family, the exon-intron organization of *MdCrRLK1Ls* was compared. As shown in Fig. 2, exon numbers varied widely (1–28). The *MdCrRLK1L* members in groups I and II were encoded by at least 8 exons, and several of them, such as MDP0000195070, had 28 exons. All the members of groups IV and V contained between 8and28 exons. A similar exon-intron organization was found within the same cluster.

Chromosomal Distribution and Duplications of *MdCrRLK1Ls*

To visualize the genome organization of *MdCrRLK1L* family members, chromosome localization maps and gene duplications were constructed using MCScanX (Fig. 3). The *MdCrRLK1L* proteins were widely distributed across16 of the 18 chromosomes. Nevertheless, the distribution and abundance of most members varied among chromosomes or along the same chromosome. Most members were distributed across chromosomes (Chr) 4, 5, 6, 9, 10, and 17. These carry 9, 9, 7, 6, 6, and 7 *MdCrRLK1L*, respectively. Additionally, six chromosomes (Chr 1, 3, 8, 11, 12, and 14) host two *MdCrRLK1Ls* each, and Chr 2 and 13 each have three *MdCrRLK1Ls*. One *MdCrRLK1L* was found on Chr 7 and four on Chr 16. We identified 34 gene pairs from segmental duplication and 11 gene clusters from tandem duplication (Fig. 3, Supplementary Table 2). Segmental duplication produces many homologous *MdCrRLK1L* genes on different chromosomes, such as*MDP0000007813*/*MDP0000945548*, and *MDP0000891518*/*MDP0000161461*. On the other hand, tandem duplication produces clusters or regions with high densities of genes, for example, *MDP0000310638*, *MDP0000451269*, and *MDP0000276599* on chromosome 6. Both segmental-and tandem-duplication have expanded the number of *MdCrRLK1L* family members.

Cis-Elements Involved in the Transcriptional Regulation of *MdCrRLK1L* Genes

A 1500 bp promoter region was analyzed for each *MdCrRLK1L* gene. The Plant CARE database was applied, and 120 different *cis*-elements were identified (Table 2, Supplemental Table 4). Forty-one *cis*-elements were involved

Fig. 1 Phylogenetic analysis of *MdCrRLK1L* proteins constructed using the neighbor-joining method with 1000 bootstrap replicates

in light-mediated transcriptional regulation. In addition, thirteen *cis*-elements were responsive to phytohormones (auxin, abscisic acid (ABA), ethylene, gibberellins, methyl jasmonate (MeJA), and salicylic acid (SA)), and four to biotic stressors (elicitor, fungal elicitor) and abiotic stressors (drought, low temperature, heat, and wounding).

The distribution of *cis*-elements involved in the response to phytohormones and stress is shown in Table 2. Most of the *cis*-elements responding to phytohormones, flavonoids (such as MBSI, MBSII, and MBSIII), and stress are ubiquitous in the *MdCrRLK1L* promoter regions. Out of 57 *MdCrRLK1L* promoter regions with *cis*-elements responsive to MeJA, 43 contained CGTCA and TGACG motifs. Moreover, TCA, ABRE, and GARE motifs were found in the promoter regions of 40, 38, and 38 *MdCrRLK1L*s, respectively. The MBS element (involved in flavonoid biosynthetic genes) and TC-rich repeats (defense and stress) were detected in 55 and 46*MdCrRLK1L* promoter regions, respectively. Four elicitor-induced *cis*-elements (ELI-box3, EIRE, AT-rich sequence, and Box-W1) were located in 5, 6, 24, and 32 *MdCrRLK1L* promoter regions, respectively. In addition, three abiotic stress-related *cis*-elements (LTR, HSE, and WUN motifs)

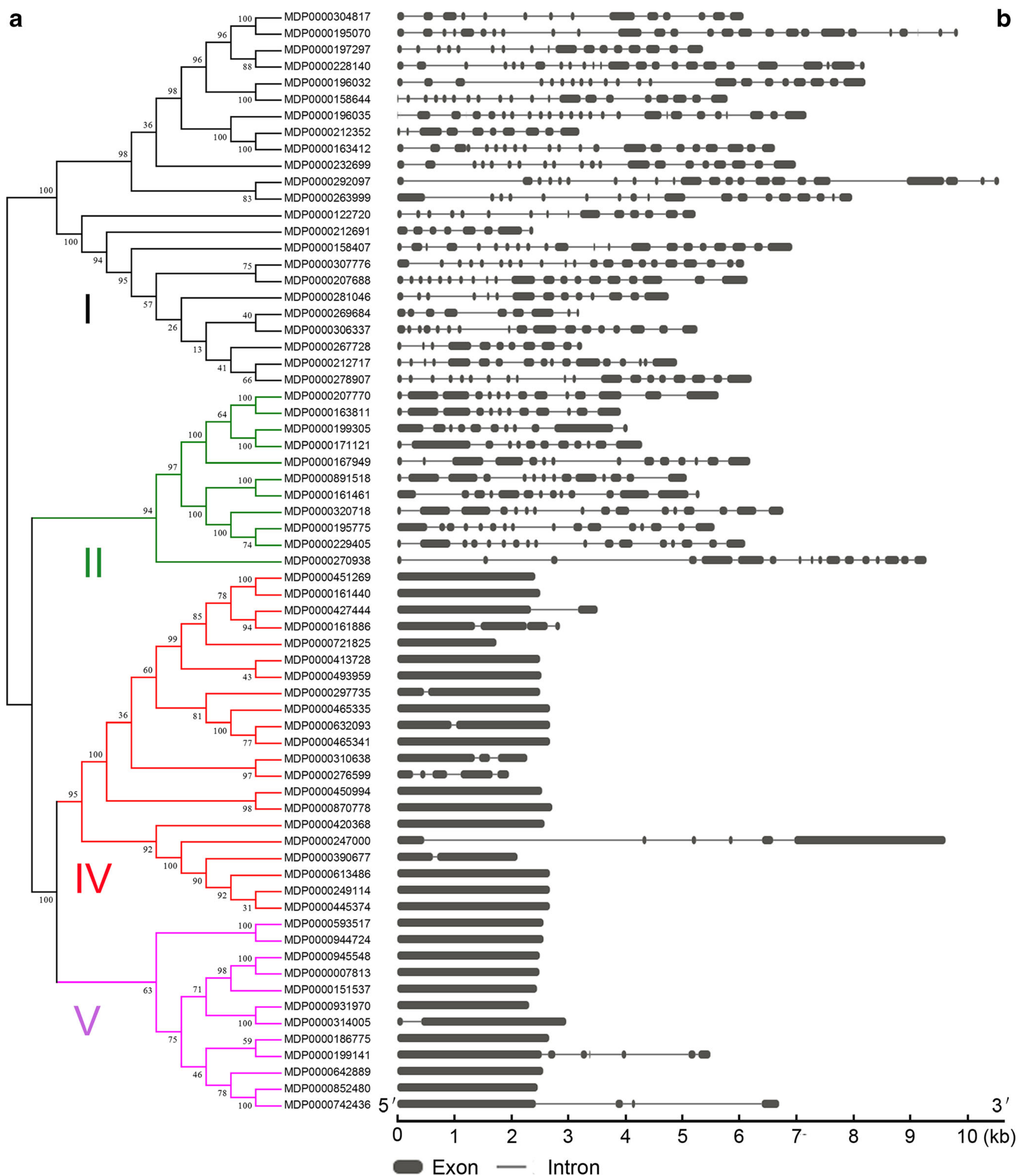

Fig. 2 Phylogenetic relationship and gene structure analysis of *MdCrRLK1L*s. a Phylogenetic tree constructed from alignments of 69 *MdCrRLK1L* genes using the neighbor-joining method in MEGA v. 5.1.2. b The exon/intron structure of each *MdCrRLK1L* gene was displayed using the Gene Structure Display Server (GSGD, http://gsds.cbi.pku.edu.cn)

were found in the promoter regions of 32, 42, and 4 *MdCrRLK1L* genes, respectively. These results indicate that the expression of many *MdCrRLK1L*s in apple may be influenced by light, phytohormones, biotic stress, or abiotic stress.

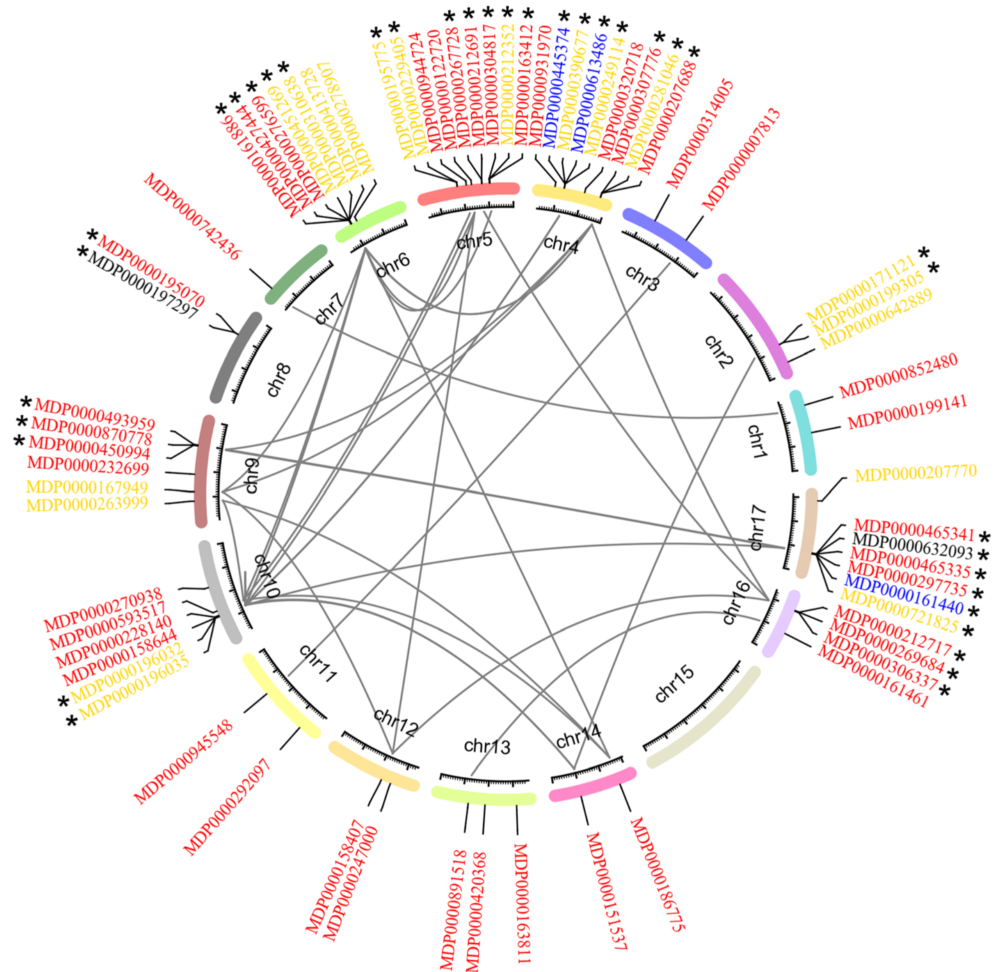

Fig. 3 Chromosomal distribution and collinearity of apple *CrRLK1L* genes. Genes arising from whole genome duplication (WGD) or segmental duplication are indicated by a black line. Genes produced by tandem duplication are labeled with "*" after each ID

Tissue-Specific Expression Patterns of *MdCrRLK1L* Genes

We searched for the expression pattern of each *MdCrRLK1L* gene in the GSE42873 dataset. It included expression profiles for seeds, seedlings, roots *in vitro*, healthy leaves, 1-year-old stems, flowers in full bloom, fruit parenchyma 100 d postanthesis, and postharvest fruit. For each tissue, the gene expression of two apple cultivars was analyzed. Expression profiles for 29 out of 69 *MdCrRLK1L*s were identified (Fig. 4(1)). *MDP0000320718* was highly expressed in fruits, whereas *MDP0000263999* was significantly expressed in flowers. *MDP0000493959*, *MDP0000450994*, and *MDP0000292097* exhibited low expression levels in all parts of the plants. *MDP0000320718* had a higher level of expression in seeds and roots compared to other organs. *MDP0000420368* was expressed mainly in the stems, leaves, and flowers. In addition, eight genes had low expression levels in the seeds but high expression levels in the seedlings, roots, stems, leaves, and flowers. In contrast, 14 *MdCrRLK1L*s had high levels of expression in almost all plant organs. The tissue-specific expression profiles of seven *CrRLK1L*s in M26 and *M. baccata* were verified with RT-qPCR. As shown in Fig. 4(2), the expression patterns of most of the *CrRLK1L*s detected by RT-qPCR resembled those found by RNA-Seq. Most *CrRLK1L*s had similar expression patterns between distinct cultivars. Taken together, our results suggest that the multiple potential regulatory responses of *MdCrRLK1L*s may be explained by the various expression patterns in different tissues.

Expression Patterns of *MdCrRLK1L* Genes in Response to Several Apple Diseases

To understand *MdCrRLK1L* transcription in response to several apple diseases, the expression profiles of apple infected with *Valsa* canker, replant disease, and scab were retrieved and analyzed (Fig. 5). Nineteen *MdCrRLK1L*s were significantly regulated by at least one of the three apple diseases. *MDP0000292097* was up-regulated by *Valsa* canker and replant disease. Five genes were up-regulated in ontogenetically resistant scab-free leaves but down-regulated in ontogenetically resistant leaves infected with apple scab and in "Fuji" apples infected with *Valsa* canker. Furthermore, two genes (*MDP0000151537* and *MDP0000891518*) were up-regulated by scab and four

Table 2 *Cis*-elements in response to hormone and stress detected in the promoter regions of *MdCrRLK1Ls*

Response	*Cis*-element name	Gene number	Sequence
Auxin	TGA-element	23	AACGAC
	AuxRR-core	7	GGTCCAT
	TGA-box	1	TGACGTGGC
Ethylene	ERE	20	ATTTCAAA
Gibberellin	GARE-motif	32	AAACAGA
	TATC-box	7	TATCCCA
	P-box	21	CCTTTTG
ABA	CE3	1	GACGCGTGTC
	ABRE	38	TACGTG
Salicylic acid	TCA-element	40	CCATCTTTTT
MeJA	CGTCA-motif;	43	CGTCA
	TGACG-motif	43	TGACG
MYB binding site involved in flavonoid biosynthetic genes	MBSI	2	aaaAaaC(G/C)GTTA
	MBSII	2	AAAAGTTAGTTA
	MBS	55	CAACTG
Defense and stress	TC-rich repeats	46	ATTTTCTTCA
Elicitor	ELI-box3	5	AAACCAATT
	EIRE	6	TTCGACC
Maximal elicitor	AT-rich sequence	4	TAAAATACT
Fungal elicitor	Box-W1	24	TTGACC
Low-temperature	LTR	32	CCGAAA
Heat stress	HSE	42	AAAAAATTTC
Wound	WUN-motif	4	TCATTACGAA
Circadian	Circadian clock	50	CAANNNNATC

(*MDP0000292097*, *MDP0000197297*, *MDP0000196032*, and *MDP0000232699*) were up-regulated by replanting disease. In contrast, these six genes were down-regulated by *Valsa* canker. *MDP0000320718* was down-regulated by both scab and *Valsa* canker, and three genes (*MDP0000163811*, *MDP0000314005*, and *MDP0000931970*) were down-regulated by both replant disease and *Valsa* canker. Three genes were exclusively down-regulated by *Valsa* canker. The expression patterns of six *MdCrRLK1Ls* in "Tian Wang No. 1" infected with *Valsa* canker were also detected using RT-qPCR and RNA-Seq; *MDP0000292097* was up-regulated, *MDP0000196032*, *MDP0000232699,* and *MDP0000320718* were down-regulated. The expression of *MdCrRLK1Ls* was significantly influenced by the aforementioned apple diseases and most genes showed distinct expressions in response to each apple pathogen.

Discussion

CrRLK1Ls are receptor-like kinases common to many plants. They have been identified and analyzed in the complete genomes of *A. thaliana* (17 members; Shiu et al. 2004), rice (16 members; Nguyen et al. 2015), and cotton (44, 40, and 79 members from diploid *G.raimondii*, diploid *G. arboretum*, and tetraploid *G. hirsutum,* respectively; Niu et al. 2016). Functional investigation of *CrRLK1L* members in *A. thaliana* suggests that they function in cell wall integrity (Hématy et al. 2007), cell-to-cell communications (Escobar-Restrepo et al. 2007), and biotic or abiotic stress response (Kessler et al. 2010; Yu et al. 2012). We systematically identified *MdCrRLK1Ls* in the apple genome. Furthermore, we analyzed their cellular localization, chromosomal mapping, phylogenetic relationships, and expression profiles *in silico*. To the best of our knowledge, this is the first study to comprehensively analyze all *CrRLK1Ls* in the *Malus* genome.

Expansion of the *MdCrRLK1L*gene Family in *Malus*

We identified 67 *CrRLK1Ls* in the genome of the economically important apple (*Malus* spp.) The total number of *CrRLK1Ls* in apple resembled that of cotton (40 to 79 members) (Niu et al. 2016), but was much higher than the number found in *Arabidopsis* (17 members) and rice (16 members) (Nguyen et al. 2015). We also confirmed many tandem and segmental duplications in *MdCrRLK1Ls*. In fact, the expansion of multiple gene classes resulted from genome-wide duplications (Velasco et al. 2010). Therefore, we speculated that the increase in the numbers of *MdCrRLK1Ls* could result from gene duplication events.

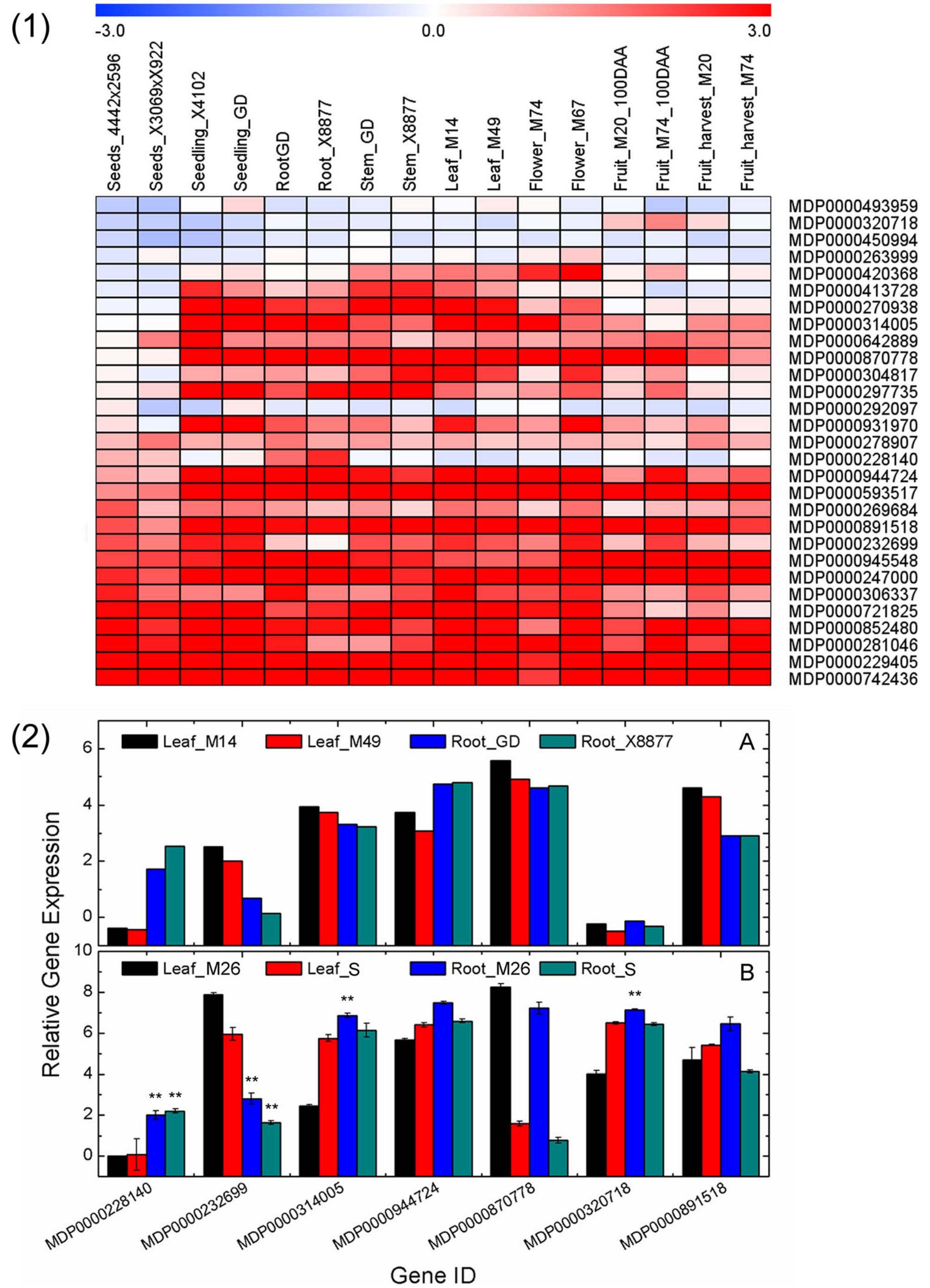

Fig. 4 (1) Expression patterns of *MdCrRLK1L* genes in seeds, seedlings, roots, stems, leaves, flowers, fruit 100 d postanthesis, and postharvest fruit from several cultivars. Data were retrieved from the GEO dataset GSE42873. (2) A Expression pattern of six *CrRLK1Ls* in the leaves and roots of the cultivars "M14," "M49," "GD," and "X8877". Data were retrieved from the GEO dataset GSE42873. B Expression patterns of the aforementioned genes from cultivars "M26" and "S" analyzed with RT-qPCR

Complex Evolutionary Relationships of *MdCrRLK1Ls*

Phylogenetic analysis revealed that most of the *MdCrRLK1Ls* were closely related to those from *Arabidopsis* and rice. The *MdCrRLK1Ls* in clusters IV and V were common to *Malus*, rice, and *Arabidopsis*, suggesting that those in the same subgroup may be derived from a common ancestor. In clusters I and II, however, the *MdCrRLK1L* members were only present in *Malus*, suggesting that they evolved to form independent branches. These results further confirm that the *MdCrRLK1L* members in clusters I and II are distinct from the *CrRLK1Ls* in *Arabidopsis* and rice. For intronless genes, duplication events may cause rapid evolution (Lecharny et al. 2003). Analysis of chromosomal distribution showed that many *MdCrRLK1Ls* in the same cluster were located in close proximity to each other (Fig. 3). The exon numbers of *CrRLK1Ls* ranged from 1 to 28 in *Malus*, 1–7 in cotton, and 1–2 in *Arabidopsis* (Niu et al. 2016), indicating that the *CrRLK1L* gene structure is more complex in *Malus* than in cotton or *Arabidopsis*. Therefore, *MdCrRLK1Ls* may have had a highly complex evolutionary process.

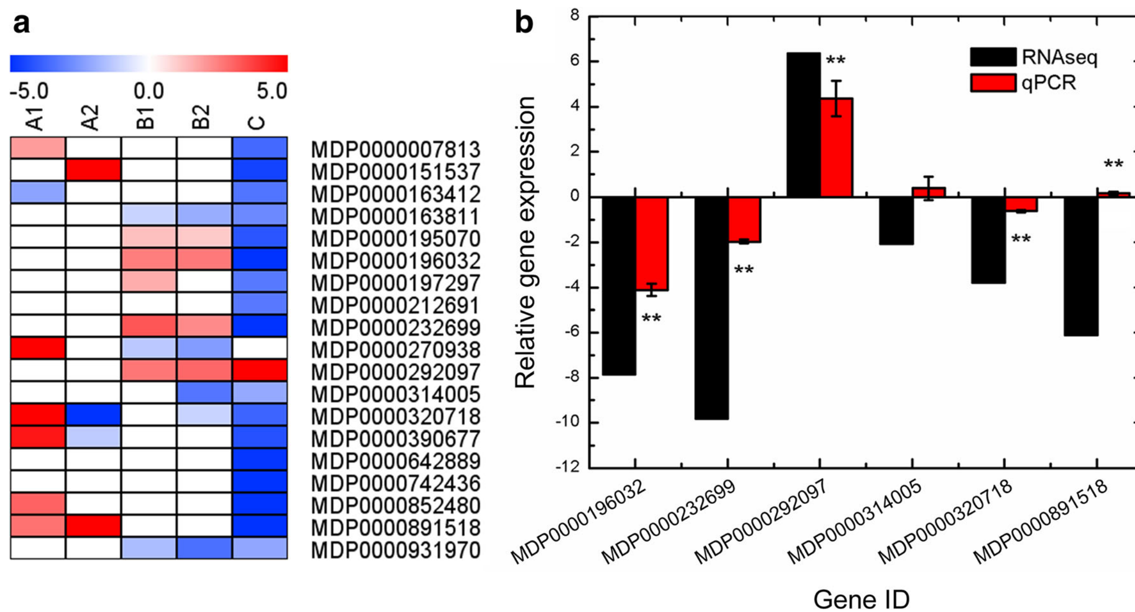

Fig. 5 **a** Expression patterns of *MdCrRLK1L* genes influenced by apple scab (Gusberti et al. 2013), apple replant disease (Shin et al. 2016), and apple *Valsa* canker (Yin et al. 2016). A1 and A2 indicate *MdCrRLK1L* expression changes in old leaves (ontogenetically resistant) compared to young leaves (susceptible), and uninfected with apple scab compared to infected for 96 h, respectively. B1 and B2 represent *MdCrRLK1L* expression in an apple plantlet derived from a cross between a replant-tolerant Geneva (G.41) scion and a replant-susceptible Malling 26 (M.26) rootstock incubated with *Pythium ultimum* for 4 and 48 h, respectively. C shows the expression of *MdCrRLK1Ls* from "Fuji" apples infected with *Valsa* canker. **b** The expression of *MdCrRLK1L* genes in response to apple *Valsa* canker were verified by RT-qPCR

Potential Regulatory Response of *MdCrRLK1Ls*

Our results showed that 29 out of 67 *MdCrRLK1Ls* were expressed in at least one tissue. Previous studies have shown that *CrRLK1L* proteins have distinct functions in different tissues. Examples include pollen tube reception (Escobar-Restrepo et al. 2007), circadian rhythm (Nguyen et al. 2015), and fungal invasion (Kessler et al. 2010). Furthermore, RALF, a family of secreted peptides, has been found to regulate *CrRLK1L* expression and function and its function in both cell elongation and plant immune responses (Haruta et al. 2014; Stegmann et al. 2017). These studies, therefore, suggest that *CrRLK1Ls* have multiple functions in cell growth and response to stress.

Cis-element analysis showed that 41 out of 120 *cis*-elements were modulated by light, and one was modulated by circadian rhythm (Table 2). Similar regulatory patterns of *CrRLK1Ls* were found in *Arabidopsis* and rice (Nguyen et al. 2015). Multiple *cis*-elements were also detected that respond to phytohormones such as auxin, ABA, ethylene, gibberellins, MeJA, and SA. Most of these elements are located in the *MdCrRLK1L* promoter region. For example, the TGA-element, ERE, GARE-motif, ABRE, TCA-element, CGTCA-motif, and TGACG-motif were detected in the promoter regions of 23, 20, 32, 38, 40, 43, and 43 *MdCrRLK1Ls*, respectively. Important roles of *CrRLK1Ls* in *Arabidopsis* cell elongation, growth, and development have been verified (Guo et al. 2009; Huck et al. 2003; Kanaoka and Torii 2010; Lindner et al. 2012; Nibau and Cheung 2011; Nissen et al. 2016). THESEUS1, a plasma-membrane-bound receptor-like kinase of the *CrRLK1L* family, is found in elongating cells and mediates their growth (Hématy et al. 2007). Therefore, regulation of circadian rhythm and modulation of growth and development may be conservative functions of *CrRLK1Ls* in plants.

Biotic stress (such as diseases) and abiotic stress (such as poor weather conditions) are major concerns in agriculture because they cause significant yield losses in many crops. To date, many stress-regulated *cis*-elements have been identified. These include TC-rich repeats (defense and stress), Box-W1 (fungal elicitor), LTR (low-temperatures), and HSE (heat stress). Analysis of *MdCrRLK1L* expression levels revealed that 19 out of 67 members were influenced by at least one of the three apple diseases (scab, replant disease, and *Valsa* canker). The expression patterns of several genes were verified by RT-qPCR (Figs. 4 and 5). Previous reports investigating the functions of *CrRLK1Ls* in other plant systems confirm that they participate in the regulation of biotic stress. For example, Flagellinsensing2 (FLS2), Ef-Tu receptor (EFR), and chitin elicitor receptor kinase 1 (CERK1) are important plasma membrane-localized pattern recognition receptors (PRR) activating pattern-triggered immunity (PTI) (Zipfel 2008). *Arabidopsis* malectin-like/LRR-RLK IOS1 plays an important role in bacterial resistance. It regulates FLS2-, EFR-, and CERK1-mediated signaling pathways and contributes to pathogen resistance (Yeh et al. 2016). An LRR/malectin RLK

mediates non-host resistance in barley and resistance to non-adapted powdery mildew fungi in wheat barley (Rajaraman et al. 2016). These results suggest that apple *CrRLK1L*s may be actively involved in disease resistance. The identification of *cis*-elements responding to low-temperature and heat stress in *MdCrRLK1L*s might be indicative of their roles in abiotic stress tolerance and requires further investigation.

Conclusions

We performed genome-wide identification, bioinformatics analysis, and gene expression profiling of *CrRLK1L*s in *Malus*. Sixty-seven members of this gene family were identified and divided into five phylogenetic branches. An increase in the number of *MdCrRLK1L*s might have occurred as a result of gene duplication events. The analysis of *cis*-elements and expression pattern profiling revealed that *MdCrRLK1L*s may participate in circadian rhythms, growth and development, and biotic and abiotic stress responses. Expressions of several members such as *MDP0000292097*, *MDP0000196032*, *MDP0000232699*, and *MDP0000320718* were significantly influenced by multiple diseases.

Authors' Contributions ZC, CB, and ZW conceived and designed the study; ZC, MZ, CM, MJ, and AZ performed the experiments, analyzed the data, and wrote the manuscript. All the authors agreed on the content of this manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Data Archiving Statement All identified amino acid and/or CDS of *MdCrRLK1L* gene sequences were deposited into the GDR (https://www.rosaceae.org/species/malus/all) and/or NCBI database (http://www.ncbi.nlm.nih.gov/). The accession numbers are listed in Table 1.

Abbreviations *RLK,* receptor-like kinase; *CrRLK1L, Catharanthus roseus* receptor-like kinase1-like kinase; *MdCrRLK1L, Malus domestica CrRLK1L*; *GDR,* Genome database of *Rosaceae* species; *GEF,* guanine nucleotide exchange factor; *RAC/ROP,* GEF-regulated Rho GTPase; *ROS,* reactive oxygen species; *RT-qPCR,* quantitative real-time PCR; *FLS2,* Flagellin sensing2; *EFR,* Ef-Tu receptor; *CERK1,* chitin elicitor receptor kinase 1; *PRR,* pattern recognition receptor; *PTI,* pattern-triggered immunity

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References

Bassett CL, Baldo AM, Moore JT, Jenkins RM, Soffe DS, Wisniewski ME, Norelli JL, Farrell RE (2014) Genes responding to water deficit in apple (*Malus× domestica* Borkh.) roots. BMC Plant Biol 14:1–12

Boisson-Dernier A, Kessler SA, Grossniklaus U (2011) The walls have ears: the role of plant CrRLK1Ls in sensing and transducing extracellular signals. J Exp Bot 62:1581–1591

Byers RE, Marini RP (1994) Influence of blossom and fruit thinning on peach flower bud tolerance to an early spring freeze. HortSci 29: 146–148

Celton JM, Gaillard S, Bruneau M, Pelletier S, Aubourg S, Martin-Magniette ML, Navarro L, Laurens F, Renou JP (2014) Widespread anti-sense transcription in apple is correlated with siRNA production and indicates a large potential for transcriptional and/or post-transcriptional control. New Phytol 203:287–299

Chen J, Yu F, Liu Y, Du C, Li X, Zhu S, Wang X, Rodriguez PL, Liu D, Chen L, Luan S (2016) FERONIA interacts with ABI2-type phosphatases to facilitate signaling cross-talk between abscisic acid and RALF peptide in Arabidopsis. Proc Natl Acad Sci U S A 113: E5519–E5527

Escobar-Restrepo JM, Huck N, Kessler S, Gagliardini V, Gheyselinck J, Yang WC, Grossniklaus U (2007) The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception. Science 317:656–660

Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer EL, Tate J, Punta M (2014) Pfam: the protein families database. Nucleic Acids Res 42:D222–D230

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9:436–442

Gallai N, Salles JM, Settele J, Vaissière BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 68:810–821

Giorno F, Guerriero G, Baric S, Mariani C (2012) Heat shock transcriptional factors in *Malus domestica*: identification, classification and expression analysis. BMC Genomics 13(1):639

Greeff CC, Roux MM, Mundy JJ, Petersen MM (2012) Receptor-like kinase complexes in plant innate immunity. Front Plant Sci 3:209

Guo H, Ye H, Li L, Yin Y (2009) A family of receptor-like kinases are regulated by BES1 and involved in plant growth in *Arabidopsis thaliana*. Plant Signal Behav 4:784–786

Gusberti M, Gessler C, Broggini GA (2013) RNA-Seq analysis reveals candidate genes for ontogenic resistance in *Malus-Venturia* pathosystem. PLoS One 8:e78457

Han YF, Yang Q, Zhang SW, Sun DY, Sun Y (2011) Receptor like kinase CrRLK1-L subfamily: novel motifs in extracellular domain and biological functions in plants. Prog Biochem Biophys 38:891–899

Hanks SK, Hunter T (1995) Protein kinases 6. The eukaryotic protein kinase super family: kinase (catalytic) domain structure and classification. FASEB J 9:576–596

Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR (2014) A peptide hormone and its receptor protein kinase regulate plant cell expansion. Science 343:408–411

Hématy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou JP, Höfte H (2007) A receptor-like kinase mediates the response of *Arabidopsis* cells to the inhibition of cellulose synthesis. Curr Biol 17:922–931

Hu R, Qi G, Kong Y, Kong D, Gao Q, Zhou G (2010) Comprehensive analysis of NAC domain transcription factor gene family in *Populus trichocarpa*. BMC Plant Biol 10:145

Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G (2014) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31: 1296–1297

Huck N, Moore JM, Federer M, Grossniklaus U (2003) The *Arabidopsis* mutant feronia disrupts the female gametophytic control of pollen tube reception. Development 130:2149–2159

Kanaoka MM, Torii KU (2010) FERONIA as an upstream receptor kinase for polar cell growth in plants. Proc Natl Acad Sci U S A 107: 17461–17462

Kellerhals M (2009) Introduction to apple (*Malus× domestica*). In: Genetics and genomics of *Rosaceae*. Springer, New York, pp 73–84

Kessler SA, Shimosato-Asano H, Keinath NF, Wuest SE, Ingram G, Panstruga R, Grossniklaus U (2010) Conserved molecular components for pollen tube reception and fungal invasion. Science 330: 968–971

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Lecharny A, Boudet N, Gy I, Aubourg S, Kreis M (2003) Introns in, introns out in plant gene families: a genomic approach of the dynamics of gene structure. J Struct Funct Genom 3:111–116

Lehti-Shiu MD, Zou C, Hanada K, Shiu SH (2009) Evolutionary history and stress regulation of plant receptor-like kinase/pelle genes. Plant Physiol 150:12–26

Lehti-Shiu MD, Zou C, Shiu SH (2012) Origin, diversity, expansion history, and functional evolution of the plant receptor-like kinase/ pelle family. In: Receptor-Like Kinases in Plants. Springer, Berlin Heidelberg, pp 1–22

Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P, Rombauts S (2002) PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res 30:325–327

Li Z, Yin Z, Fan Y, Xu M, Kang Z, Huang L (2015) Candidate effector proteins of the necrotrophic apple canker pathogen *valsa mali* can suppress bax-induced PCD. Front Plant Sci 6:579

Lindner H, Muller LM, Boisson-Dernier A, Grossniklaus U (2012) CrRLK1L receptor-like kinases: not just another brick in the wall. Curr Opin Plant Biol 15:659–669

Nguyen QN, Lee YS, Cho LH, Jeong HJ, An G, Jung KH (2015) Genome-wide identification and analysis of *Catharanthus roseus* RLK1-like kinases in rice. Planta 241:603–613

Nibau C, Cheung A (2011) New insights into the functional roles of CrRLKs in the control of plant cell growth and development. Plant Signal Behav 6:655–659

Nissen KS, Willats WG, Malinovsky FG (2016) Understanding CrRLK1L function: cell walls and growth control. Trends Plant Sci 21:516–527

Niu E, Cai C, Zheng Y, Shang X, Fang L, Guo W (2016) Genome-wide analysis of CrRLK1L gene family in *Gossypium* and identification of candidate CrRLK1L genes related to fiber development. Mol Gen Genomics 291:1137–1154

Rajaraman J, Douchkov D, Hensel G, Stefanato FL, Gordon A, Ereful N, Caldararu OF, Petrescu A, Kumlehn J, Boyd LA, Schweizer P (2016) An LRR/malectin receptor-like kinase mediates resistance to non-adapted and adapted powdery mildew fungi in barley and wheat. Front Plant Sci 7:1836

Reed A, Mazzola M (2015) Characterization of apple replant disease-associated microbial communities over multiple growth periods using next-generation sequencing. Phytopathology 105:S4

Rodrigo J (2000) Spring frosts in deciduous fruit trees-morphological damage and flower hardiness. Sci Hortic 85:155–173

Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. Proc Natl Acad Sci U S A 95:5857–5864

Schulze-Muth P, Irmler S, Schröder G, Schröder J (1996) Novel type of receptor-like protein kinase from a higher plant (*Catharanthusroseus*) cDNA, gene, intramolecular autophosphorylation, and identification of a threonine important for auto- and substrate phosphorylation. J Biol Chem 271:26684–26689

Shin S, Zheng P, Fazio G, Mazzola M, Main D, Zhu Y (2016) Transcriptome changes specifically associated with apple (*Malus domestica*) root defense response during *Pythium ultimum* infection. Physiol Mol Plant Pathol 94:16–26

Shiu SH, Karlowski WM, Pan R, Tzeng YH, Mayer KF, Li WH (2004) Comparative analysis of the receptor-like kinase family in *Arabidopsis* and rice. Plant Cell 16:1220–1234

Stegmann M, Monaghan J, Smakowska-Luzan E, Rovenich H, Lehner A, Holton N, Belkhadir Y, Zipfel C (2017) The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. Science 355:287–289

Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczyglowski K, Parniske M (2002) A plant receptor-like kinase required for both bacterial and fungal symbiosis. Nature 417:959–962

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A, Fontana P, Bhatnagar SK, Troggio M, Pruss D, Salvi S, Pindo M, Baldi P, Castelletti S, Cavaiuolo M, Coppola G, Costa F, Cova V, Ri AD, Goremykin V, Komjanc M, Longhi S, Magnago P, Malacarne G, Malnoy M, Micheletti D, Moretto M, Perazzolli M, Si-Ammour A, Vezzulli S, Zini E, Eldredge G, Fitzgerald LM, Gutin N, Lanchbury J, Macalma T, Mitchell JT, Reid J, Wardell B, Kodira C, Chen Z, Desany B, Niazi F, Palmer M, Koepke T, Jiwan D, Schaeffer S, Krishnan V, Wu C, Chu VT, King ST, Vick J, Tao Q, Mraz A, Stormo A, Stormo K, Bogden R, Ederle D, Stella A, Vecchietti A, Kater MM, Masiero S, Lasserre P, Lespinasse Y, Allan AC, Bus V, Chagné D, Crowhurst RN, Gleave AP, Lavezzo E, Fawcett JA, Proost S, Rouzé P, Sterck L, Toppo S, Lazzari B, Hellens RP, Durel C, Gutin A, Bumgarner RE, Gardiner SE, Skolnick M, Egholm M, Peer YV, Salamin F (2010) The genome of the domesticated apple (*Malus* [times] *domestica* Borkh.). Nat Genet 42:833–839

Walker JC (1994) Structure and function of the receptor-like protein kinases of higher plants. Plant Mol Biol 26:1599–1609

Walker JC, Zhang R (1990) Relationship of a putative receptor protein kinase from maize to the S-locus glycoproteins of *Brassica*. Nature 345:743–746

Wang G, Ellendorff U, Kemp B, Mansfield JW, Forsyth A, Mitchell K, Bastas K, Liu C, Woods-Tör A, Zipfel C, de Wit PJ, Jones JD, Tör M, Thomma BP (2008) A genome-wide functional investigation into the roles of receptor-like proteins in *Arabidopsis*. Plant Physiol 147:503–517

Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H, Kissinger JC, Paterson AH (2012) MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res 40:e49

Wang S, Hu T, Wang Y, Luo Y, Michailides TJ, Cao K (2016) New understanding on infection processes of *Valsa* canker of apple in China. Eur J Plant Pathol 146:531–540

Wu R, Wang Y, Wu T, Xu X, Han Z (2017) MdMYB4, an R2R3-type MYB transcription factor, plays a crucial role in cold and salt stress in apple. J Am Soc Hortic Sci 142:209–216

Yamaguchi H, Matsushita M, Nairn AC, Kuriyan J (2001) Crystal structure of the atypical protein kinase domain of a TRP channel with phosphotransferase activity. Mol Cell 7:1047–1057

Yeh YH, Panzeri D, Kadota Y, Huang YC, Huang PY, Tao CN, Roux M, Chien HC, Chin TC, Chu PW, Zipfel C, Zimmerli L (2016) The

Arabidopsis malectin-like/LRR-RLK IOS1 is critical for BAK1-dependent and BAK1-independent pattern-triggered immunity. Plant Cell 28:1701–1721

Yin Z, Ke X, Kang Z, Huang L (2016) Apple resistance responses against *valsa mali*, revealed by transcriptomics analyses. Physiol Mol Plant 93:85–92

Yu F, Qian L, Nibau C, Duan Q, Kita D, Levasseur K, Li X, Lu C, Li H, Hou C, Li L, Buchanan BB, Chen L, Cheung AY, Li D, Luan S (2012) FERONIA receptor kinase pathway suppresses abscisic acid signaling in *Arabidopsis* by activating ABI2 phosphatase. Proc Natl Acad Sci U S A 109:14693–14698

Zipfel C (2008) Pattern-recognition receptors in plant innate immunity. Curr Opin Immunol 20:10–16