

Note

## Diurnal and circadian expression of clock-associated pseudo-response regulators in *Populus trichocarpa*

Wushuang Liu<sup>1</sup>, Weina Zhang<sup>1</sup>, Minghui Du<sup>1</sup>, Yuqing Sha<sup>1</sup>, Xiang Yu<sup>2</sup>, Misato Ohtani<sup>2</sup>, Taku Demura<sup>2</sup>, Qiang Zhuge<sup>1,\*</sup>

<sup>1</sup>Key Laboratory of Forest Genetics & Biotechnology, Ministry of Education, Nanjing Forestry University, Nanjing 210037, China; <sup>2</sup>RIKEN Biomass Engineering Program, Yokohama, Kanagawa 230-0045, Japan

\* E-mail: qzhuge@njfu.edu.cn Fax: +86-25-8542-8701

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**Abstract** The circadian clock is an autonomous oscillator that produces endogenous biological rhythms with a period of approximately 24 h. A number of circadian clock-associated factors have been intensely studied in the model plant *Arabidopsis thaliana* (*At*), including pseudo-response regulators (PRRs), which are key regulators of the circadian clock. In *Populus trichocarpa* (*Pt*), seven orthologs of the *At* PRR genes have been identified. Here, the *Pt* PRR family of genes, *Pt**PRR1*, *Pt**PRR37*, *Pt**PRR5a*, *Pt**PRR5b*, *Pt**PRR73*, *Pt**PRR9la*, and *Pt**PRR9lb*, were analyzed for circadian expression at the transcriptional level. These genes were expressed diurnally in the following order: *Pt**PRR9la*/*Pt**PRR9lb* → *Pt**PRR37*/*Pt**PRR73* → *Pt**PRR5a*/*Pt**PRR5b* and *Pt**PRR1*, with the *Pt* PRR mRNAs starting to accumulate sequentially in 2–3-h intervals. These sequential transcriptional events, termed ‘circadian waves of *Pt* PRR’, were not significantly affected by the photoperiod conditions. All *Pt* PRR genes were shown to be primarily expressed in mature leaves. These results suggest that members of the *Pt* PRR family play important roles in mechanisms underlying the poplar circadian clock.

**Key words:** *Populus*, circadian clock, pseudo-response regulator, photoperiod, expression.

Circadian rhythms, endogenous biological oscillations with a period of approximately 24 h, are generated and controlled by an autonomous oscillator, the circadian clock (Edmunds 1988; Harmer 2009). This clock allows organisms to anticipate predicted daily changes in the environment (e.g., light and temperature, which cycle with the rotation of the earth) and to regulate their physiology, metabolism, and development accordingly (Michael et al. 2003; Ni et al. 2009). In plants, circadian clock performance can increase growth and photosynthetic capacity while strengthening survival and competitive advantages (Dodd et al. 2005). Genes associated with transcriptional/translational feedback loops in the eukaryotic circadian clock are core components that control the form of the central oscillator (Bell et al. 2005; Locke et al. 2006; McClung 2006). In the dicot *Arabidopsis thaliana* (*At*), identification of the circadian clock’s molecular components has revealed the basic workings of the plant circadian clock (Harmer 2009; Takata et al. 2010). Reciprocal transcriptional regulation between *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) and the homologous gene *LATE ELONGATED HYPOCOTYL* (*LHY*) and *TIMING OF CAB EXPRESSION1* (*TOC1*), also called *PSEUDO-RESPONSE REGULATOR1* (*PRR1*), has been proposed as the main

feedback loop (Alabadí et al. 2001; Mizoguchi et al. 2002; Perales and Mas 2007). In addition to *TOC1*, numerous genetic studies have demonstrated that other members of the PRR gene family are involved in the functioning of the central oscillator (Eriksson et al. 2003; Farré et al. 2005; Ito et al. 2009; Kaczorowski and Quail 2003; Michael et al. 2003; Nakamichi et al. 2005; Para et al. 2007; Salome and McClung 2005; Yamamoto et al. 2003), indicating that the PRR gene families play key roles in the regulatory network of the plant clock system.

In woody plants, circadian clock factors have been investigated from the viewpoint of seasonal growth regulation. The detailed expression analysis of *CsTOC1* and *CsLHY1*, the genes homologous to *AtTOC1* and *AtLHY1* in the chestnut tree (*Castanea sativa*), revealed that *CsTOC1* and *CsLHY* were constantly expressed under cold temperature conditions (Ramos et al. 2005). Other *CsPRR* genes were also affected by the low temperature, resulting in the disruption of circadian behavior for gene expression (Ibañez et al. 2008). These findings suggested the involvement of the circadian clock in winter dormancy. Functional analysis of *Populus tremula* × *tremuloides* (*Ptt*) *TOC1* and *PttLHY* genes performed by Ibañez et al. (2010) provided further evidence; the downregulation of these genes by RNA

interference affected clock-controlled gene expression patterns and entry into winter dormancy. Moreover, the downregulation of *PtLHY* altered freezing tolerance and timing of bud burst in response to warmer temperatures. Therefore, the timing of seasonal traits likely also depends on circadian clock components in deciduous plant species (Cooke et al. 2012).

In the *Populus trichocarpa* (*Pt*) genome (Tuskan et al. 2006), seven genes homologous to *AtPRRs* including *PtPRR1/TOC1* have been identified (Takata et al. 2010; Supplemental Data 1 and 2). To ascertain whether all the *PtPRR* genes are involved in the poplar circadian rhythm, we assessed their expression patterns under different light conditions by quantitative RT-PCR. Young plants of *P. trichocarpa* (strain Nisqually-1), referred to as “poplar” hereafter, were cultured in a plant culture box (MICRO BOX OS140-15, BM Equipment Co. Ltd., Tokyo, Japan) on an agar medium containing 1.23 g l<sup>-1</sup> McCown’s Woody Plant Basal Salt Mixture (Duchefa, Haarlem, The Netherlands) and 2.7 g l<sup>-1</sup> gellan gum (Wako, Tokyo, Japan) at pH 5.8 under 16:8-h light/dark (16:8LD) conditions at 24°C for 1 month. For analysis of tissue-specific expression, the medium-sized plants were transplanted into pots of soil and grown in a confined greenhouse under 16:8LD conditions at 24°C for 3 months, then subjected to tissue-specific expression analysis. Shoot apices, young (the first and second leaves from the top of the plant) and mature (the third and down leaves from the top of the plant) leaves, stems (upper half region of the whole stem and lower half region of the whole stem), and roots were collected separately, and immediately frozen in liquid nitrogen. Total RNA was isolated using Plant RNA Reagent (Life Technologies, Paisley, UK) followed by purification using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Total RNA (2 µg) was reverse-transcribed using SuperScript-III Reverse Transcriptase (Invitrogen, Paisley, UK) with oligo(dT)<sub>12-16</sub> primers, following DNaseI treatment. Based on the sequence information available in the *Pt* genome database (<http://www.phytozome.net/search.php>), we designed primer sets to amplify the coding region of the *PtPRR* gene (Supplemental Data 3), and performed molecular cloning and sequence analyses to determine the sequences experimentally, using the synthesized complementary DNA derived from the total RNA from the leaves. Of the seven members of the *PtPRR* gene family, the determined amino acid sequences of three (*PtPRR73*, *PtPRR9la* and *PtPRR9lb*) were identical to the reported sequences, while the remaining four members (*PtPRR1*, *PtPRR37*, *PtPRR5a* and *PtPRR5b*) were largely consistent with sequences reported previously (Takata et al. 2010; Supplemental Data 4). Quantitative PCR analysis was performed with a Lightcycle 480 II instrument (Roche, Indianapolis, IN, USA) and FS Universal SYBR® Green

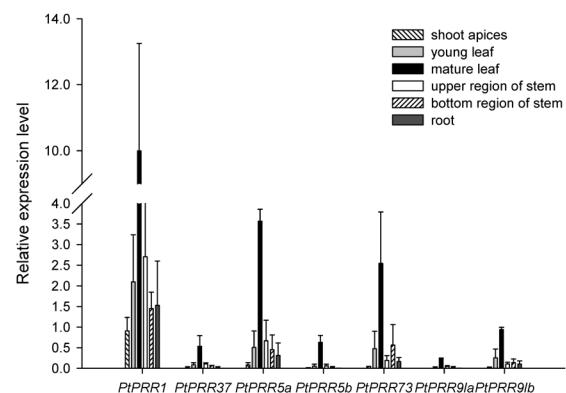


Figure 1. Expression patterns of *PtPRR* genes in different tissues. Expression analysis of *PtPRR* genes using qRT-PCR analysis. Parts of the shoot apices, young and mature leaves, upper and bottom regions of stems, and roots were separately sampled and subjected to total RNA extraction. The relative mRNA abundance of *PtPRR* genes was normalized with respect to the reference gene *ELF4A* in different tissues. The bars are the standard deviations (SD) based on three biological replicates.

Master Mix (Roche). Aliquots (0.5 µl) of the reaction solution were used as a template for PCR amplification with gene-specific primers (Supplemental Data 5). The reaction conditions were 50°C for 2 min, 95°C for 10 min, and 45 cycles of 95°C for 2 min, 62°C for 30 s, and 72°C for 30 s. The *ELF4A* (*Elongation Factor 4A*) gene was used as an internal control. The results showed that expression of the seven members of the *PtPRR* gene family differed among the various tissues, but all members were expressed preferentially in mature leaves (Figure 1). *PtPRR1* expression was much greater than that of the other *PtPRR* gene family members.

To test the diurnal and circadian behavior of the *PtPRR* genes, we analyzed their expression in poplars grown under different light conditions. The plants were grown in the culture box as described above under 12:12-h light/dark (12:12LD) conditions at 25°C for 6 weeks, and were subsequently transferred to continuous light (LL) or continuous dark (DD) conditions. The plants grown under 12:12LD conditions were harvested every 2 h for 1 day, while the plants grown under LL and DD conditions were harvested every 4 h for 2 days. Samples of the whole plants after treatment in the different light conditions were immediately frozen in liquid nitrogen and stored at -80°C until use, and the quantification of mRNA expression was performed by quantitative RT-PCR, as described above, using gene-specific primers (Supplemental Data 5). Under 12:12LD conditions, all genes showed high-amplitude mRNA rhythms with a period of 1 day, which peaked in the light phase (Figure 2). These *PtPRR* transcripts started to accumulate after subjective dawn in the following order: *PtPRR9la/PtPRR9lb* → *PtPRR37/PtPRR73* → *PtPRR5a/PtPRR5b* and *PtPRR1*, with the *PtPRR* mRNAs starting to accumulate sequentially in 2–3-h intervals. This order

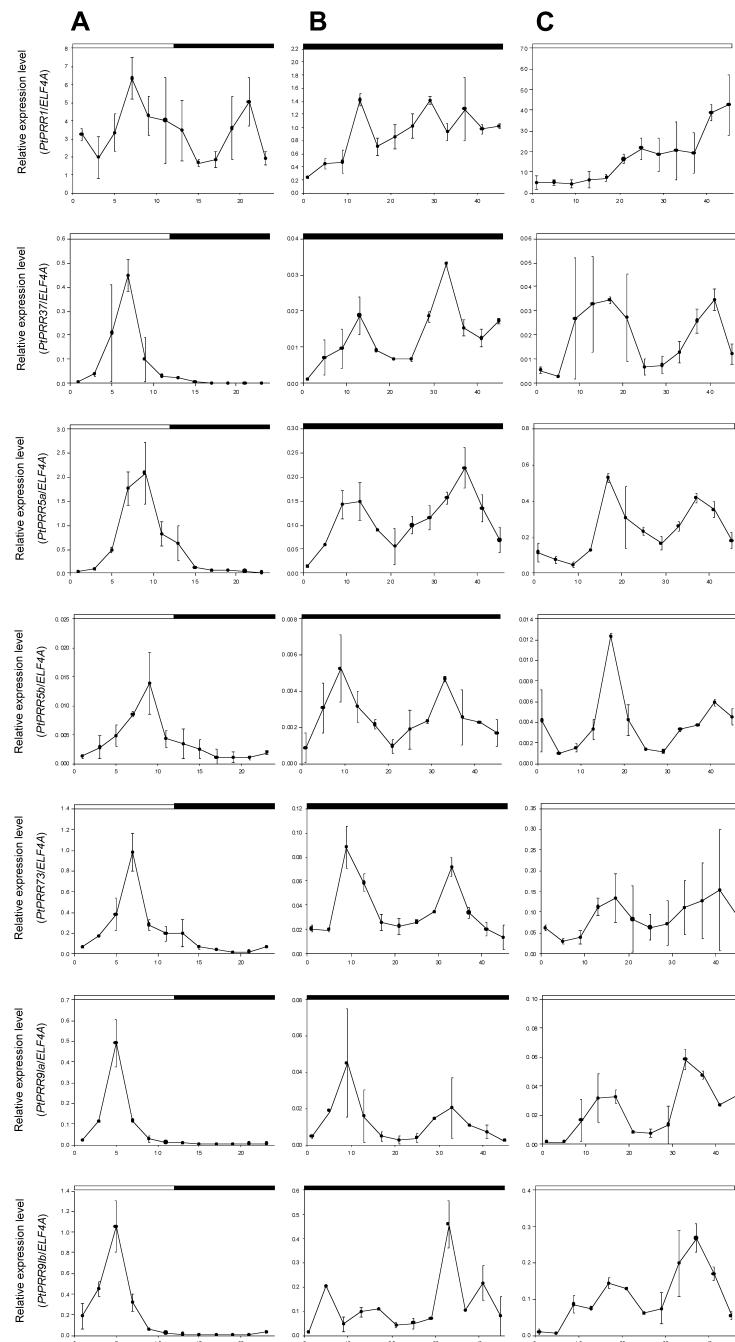


Figure 2. *PtPRR* gene expression in whole young plants grown under 12:12LD, DD, and LL conditions. *PtPRR* gene expression rhythms observed in 6-week-old poplar seedlings grown under standard conditions (12:12LD) and subsequently transferred to LL and DD conditions. Samples were collected at 2-h (12:12LD) and 4-h (LL and DD) intervals. The results of our qRT-PCR analysis and relative transcript abundances are shown in the graphs. The results are the mean  $\pm$  S.D., based on three biological replicates. The open and shaded bars above the graphs represent subjective day and night lengths, respectively. (A) 12:12LD condition, (B) DD condition, and (C) LL condition.

of expression closely resembles that of the *AtPRR* and *CsPRR* genes (*PRR9* $\rightarrow$ *PRR7* $\rightarrow$ *PRR5* $\rightarrow$ *PRR1*) observed in rice (*OsPRR73/OsPRR37* $\rightarrow$ *OsPRR95/OsPRR59* $\rightarrow$ *OsPRR1*) (Ibañez et al. 2008; Matsushika et al. 2000; Murakami et al. 2003). Under DD conditions, all genes except *PtPRR1* showed endogenous rhythms with damping (Figure 2B). The rhythms under LD or DD conditions showed phase relationships roughly similar to that of *AtPRR3*, *AtPRR5*, or *AtPRR7* (Mizuno et al. 2005; Mizuno and Nakamichi

2005). Under LL conditions, *PtPRR1* exhibited no sign of circadian regulation, whereas the other *PtPRR* genes showed a sustained rhythm (Figure 2C), suggesting that *PtPRR1* expression is highly correlated with light conditions.

In this paper, we reveal that the expression of seven members of the *PtPRR* gene family followed a circadian rhythm pattern. These findings suggest that not only *PRR1/TOC1* but also other *PRR* genes are associated with

the circadian clock system in *Populus*, as reported for the *AtPRR* genes (Gendron et al. 2012; Huang et al. 2012; Nakamichi et al. 2010; Nakamichi et al. 2012; Wang et al. 2013). Our results provide insight into further analyses of the poplar circadian clock system and will facilitate the study of clock systems in woody plants.

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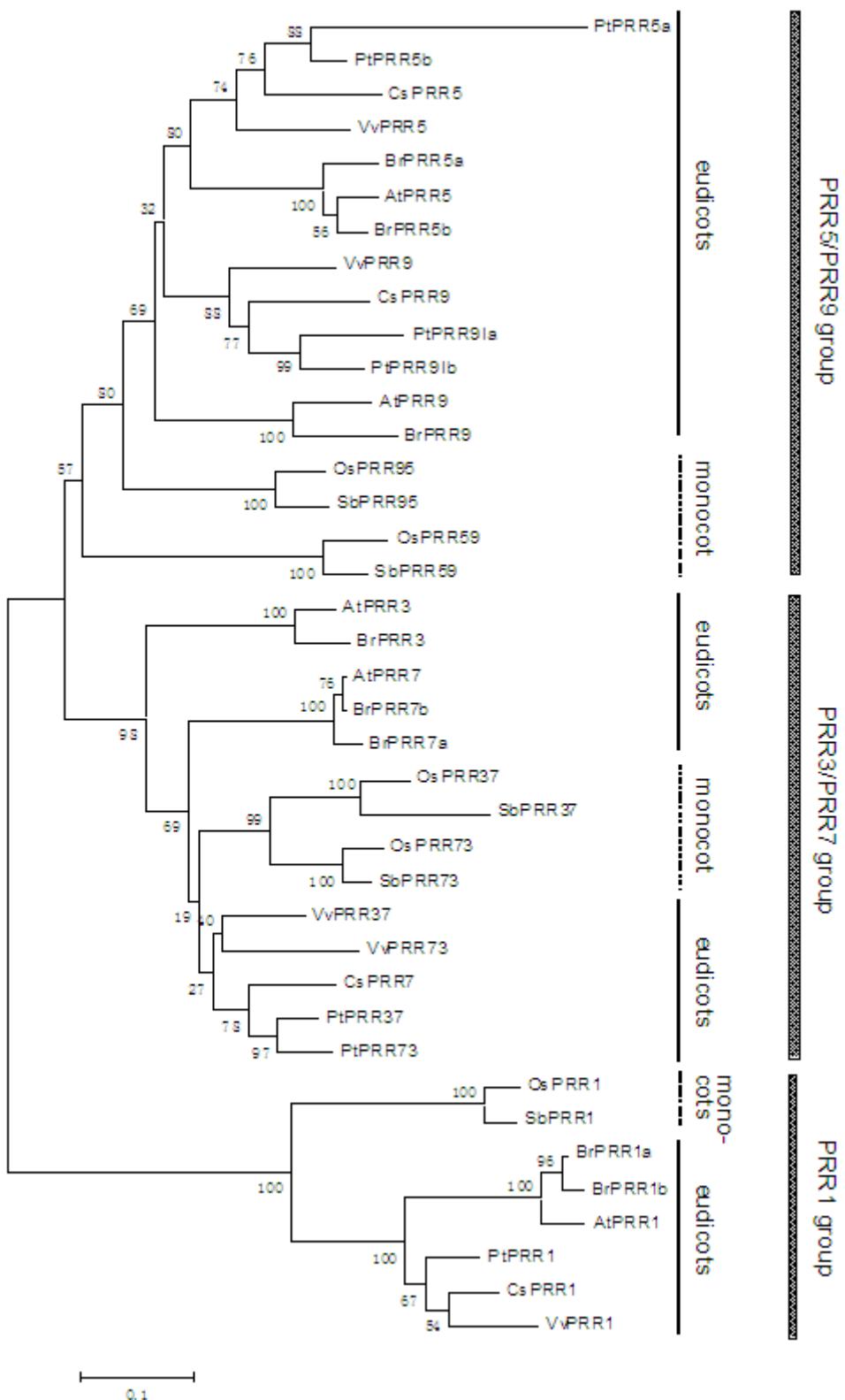
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Data 1 Characteristics of *PRR* genes in *Populus trichocarpa*

<b>Gene</b>	<b>Accession number</b>	<b>Locus</b>	<b>Location</b>	<b>Amino acid</b>	<b>Pi</b>	<b>Molecular weight</b>
<i>PtPRR1/TOC1</i>	KF359505	POPTR_0015s07310	scaffold_15: 8453092 - 8458518	558	5.72	61.88 KDa
<i>PtPRR37</i>	KF359506	POPTR_0008s04610	scaffold_8: 2634239 - 2639073	620	8.15	67.29 KDa
<i>PtPRR5a</i>	KF359507	POPTR_0012s00600	scaffold_12: 293665 - 298881	703	6.97	77.87 KDa
<i>PtPRR5b</i>	KF359508	POPTR_0015s00440	scaffold_15: 243472 - 248849	686	6.91	75.78 KDa
<i>PtPRR73</i>	KF359509	POPTR_0010s22230	scaffold_10: 19212422 - 19220597	763	6.58	84.08 KDa
<i>PtPRR9la</i>	KF359510	POPTR_0002s18050	scaffold_2: 13928322 - 13933096	694	7.18	76.42 KDa
<i>PtPRR9lb</i>	KF359511	POPTR_0014s10160	scaffold_14: 7616960 - 7621698	717	6.79	79.09 KDa



Data 2 The Phylogenetic tree of the PRR genes. Amino acid sequences of PRR homologues were aligned using ClustalW program and the phylogenetic tree was established by the neighbor-joining method. GenBank accession numbers were provided for other PRR proteins than PtPRR. Bars, 0.1 amino acid substitutions per site.

Data 3 The primers for coding region of *PtPRR* genes

<b>Gene</b>	<b>Primer sequence</b>
<i>PtPRR1</i>	F: 5'-ATGAAGGAGAGTGGTAATGGTAAAAG-3' R: 5'-TTAAGATCCTGAAGCATCATCCTC-3'
<i>PtPRR37</i>	F: 5'-GCCATGCCTGTGTTATCAGG-3' R: 5'-TTAGCTACGTGCATCCTCATCTT-3'
<i>PtPRR5a</i>	F: 5'-GGATCCATGGGGGAGGTAGTGATTAGTAGT-3' R: 5'-GAGCTCTCACTCTAGGACGCTGCTCAG-3'
<i>PtPRR5b</i>	F: 5'-GGATCCATGGGAGTGGTAGTGGTTAGTAGT-3' R: 5'-GAGCTCCTACTGGTCAGTTCAGCAGGT-3'
<i>PtPRR73</i>	F: 5'-ATGCTCTCAATGAACAACGGG-3' R: 5'-TTAGCTCTGTGCATCCTCATCT-3'
<i>PtPRR9la</i>	F: 5'-ATGGGTAAGGTGGTGGTAGT-3' R: 5'-TCAACCATTAGCAACTGGGCA-3'
<i>PtPRR9lb</i>	F: 5'-ATGGGTGAGGTTGTGGTGAG-3' R: 5'-GAATTTCATGGTCAACCATTAGCAATT-3'

#### Data 4 Amino acid alignment between the PtPRR and the related phytozome PtPRR

PtPRR1	MKESGNKGSKVGGGGAGDFVDRSKVRILLCDNDAKSSQEVFTLLKCSYQVTSVRSARQVIDALNAEGPEIDIILSEVDIPMTKGKMLKYIMRDKDRLR	100
Phytozome PtPRR1	MKESGNKGSKVGGGGAGDFVDRSKVRILLCDNDAKSSQEVFTLLKCSYQVTSVRSARQVIDALNAEGPEIDIILSEVDIPMTKGKMLKYIMRDKDRLR	100
Consensus	mkesgnkgskvgggagdgfvdrskvrillcdndakssqevftllkcsyqvtvsrsarqvidalnaegpeidiilsevdipmtkgmklkyimrdkdrlr	
PtPRR1	IPVIMMSAQDEVSIVVKCLRLGAADYLVKPLRTNEELLNLWTHMWRRLHMLGLAEKNILNQYDFDPVASPDSANTNSTLFSDDTDLSSRRSTNPENGMST	200
Phytozome PtPRR1	IPVIMMSAQDEVSIVVKCLRLGAADYLVKPLRTNEELLNLWTHMWRRLHMLGLAEKNILNQYDFDPVASPDSANTNSTLFSDDTDLSSRRSTNPENGMST	200
Consensus	ipvimsaqdevisvkkclrlgaadylvkplrtneellnlwthmwrmlglaeknlnydfdpvaspdsantnstlfsddtddlsrrstnpemgmst	
PtPRR1	HQEDE SAAAASASASAAAAAEPSPGDPQKYRPDVPGISDRRTGHLSSGPKKSELKGESSAFFTYVKPSTVKNNSQVALIEDNTNQNLRMEEKLVQCG	300
Phytozome PtPRR1	HQEDE SAAAASASASAAAAAEPSPGDPQKYRPDVPGISDRRTGHLSSGPKKSELKGESSAFFTYVKPSTVKNNSQVALIEDNTNQNLRMEEKLVQCG	283
Consensus	hqede pspgdpgkyrpdpvgipsdrrtghlssgpkkselkgessafftyvkpstknnsgvaliedntnqn1rmeeklvqcg	
PtPRR1	EQMLNDLAHQENGEALEIHSQVDDFRSSSIPIDSLERSCTPPMSREFPQRNFKDDRVLMHQTNEPQLDASSLTSQSVPYFMSGVNVQVMSSSAQLY	400
Phytozome PtPRR1	EQMLNDLAHQENGEALEIHSQVDDFRSSSIPIDSLERSCTPPMSREFPQRNFKDDRVLMHQTNEPQLDASSLTSQSVPYFMSGVNVQVMSSSAQLY	383
Consensus	eqmlndlahqengealeihsqvddfrsssipldslersctppmsrefpqrnfkddrvlmhqtnepql dassltsqsvpyfmsgvvnqvmsssaqly	
PtPRR1	QKNLHEQSLIGTSAMLPQGYNHLPQCPFMHSMASFPYFPVSCICLQPQMPPTFSWSPSFGSTSADVTKLNKVDREAAALKFRQKRKERCFDKKIRYVNRK	500
Phytozome PtPRR1	QKNLHEQSLIGTSAMLPQGYNHLPQCPFMHSMASFPYFPVSCICLQPQMPPTFSWSPSFGSTSADVTKLNKVDREAAALKFRQKRKERCFDKKIRYVNRK	483
Consensus	qknlheqlsgtsamlpqgynhlpqcpfmhsmasfpypvsciclgpgqmpptfspsfgstsadvtklnkvdreaaalnkfrqkrkercfdkkiryvnrk	
PtPRR1	KLAERRPRVRGQFVVRKVNGVNVLDLNQOPASTDYDEEEDGDEQASRDSSPEDDASGS	558
Phytozome PtPRR1	KLAERRPRVRGQFVVRKVNGVNVLDLNQOPASTDYDEEEDGDEQASRDSSPEDDASGS	541
Consensus	klaerrprvrqgfvrkvngvnlvdlnqopastdydedeedgdeqasrdsspeddags	
PtPRR37	MPCLSGIGLLSKIMSHKTCRNIPVIMMSSHSDSMNVVFCKLSKGAVDFLVKPIRKNELKILWQHWRKCHSASGSGSESAVRTQKSTKSNGADESDNDTG	100
Phytozome PtPRR37	MPCLSGIGLLSKIMSHKTCRNIPVIMMSSHSDSMNVVFCKLSKGAVDFLVKPIRKNELKILWQHWRKCHSASGSGSESAVRTQKSTKSNGADESDNDTG	100
Consensus	mpclsigllskimshktcrnipvimmshsmvvfkclskgavdflvkpirknelkilwqhnwrvrkchisasgsgsesavrtqkstksngadesdndtg	
PtPRR37	NDDDGIGSVGLNARDGSNDGSTQSSWTKRAVEVESPKPMSPWDQHLDSPPDSTCAQVIHSRPEACDNWSVPLATMKKGEGQDDELNIVMGKDLIEGV	200
Phytozome PtPRR37	NDDDGIGSVGLNARDGSNDGSTQSSWTKRAVEVESPKPMSPWDQHLDSPPDSTCAQVIHSRPEACDNWSVPLATMKKGEGQDDELNIVMGKDLIEGV	200
Consensus	ndddigsvglnardgsndgstqsswtkraevevespkpmspwdqhdlsppdstcaqvihsrpeacdnswvplatmkkgcgeqddelndnivmgkdliegv	
PtPRR37	PRIPNLQLKDPIKRVPNTIAQDGEKFPEIKSKHDGQHLEKRQOELNSEKCNTELNRQNDLKGGITNSANPRMDSLVLDVPGNGLSSRNKNEVITYETKE	300
Phytozome PtPRR37	PRIPNLQLKDPIKRVPNTIAQDGEKFPEIKSKHDGQHLEKRQOELNSEKCNTELNRQNDLKGGITNSANPRMDSLVLDVPGNGLSSRNKNEVITYETKE	300
Consensus	pripnlqlkdpikrvpntiadndgekfpeikskhdggglekrqgqelnsenkctelnrgndlkggqitnsanprmdslvldvpgnlgssrnknevityetke	
PtPRR37	VPSFEFLSKRLRDIGDAGASSHDRNVLRHSDLASFYSRNSASTADQAPTGNVGSCSPLDNSSEAAKTESMQNLQSNNSNTPNQRSGSSHNNDMGSTNN	400
Phytozome PtPRR37	VPSFEFLSKRLRDIGDAGASSHDRNVLRHSDLASFYSRNSASTADQAPTGNVGSCSPLDNSSEAAKTESMQNLQSNNSNTPNQRSGSSHNNDMGSTNN	400
Consensus	vpsfeflskrlrdigdaggshdrnvlrhlsdafsrysntadqaptgnvgscpldnsseaaakteesmqnlqsnestppnqrsgsshnnmdgstnn	
PtPRR37	ITFAKPSVISDKPTLKPTVKCHYPSAFQPVQNDHTALPQPVICQKGDAPIANTTLVKSRGVNQQGQVHHNHCVHNMPQQQLTNHDDLSLNMPAAAPQC	500
Phytozome PtPRR37	ITFAKPSVISDKPTLKPTVKCHYPSAFQPVQNDHTALPQFVICQKGDAPIANTTLVKSRGVNQQGQVHHNHCVHNMPQQQLTNHDDLSLNMPAAAPQC	449
Consensus	itfakpsvisdkptlkptvkhcypqvdhtalpqpovicqkgdaapianttlvksrgvnqqgvhhnhcvhnmpqqqltnhddlslnmpaaapqc	
PtPRR37	GSSNMLSTPTQGNAGDYSILNGSDHGSNQNQNGSSIALSGAVEKGTTGPGDESGSRSGVGRNRFALREAALKFRQKRKERCFKKVRYQSRKKLAEQPR	600
Phytozome PtPRR37	GSSNMLSTPTQGNAGDYSILNGSDHGSNQNQNGSSIALSGAVEKGTTGPGDESGSRSGVGRNRFALREAALKFRQKRKERCFKKVRYQSRKKLAEQPR	549
Consensus	gssnmlstptqgnagdysilngsdhgngqngssialsgavekgttgpgdsgsrsgvnrnfalreaalkfrqkrkercfkkvryqsrkklaeqpr	
PtPRR37	IRGFVROVGPBEPHKDEDARS	620
Phytozome PtPRR37	IRGFVROVGPBEPHKDEDARS	569
Consensus	irggfvrqvgpehkdedars	
PtPRR5a	MGEVVISSGEELEVRSKSEREEEKQRQSKSEETGEVKKKKKKKEGEGLNDLGLVRWDGFLPMLRVLVLLVEADDSTQIIALLRKCSYRVSVSPDGLKA	100
Phytozome PtPRR5a	MGEVVISSGEELEVRSKSEREEEKQRQSKSEETGEVKKKKKKKEGEGLNDLGLVRWDGFLPMLRVLVLLVEADDSTQIIALLRKCSYRVSVSPDGLKA	100
Consensus	mgevivissgeelevrsksereeeekqrqskseetgevkkkkkkkegeglndglvrvwdgflprmvlrvllveaddstrqiiallrkcsyrvsvpdglka	
PtPRR5a	WEILKGRPHGIDLILTEVLDLPSISGYPLLTIIIMEHEICKNIPVIMMSSQDSISTVVKCMLRGAADYLVKPLRKNELRNLWQHWRQSSLAGGNGPQDES	200
Phytozome PtPRR5a	WEILKGRPHGIDLILTEVLDLPSISGYPLLTIIIMEHEICKNIPVIMMSSQDSISTVVKCMLRGAADYLVKPLRKNELRNLWQHWRQSSLAGGNGPQDES	200
Consensus	weilkgrphgidliltedvlpsisgyplltiimeheicknipyimmssqdsistvykcmrlrgaadylkplrknelrnliwqhwrrqsslaggngpdes	
PtPRR5a	VGGDKIATESENPSAASNHASGEIASIORSKGOTKEKGSDAQSSCTKPDLEAESSHMMNQEPLOVFRSISLTDMNNQKREMFVNILGOKLILLHDRAEGSA	300
Phytozome PtPRR5a	VGGDKIATESENPSAASNHASGEIASIORSKGOTKEKGSDAQSSCTKPDLEAESSHMMNQEPLOVFRSISLTDMNNQKREMFVNILGOKLILLHDRAEGSA	240
Consensus	vggdkiateenspashasgemasiqrskgqtekgsdq	
PtPRR5a	AAAREDANIMDVDEIISPNGRGTGAYVAIESCDNDVALANSHREAFDFMGASTNRSSFFNNVINKFDSSPHLDLSLRRSHPSGFEIRDTEERRALWHSNA	400
Phytozome PtPRR5a	AAAREDANIMDVDEIISPNGRGTGAYVAIESCDNDVALANSHREAFDFMGASTNRSSFFNNVINKFDSSPHLDLSLRRSHPSGFEIRDTEERRALWHSNA	326
Consensus	eispngnrtgayvaiescdndvalanshreafdfmgastnrssfnvinkfdssphldlsrrshpsgfeirdteerralwhsna	
PtPRR5a	SAFTQYINRPLQPLPHSALESTGNQKEGLTNYDRKISSTGYNSDALSLAPSTQKSEISLAAQGQTEKESEIATSSPGQRVFPQIPIAKETRLNNLCNSYGSVF	500
Phytozome PtPRR5a	SAFTQYINRPLQPLPHSALESTGNQKEGLTNYDRKISSTGYNSDALSLAPSTQKSEISLAAQGQTEKESEIATSSPGQRVFPQIPIAKETRLNNLCNSYGSVF	426
Consensus	saftqyinrplqlphsalestgnqkelgtndrkisstgynsdalslapstqkseislaagqtkeseiatsppgqrvfpiqipaketrlnnlcnseygsvf	
PtPRR5a	PPIFCKQSGLSPMMSPSPSACQQEPETYKVNQFQHSNHGSTSEQNRLQKQEDRLDSLDRLGLISPATDQSASSFCNGAASHFNNSMGYGS	600
Phytozome PtPRR5a	PPIFCKQSGLSPMMSPSPSACQQEPETYKVNQFQHSNHGSTSEQNRLQKQEDRLDSLDRLGLISPATDQSASSFCNGAASHFNNSMGYGS	466
Consensus	ppifckqs	
PtPRR5a	TSGSGNGNDQVAIVRDASESKNEEGAFTHSYSHRSIQCRAALTKFRLKRKERCYKEKKVCHHLHSSSPLLQHNSNTISIDYCLVTVTKFGMRAEKNLSS	700
Phytozome PtPRR5a	TSGSGNGNDQVAIVRDASESKNEEGAFTHSYSHRSIQCRAALTKFRLKRKERCYKEKKVCHHLHSSSPLLQHNSNTISIDYCLVTVTKFGMRAEKNLSS	566
Consensus	tsgsgngndqvaivrdaseskeegafthsyshrsigrealtkfrkrkercyekvcchlhlssspqllqghsntisidycltvtkfgmraechnllss	
PtPRR5a	VLB	703
Phytozome PtPRR5a	VLE	569
Consensus	vle	

PtPRR5b	<b>MGVVVVSSGEELEVKTGSETPEEKQSKEETSESETGEVKRKRRKKEGEGSNDGLVRWERFLPRMVLRLVLLVEADDSTROQIIAALLRKCSYK</b>	VATVSDGLK 99
Phytozome_PtPRR5 Consensus	mgvvvvssgeellevktgsetpeekqskeetsesetgevkrrkkekgegsndglvrwerflprmlrlvllveaddstrqiaallrkcsyk vatvsdglk	100
PtPRR5b	<b>AWEILKERPHNIDLITLVEVDLPSVSGYALLTLMIEHEICKNPVIMMSSQDSIKTVKCMRLRGAADYLVKPIRKNELRNLWQHWRKQSSLGGGNPHDE</b>	199
Phytozome_PtPRR5 Consensus	aweiikerpnhnidlitelvdpsvsgyalltlimeheicknlpvimmsqdsiktvkcmrlrgaadylvkpirknelrnlgwqhvwrkqsslgggnphde	200
PtPRR5b	<b>SVGQDKTEATSENNADGNHSSGEMASIORSKKEQAVKRSDSQSSCTKPGLEAEAGAHMENMQEFLQPWWSKFLTDTNMQKHEEHVNLCQKLIVRDSEAGS</b>	299
Phytozome_PtPRR5 Consensus	svgqdktteatsennadgnhssgemaqrskeavkrssqscstkpgleaeaghmennmeflqpwwskfltdtnmqkheehvnlcqklivrdseags	300
PtPRR5b	<b>ATAVCEDSNKITVDKEITPGSGRVTANIAIEGCDKIGALANSPREAIDFMGASTNHSSFNNVIEIHCSSPHLDLSLRSHSPGFETOVTEERHTLRHSNA</b>	399
Phytozome_PtPRR5 Consensus	atavcedsnkitvdkeitpgsgrvtanaiiegcdkgalanspreaidfmgastnhsfnnveihcscphldisirrshspgfetcgvteerhtlrhsna	400
PtPRR5b	<b>SAFTWYTNRASQLPHSALANTIGQEEFRNGKISSNNGYNSDALSLAPSTRRSALISLAQGOTKEYEIVTSSSGEKVFPPIHVPKDTRFNNLCSYGA</b>	499
Phytozome_PtPRR5 Consensus	saftwytnrasqlphsalantagqneefranydgkissnngynsdalslapstrrsalslaaggckeyievtssgekvpilipwkdrfnnlcsyga	500
PtPRR5b	<b>VLPPMMSQSSASAKPEIHKVNPPQCSNYGSTSVOLCDRLGQNANDSINGSLQKQENKLDSLEGREHISSATDQSASSFCNGAASHFNSIGYGSAQSYS</b>	599
Phytozome_PtPRR5 Consensus	vlppmmsqssasakpeihkvnpfqcsnygstsvolcdrlgqnandsingslkqkenklldslslegrehiatdqsassfcngaaashfnlsigygssys	600
PtPRR5b	<b>NADQIATVSAASESKNEEGVFTHNSNSHRIQREAALTFKRKCERYKKVSRKLAQPRFQVHRVQHIDPSPAEVDQ</b>	686
Phytozome_PtPRR5 Consensus	nadqiatvsaaseskneegvfthnsnshriqreaaltfkrlkcryekvreskklaeqrprvkgqfvrvqhidpstaedq	687
PtPRR73	<b>MLSMNNNGFAEQNHIVEDEQKKIRGDGIMGEDQELSEEAGESQINEDEKDVNDKGMEISQLVLTDAQVVIQSOHQSQQGPLVHWERFLPRLRSLKVLLVENDST</b>	100
Phytozome_PtPRR73 Consensus	mlsmnnngfaeqnhivedekqkirgdgimedqelseeagesqinedekdvndkgmesqlqvldaqvqisqhqgpqlvhwerflprrlsklvllvendst	100
PtPRR73	<b>RHVVSALLRNCGYEATAVANLQLQAWKLLQDLTNHIDLVLTEVAMPCLSGIGLLSNMSMHCKTRCNIPVIMMSSHDMNVVFRCLSKGAVDFLVKPIRKNEL</b>	200
Phytozome_PtPRR73 Consensus	rhvvsallrnagyeatavanlqlawkllqdltnhidlvtlevampclsgigllsnmsmhcktrcnipvimmsshdmnvvfrcleskgavdflvkpknel	200
PtPRR73	<b>KILWQHWWRCHSASSGSESAVRIQKSLKSGADESDNDTSDNDDDIISIGLNARDGSNGSGTQSSWTKRAVEVDSPKPMPLPWQDQADPPDSTFAQV</b>	300
Phytozome_PtPRR73 Consensus	kilwqhwrrchsassgsesavriqkslksgadesndtdndddiisiglnardgsngstqsswtkraevevdspkpmplpwqdadppdstfaqv	300
PtPRR73	<b>IHSRSEACDNWVPLATTKKFGKQDDELDNFVMGKDLIEGVPRIPNLQHKDLSKEVLTNIAGNNGEKFREIKESEQDSGHLEKGQLELNESEKHNTIELRNQGN</b>	400
Phytozome_PtPRR73 Consensus	ihsrseacdnwvplattkkfgkqddelnfmvgkdleigvpriplqhkdlsekvltniagnngekfrikeqseqdsghlekgqlelnesekhntrnqgn	400
PtPRR73	<b>DLKGVSTNITNPQLESEVVDDISNLSLSSNNKKEVITYETKEMPSLEVLKRLRDTGDAWASANDRNVLRLHDLSLASFYSRNSASTAYCPTGNVGSCSLLKDCK</b>	500
Phytozome_PtPRR73 Consensus	dlkgvstnitnpqlesevvddisnlssnnknevityetkempslevlkrlrtdtgdaawasandrnlvrhdsalsfsrysastaycptgnvgscsilkdc	500
PtPRR73	<b>SEAAKTESMQNLOQNSNSNTPRNLCSCNGSNNSNNNDVGTTTNNAPAKPLVIRDKPDKPKSTVKCLHPSFSAFQPVQNDQTLIHAQPVQIQQKGKDAPIANTLILAOSRG</b>	600
Phytozome_PtPRR73 Consensus	seaaktesmqnlqsnstprnlcsngsnnmdvgtttnafakplvirkdkpdkstvcklchpsfafqpvqndqltlhaqpvqiqkgkdapiantilaosrg	600
PtPRR73	<b>MNQGQVQHRRHCVHNMPLTIRNDLSLKNMAAAAGPRFGSSNNLSTPMBGNAQNYSMNGNSNGQESIALPNRGINLESNSGAAGKDENPGTDESGSRSG</b>	700
Phytozome_PtPRR73 Consensus	mnqgqvqhrrhcvhnmpltirndlslknmaaadagprfgssnnlstmpegnagnyqsmngsngqesialpnrginlesnsgaagkdengptdesgrsg	700
PtPRR73	<b>GGQNCFALREAALNKFRQKRKCERCFEKVRYQSRKLAEHRPRVRQGPVQRFPEHKKDEDAQS</b>	763
Phytozome_PtPRR73 Consensus	ggqncfalreaalnkfrqkrkcercfekvryqsrklaeprvrgfqrqvpfehkkdedaqs	763
PtPRR91a	<b>MGKVLSSSSEEAGMVVELETEKDDIGSSEVRWEKFLPKMVLRLVLLVEADDSTROQIIVALLRKCGYRVSAVPDGLMAWETLKERPHSIDLILEVELP</b>	100
Phytozome_PtPRR91a Consensus	mgkvvlsssseagmvveletekddigssesvrwekflpkmlrlvllveaddstrqivallrkcgryrvsavpdglmawetlkerphsidlilevelp	100
PtPRR91a	<b>LISGYAFLALVMEHDKCKNIPVIMMSSHDISVVLKCMLGKSDFLVLPVKRNLNWLQHVWRRQQTAGKIPRNSRNRVEASSENNAASSDFATSLQKN</b>	200
Phytozome_PtPRR91a Consensus	lisgyaflalvmehdkcknipsvmmsshdslsikmlgksdflvlpvkernelnlqhvwrqqttagkiprnsrnrveassemnaassdfatslqkn	200
PtPRR91a	<b>KDCSEKGSDAQSSCTTCPLEAESAHMQNIQQLSYLKVRASNLSADNEKYEDYALKNPKSPNPESTKGVFAERSNRTRPDREPYYGAYNPTASRLVEE</b>	300
Phytozome_PtPRR91a Consensus	kdcsekgsdqsscttcpcleaesahmqnigglisylikvrasnlidnadekyediyalknpsvnpestkgfvfaersnrtrpdrepyygavnpasrlvee	300
PtPRR91a	<b>HACAKSIAHDENSRPENDREHANSFGHDVLAETSTGSAIDLIGSFNQNPQKHTVAYSSLHDATAKTFEEPPPLLELSLRLRILYPSSSKNQGLDERHALHNSNS</b>	400
Phytozome_PtPRR91a Consensus	hacaksiahdensrpndrehansfgdvltaetstgsgaidlgsfnqnpqkhtvaysslhdataktfeppllelsrlrlypsssknqgliderhalhnsns	400
PtPRR91a	<b>SAFSLYNSKTLLOSLEPTSAENGDSKEEAKSPDPSSNOLAQNVGTLSQIHDASLSCNQEIIITPVIGOSKGVELAHPSPQQLPVLGTRLDNISTCG</b>	500
Phytozome_PtPRR91a Consensus	safslynsktlqslfptsaengdskeeakspdpssnolqnvgtlsqiqhdaslsqngeiittpvigqsgkvelahpsqqlipvgtrldninstcg	500
PtPRR91a	<b>HVFSPLCYTSQNSAAWNPNLAGRQSPPFITAVSHSNPEVLDLSQNHKCYVIDQNDLQOQNRRPVEDMRHDSPAAGCSTSSSLNRVANNNSSAYESFGSG</b>	600
Phytozome_PtPRR91a Consensus	hvfsplcytqsnawnpnlagrqspfpitasvhsnpevldskqnhkcyvdqndlqnnpnrepvdemrhdspaggstssllcnrvannnssayesfgsg	600
PtPRR91a	<b>NDVNASSVGTAEKSMQAENLNNGNHNHDGFFGSDSYRSSQREALTFRKRLKRDRCYEVKRVYQSRKLAERQRPRVKQFVRQAQNDCPVANG</b>	694
Phytozome_PtPRR91a Consensus	ndvnassvgtaeqsmqenlnngnfnhdfgqssdsyrsqrealtfkrlkrkdrckyekrvryqsrkrlaeqrprvkgqfvrqaqndcpvang	694

PtPRR9lb	MGEVVVSSSSEEVEGMAVELETEKKDIDSSEVVWEEKFLPRMVLSQLVEADDSTROIIAALLRKCSYRVAAVPDGLMAWETLKGGPHNIDLILTEVELP	100
Phytozome PtPRR9lb	MGEVVVSSSSEEVEGMAVELETEKKDIDSSEVVWEEKFLPRMVLSQLVEADDSTROIIAALLRKCSYRVAAVPDGLMAWETLKGGPHNIDLILTEVELP	100
Consensus	mgvvvvssssevegmaeveletekkdigssevvwekfplprmvlsvlveaddstrgiaallrkcsyrvaaavpdglmawetlkggphnidliitevelp	
PtPRR9lb	LISGVALLTLVTEHAVCKNIPVIMMSSQDSISMVLKCMKGAADEFLIKPKVRKNELRNWLQHWRQTLTSAGQIPCNLHKVEASSEINAASNGSSSDSVMS	200
Phytozome PtPRR9lb	LISGVALLTLVTEHAVCKNIPVIMMSSQDSISMVLKCMKGAADEFLIKPKVRKNELRNWLQHWRQTLTSAGQIPCNLHKVEASSEINAASNGSSSDSVMS	200
Consensus	lisgalltlvtehavcknipvimmsqdsismvlkcmkgaadeflikpkvrknelrnwghwrrqtltsagqipgnlhkveasseinaasngsssdsvms	
PtPRR9lb	RKNKDCSEKGCDAQSSCTTPCLEAESAHMQNMQGLSQMKYRSASNLSNTDREEFEECAKLKSPVTPENKTVFVPERPNRMESDGEPGSGAYNPTSLRL	300
Phytozome PtPRR9lb	RKNKDCSEKGCDAQSSCTTPCLEAESAHMQNMQGLSQMKYRSASNLSNTDREEFEECAKLKSPVTPENKTVFVPERPNRMESDGEPGSGAYNPTSLRL	300
Consensus	rknkdcsekgcdagscttpcleaesahmqnmglsqmkyrsasnlsntdreefecaclkspvtpeenktvfvperpnrmesdgepgsgaynptsrl	
PtPRR9lb	LEEHACAKSAIQDENSRPENDRGLANSSFGCDDVPFESSSGAIDLIGTLNNNGPKTTVHSSLHYGTNKEFAPQLESLKRLYPSSSKNQGVDERHALNH	400
Phytozome PtPRR9lb	LEEHACAKSAIQDENSRPENDRGLANSSFGCDDVPFESSSGAIDLIGTLNNNGPKTTVHSSLHYGTNKEFAPQLESLKRLYPSSSKNQGVDERHALNH	400
Consensus	leehacaksaiqdensrpndrglanssfgcddvpfesssgaidlgtlnngpkttvhsslhygtnkfeafqlelslkrlypsssknqgvderhalnh	
PtPRR9lb	SHASAFSWKKQGCWDGSRDGIGGSDFRRYNSKTLQPPFPASAENGSDKEEASKSPELSSNQHAQNINSISQRHGATLSGNQDMTIPPIIGQSGKAELAYP	500
Phytozome PtPRR9lb	SHASAFSWKKQGCWDGSRDGIGGSDFRRYNSKTLQPPFPASAENGSDKEEASKSPELSSNQHAQNINSISQRHGATLSGNQDMTIPPIIGQSGKAELAYP	500
Consensus	shasafswkkqgcwdsgrdiggsdfrryntsctlqppfpasaengsdkeeaskspelssnqhaqninsisqrhgatlsngndmtipiigqsgkaelayp	
PtPRR9lb	SPRHGLIPVVRGMLDNISTEYGHDFSPLYTQSSAAWSPLKLAGWQQSPYPLSTSIIHSNPDIHDESEKNHRCSEDETTYNVDQNDHQNNKGPADEVRHDSP	600
Phytozome PtPRR9lb	SPRHGLIPVVRGMLDNISTEYGHDFSPLYTQSSAAWSPLKLAGWQQSPYPLSTSIIHSNPDIHDESEKNHRCSEDETTYNVDQNDHQNNKGPADEVRHDSP	600
Consensus	sprhglipvrrgmldnisteyghdfsplytqssaaawspklagwqqspypilstsihsnpdihdeknhrccsdeettynsvdqndhqnnkgpaudevrdsp	
PtPRR9lb	AAGQSTGGICNGVVINHNKSSAYESFGSPDDGNAKEKAMAQDNLDGDNFNRDGRGIDSLRQQREAAALTKFRLKRKDRCYEVKKVRYQSRKRLAEQRPRV	700
Phytozome PtPRR9lb	AAGQSTGGICNGVVINHNKSSAYESFGSRDDGNAKEKAMAQDNLDGDNFNRDGRGIDSLRQQREAAALTKFRLKRKDRCYEVKKVRYQSRKRLAEQRPRV	700
Consensus	aagqstggicngvinhnkssayesfgsrddgnakekamaqdnlndgdnfnrdgrgidslrqqreaultkfrlkrdrcyekkvryqsrkrlaeqrprv	
PtPRR9lb	KGOFVROVQNDSPPIANG	717
Phytozome PtPRR9lb	KGOFVROVQNDSPPIANG	717
Consensus	kggfvrqvnqndspiang	

Data 5 The gene-specific primers of *PtPrr* genes for quantitative RT-PCR analysis

<b>Gene</b>	<b>Primer sequence</b>
<i>PtPrr1</i>	F: 5'-AGATGAGATCAAGCTGCGAGTG-3' R: 5'-ACCACAGACCTAGTCAACAGATTAG-3'
<i>PtPrr37</i>	F: 5'-TCCAATTGAGATCGGTTGGATTATG-3' R: 5'-CCCATTACTGGAGAAGGCTACC-3'
<i>PtPrr5a</i>	F: 5'-CCCTCACCTGCGGAAACTG-3' R: 5'-GTTATATCTACAAATCGGCTCAAGC-3'
<i>PtPrr5b</i>	F: 5'-TGTGAAGTCTAGAGGAAACAATATG-3' R: 5'-CTATATTGAGACGTGAAGATGGC-3'
<i>PtPrr73</i>	F: 5'-CCTTGGGAATTTCGTGCTTGAG-3' R: 5'-CGTGGCAGTAACATGTATATTAGTC-3'
<i>PtPrr9la</i>	F: 5'-TCTGAATCCTCTTACTCTGG-3' R: 5'-TCCACTTCTCTTAATTGATC-3'
<i>PtPrr9lb</i>	F: 5'-CCATGAAATTCATCATCACACG-3' R: 5'-GACCCAAAAATTACAGTTAGAACCC-3'
<i>ELF4A</i>	F: 5'-ACACAGTCTCAGCTACTCATGGAGA-3' R: 5'- ATTATGACAAGGGACACTTGCTG-3'