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Note

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

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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 *AtPRR* genes have been identified. Here, the *PtPRR* family of genes, *PtPRR1*, *PtPRR37*, *PtPRR5a*, *PtPRR5b*, *PtPRR73*, *PtPRR9la*, and *PtPRR9lb*, were analyzed for circadian expression at the transcriptional level. These genes were expressed diurnally 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. These sequential transcriptional events, termed 'circadian waves of *PtPRR*,' were not significantly affected by the photoperiod conditions. All *PtPRR* genes were shown to be primarily expressed in mature leaves. These results suggest that members of the *PtPRR* 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 preformed by Ibañez et al. (2010) provided further evidence; the downregulation of these genes by RNA

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interference affected clock-controlled gene expression patterns and entry into winter dormancy. Moreover, the downregulation of *PttLHY* 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^{-1} McCown's Woody Plant Basal Salt Mixture (Duchefa, Haarlem, The Netherlands) and 2.7 g l^{-1} 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$(dT)_{12–16}$ 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

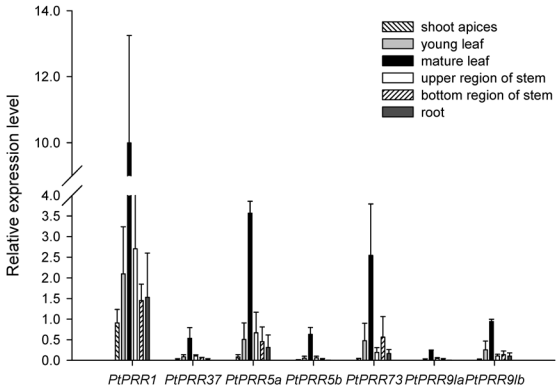

Figure 1. Expression patterns of *PtPRR* genes in different tissues. Expression analysis of *PtPRR* genes using qRT-PCR analysis. Parts of the shoot apices, young and mature leaves, upper and bottom regions of stems, and roots were separately sampled and subjected to total RNA extraction. The relative 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

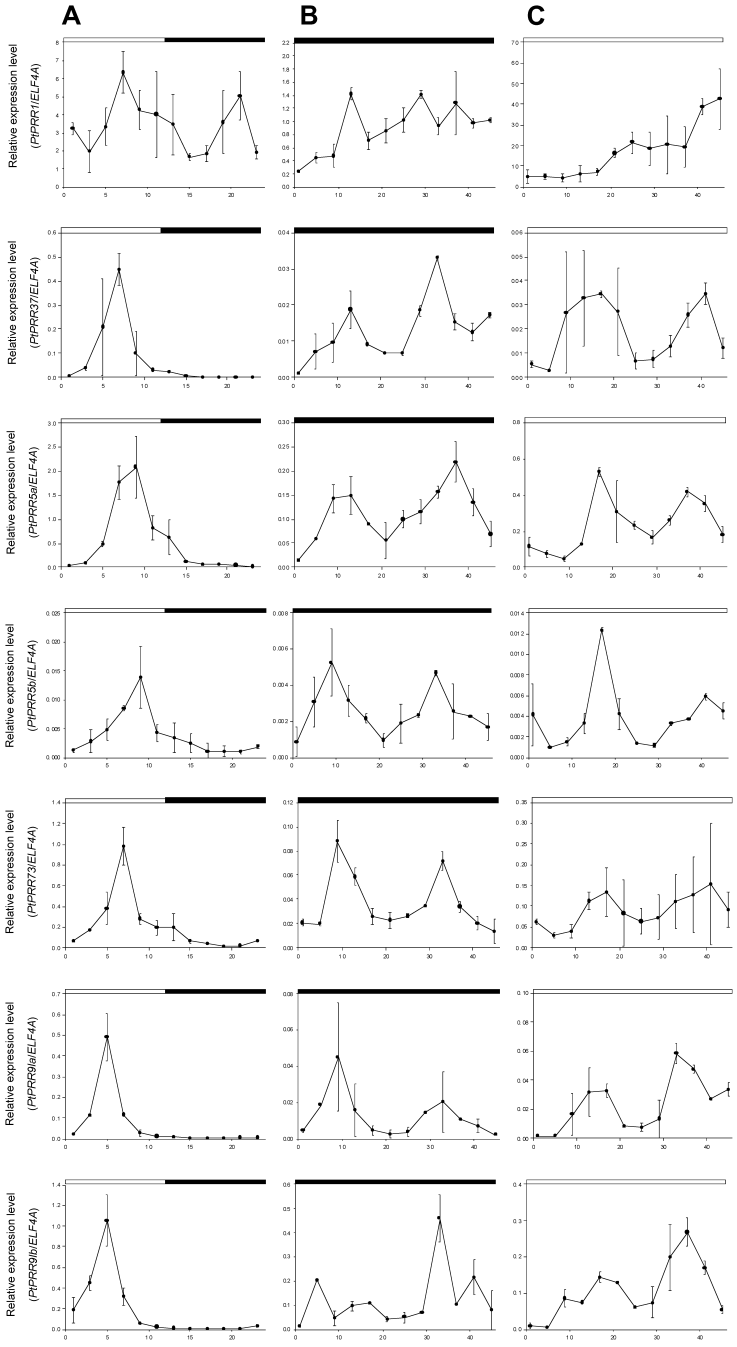

Figure 2. *PtPRR* gene expression in whole young plants grown under 12 : 12LD, DD, and LL conditions. *PtPRR* gene expression rhythms observed in 6-week-old poplar seedlings grown under standard conditions (12 : 12LD) and subsequently transferred to LL and DD conditions. Samples were collected at 2-h (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±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→PRR7→PRR5→PRR1*) observed in rice (*OsPRR73*/*OsPRR37→OsPRR95*/*OsPRR59→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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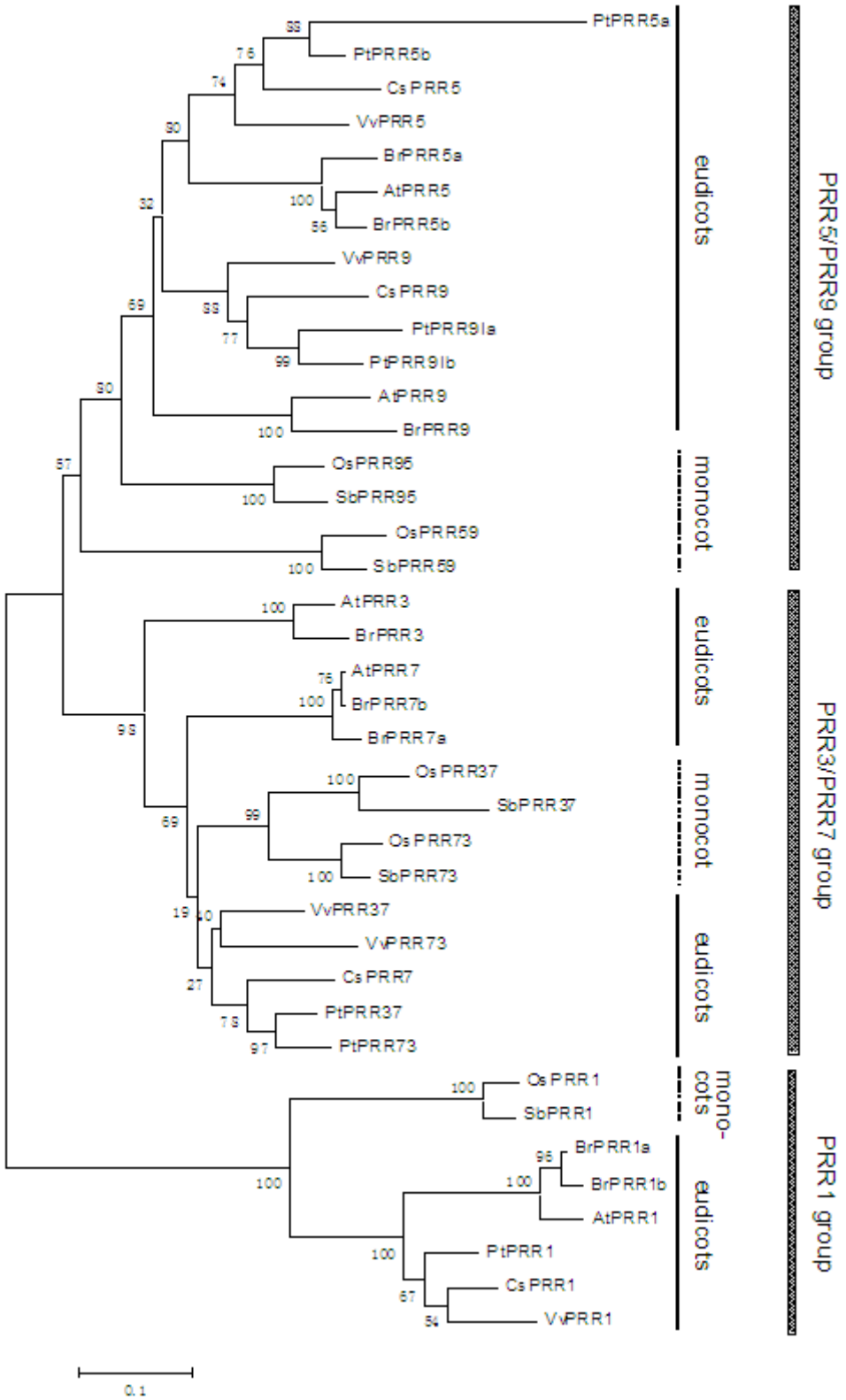
Data 1 Characteristics of *PRR* genes in *Populus trichocarpa*

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

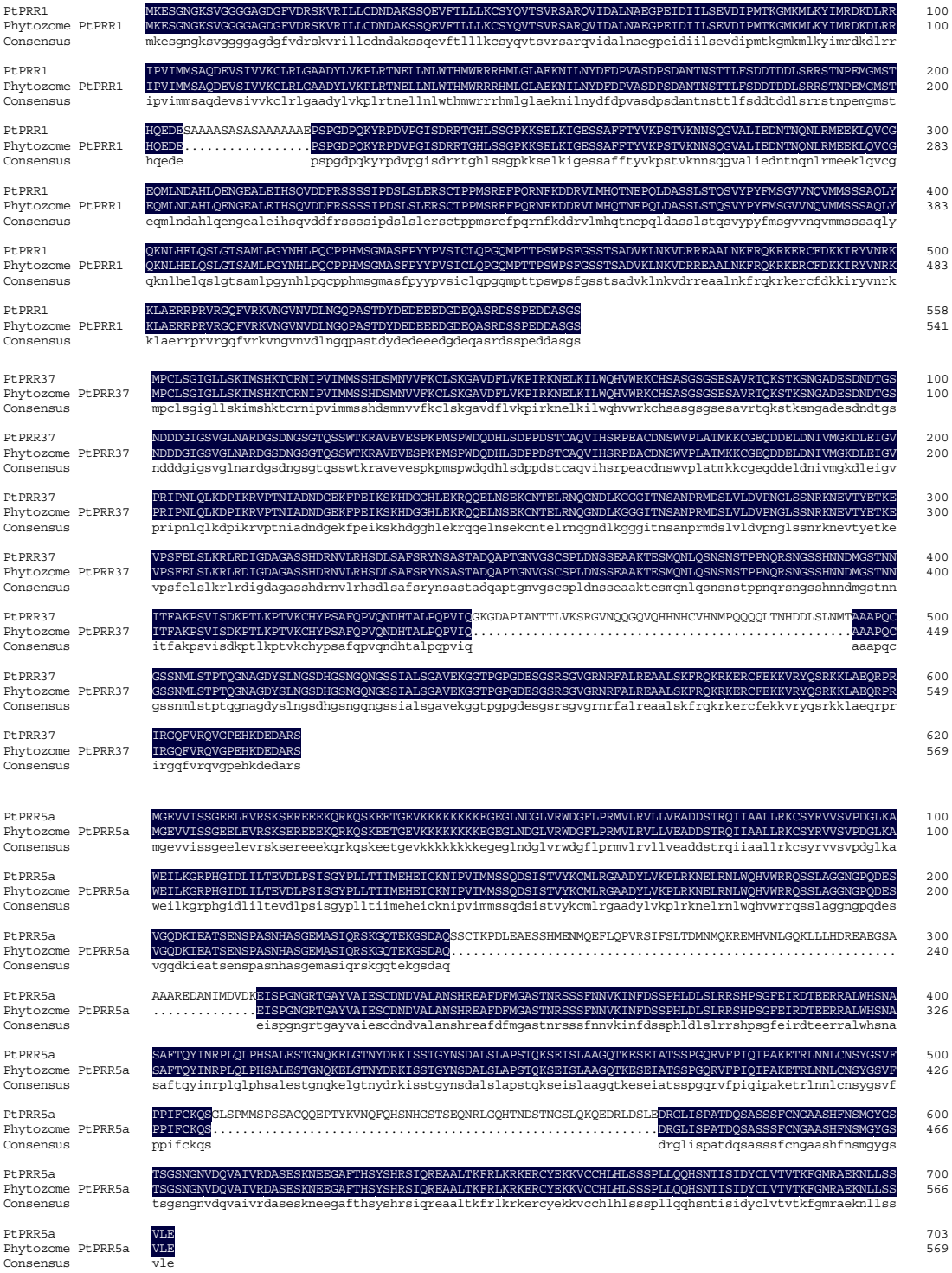


Data 2 The Phylogenetic tree of the PRR genes. Amino acid sequences of PRR homologues were aligned using ClustalW program and the phylogenetic tree was established by the neighbor-joining method. GenBank accession numbers were provided for other PRR proteins than PtPRR. Bars, 0.1 amino acid substitutions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

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

Gene	Primer sequence
PtPRR1	F: 5'-ATGAAGGAGAGTGGTAATGGTAAAAG-3'
	R: 5'-TTAAGATCCTGAAGCATCATCCTC-3'
PtPRR37	F: 5'-GGCCATGCCTTGTTTATCAGG-3'
	R: 5'-TTAGCTACGTGCATCCTCATCTT-3'
PtPRR5a	F: 5'-GGATCCATGGGGGAGGTAGTGATTAGTAGT-3'
	R: 5'-GAGCTCTCACTCTAGGACGCTGCTCAG-3'
PtPRR5b	F: 5'-GGATCCATGGGAGTGGTAGTGGTTAGTAGT-3'
	R: 5'-GAGCTCCTACTGGTCAGTTTCAGCAGGT-3'
PtPRR73	F: 5'-ATGCTCTCAATGAACAACGGG-3'
	R: 5'-TTAGCTCTGTGCATCCTCATCT-3'
PtPRR9la	F: 5'-ATGGGTAAGGTGGTGTTGAGTAG-3'
	R: 5'-TCAACCATTAGCAACTGGGCA-3'
PtPRR9lb	F: 5'-ATGGGTGAGGTTGTGGTGAG-3'
	R: 5'-GAATTTCATGGTCAACCATTAGCAATT-3'

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



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PtPRR5b              MGVVVVSSGEELEVKTGSETEEEKQSKEETESETGEVKRKRKKKEGEGSDNGLVRWERFLPRMVLRVLLVEADDSTRQIIAALLRKCSYK VATVSDGLK   99
Phytozome_PtPRR5     MGVVVVSSGEELEVKTGSETEEEKQSKEETESETGEVKRKRKKKEGEGSDNGLVRWERFLPRMVLRVLLVEADDSTRQIIAALLRKCSYKAVATVSDGLK  100
Consensus            mgvvvvssgeelevktgseteeekqskeetesetgevkrkrkkkegegsdnglvrwerflprmvlrvllveaddstrqiiaallrkcsyk vatvsdglk

PtPRR5b              AWEILKERPHNIDLILTEVDLPSVSGYALLTLIMEHEICKNIPVIMMSSQDSIKTVYKCMLRGAADYLVKPIRKNELRNLWQHVWRKQSSLGGGNGPHDE  199
Phytozome_PtPRR5     AWEILKERPHNIDLILTEVDLPSVSGYALLTLIMEHEICKNIPVIMMSSQDSIKTVYKCMLRGAADYLVKPIRKNELRNLWQHVWRKQSSLGGGNGPHDE  200
Consensus            aweilkerphnidliltevdlpsvsgyalltlimeheicknipvimmssqdsiktvykcmlrgaadylvkpirknelrnlwqhvwrkqsslgggngphde

PtPRR5b              SVGQDKTEATSENNADGNHSSGEMASIQRSKEQAVKRSDSQSSCTKPGLEAEGAHMENMQEFLQPVWSKFSLTDTNMQKHEEHVNLGQKLLVRDSEAEGS  299
Phytozome_PtPRR5     SVGQDKTEATSENNADGNHSSGEMASIQRSKEQAVKRSDSQSSCTKPGLEAEGAHMENMQEFLQPVWSKFSLTDTNMQKHEEHVNLGQKLLVRDSEAEGS  300
Consensus            svgqdkteatsennadgnhssgemasiqrskeqavkrsdsqssctkpgleaegahmenmqeflqpvwskfsltdtnmqkheehvnlgqkllvrdseaegs

PtPRR5b              ATAVCEDSNKITVDKEITPGSGRVTANIAIEGCDKIGALANSPREAIDFMGASTNHSSFNNVEIHFCSSPHLDLSLRRSHPSGFETQVTEERHTLRHSNA  399
Phytozome_PtPRR5     ATAVCEDSNKITVDKEITPGSGRVTANIAIEGCDKIGALANSPREAIDFMGASTNHSSFNNVEIHFCSSPHLDLSLRRSHPSGFETQVTEERHTLRHSNA  400
Consensus            atavcedsnkitvdkeitpgsgrvtaniaiegcdkigalanspreaidfmgastnhssfnnveihfcssphldlslrrshpsgfetqvteerhtlrhsna

PtPRR5b              SAFTWYTNRASQLPHSALANTGNQEEFRANYDGKISSNVNGYNSDALSLAPSTRRSAISLAAGQTKEYEIVTSSSGEKVFPIHIPVKDTRFNNLCNSYGA  499
Phytozome_PtPRR5     SAFTWYTNRASQLPHSALANTGNQEEFRANYDGKISSNVNGYNSDALSLAPSTRRSAISLAAGQTKEYEIVTSSSGEKVFPIHIPVKDTRFNNLCNSYGA  500
Consensus            saftwytnrasqlphsalantgnqeefranydgkissnvngynsdalslapstrrsaislaagqtkeyeivtsssgekvfpihipvkdtrfnnlcnsyga

PtPRR5b              VLPPMMSQSSASQKEPIHKVNPFQCSNYGSTSVQLCDRLGQNANDSINGSLQKQENKLDSLEGREHISSATDQSASSSFCNGAASHFNSIGYGSASGSYS  599
Phytozome_PtPRR5     VLPPMMSQSSASQKEPIHKVNPFQCSNYGSTSVQLCDRLGQNANDSINGSLQKQENKLDSLEGREHISSATDQSASSSFCNGAASHFNSIGYGSASGSYS  600
Consensus            vlppmmsqssasqkepihkvnpfqcsnygstsvqlcdrlgqnandsingslqkqenkldslegrehissatdqsasssfcngaashfnsigygsasgsys

PtPRR5b              NADQIATVSAASESKNEEGVFTHNSNSHRSIQREAALTKFRLKRKERCYEKKVRYESRKKLAEQRPRVKGQFVRQVHIDPSPAETDQ                686
Phytozome_PtPRR5     NADQIATVSAASESKNEEGVFTHNSNSHRSIQREAALTKFRLKRKERCYEKKVRYESRKKLAEQRPRVKGQFVRQVHIDPSPAETDQ                687
Consensus            nadqiatvsaasesknеegvfthnsnshrsiqreaaltkfrlkrkercyekkvryesrkklaeqrprvkgqfvrqvhidpspaetdq
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PtPRR73              MLSMNNGFAEQNHIVEDEQKKIRDGIMGEDQELSEEGESQINEDEKDVNDKGMESLQVLTDAQVVIQSQHQQSQGPLVHWERFLPRRSLKVLLVENDDST  100
Phytozome_PtPRR73    MLSMNNGFAEQNHIVEDEQKKIRDGIMGEDQELSEEGESQINEDEKDVNDKGMESLQVLTDAQVVIQSQHQQSQGPLVHWERFLPRRSLKVLLVENDDST  100
Consensus            mlsmnngfaeqnhivedeqkkirdgimgedqelseegesqinedekdvndkgmeslqvltdaqvviqsqhqqsqgplvhwerflprrslkvllvenddst

PtPRR73              RHVVSALLRNCGYEATAVANGLQAWKLLQDLTNHIDLVLTEVAMPCLSGIGLLSNIMSHKTCRNIPVIMMSSHDSMNVVFRCLSKGAVDFLVKPIRKNEL  200
Phytozome_PtPRR73    RHVVSALLRNCGYEATAVANGLQAWKLLQDLTNHIDLVLTEVAMPCLSGIGLLSNIMSHKTCRNIPVIMMSSHDSMNVVFRCLSKGAVDFLVKPIRKNEL  200
Consensus            rhvvsallrncgyeatavanglqawkllqdltnhidlvltevampclsgiglls nimshktcrnipvimmsshdsmnvvfrclskgavdflvkpirknel

PtPRR73              KILWQHVWRRCHSASGSGSESAVRIQKSLKSKGADESDNDTDSNDDDDIGSIGLNARDGSDNGSGTQSSWTKRAVEVDSPKPMLPWDQLADPPDSTFAQV  300
Phytozome_PtPRR73    KILWQHVWRRCHSASGSGSESAVRIQKSLKSKGADESDNDTDSNDDDDIGSIGLNARDGSDNGSGTQSSWTKRAVEVDSPKPMLPWDQLADPPDSTFAQV  300
Consensus            kilwqhvwrrchsasgsgsesavriqkslkskgadesdndtdsndddigsiglnardgsdngsgtqsswtkravevdspkpmlpwdqladppdstfaqv

PtPRR73              IHSRSEACDNWVPLATTKKFGKQDDELDNFVMGKDLEIGVPRIPNLQHKDLSKEVLTNIAGNNGEKFREIKSEQDSGHLEKGQLELNSEKHNTELRNQGN  400
Phytozome_PtPRR73    IHSRSEACDNWVPLATTKKFGKQDDELDNFVMGKDLEIGVPRIPNLQHKDLSKEVLTNIAGNNGEKFREIKSEQDSGHLEKGQLELNSEKHNTELRNQGN  400
Consensus            ihsrseacdnwvplattkkfgkqddeldnfvmgkdleigvpripnlqhkdlskevltniagnngekfreiksеqdsghlekgqlelnsekhntelrnqgn

PtPRR73              DLKGVSTNITNPQIESEVVDISNSLSSNKKNEVIYETKEMPSLELVLKRLRDTGDAWASANDRNVLRHSDLSAFSRYNSASTAYQAPTGNVGSCSLLDKC  500
Phytozome_PtPRR73    DLKGVSTNITNPQIESEVVDISNSLSSNKKNEVIYETKEMPSLELVLKRLRDTGDAWASANDRNVLRHSDLSAFSRYNSASTAYQAPTGNVGSCSLLDKC  500
Consensus            dlkgvstnitnpqiesevvdisnslssnkkneviyetkempslelvlkrlrdtgdawasandrnvlrhsdlsafsrynsastayqaptgnvgscslldkc

PtPRR73              SEAAKTESMQNLQSNSNSTPRNLCSNGSSNNNDVGTTTNNAFAKPLVIRDKPTPKSTVKCLHPSSAFQPVQNDQTLHAQPVIQGKGDAPIANTILAQSRG  600
Phytozome_PtPRR73    SEAAKTESMQNLQSNSNSTPRNLCSNGSSNNNDVGTTTNNAFAKPLVIRDKPTPKSTVKCLHPSSAFQPVQNDQTLHAQPVIQGKGDAPIANTILAQSRG  600
Consensus            seaaktesmqnlqsnsnstprnlcsngssnnndvgtttnnafakplvirdkptpkstvkclhpssafqpvqndqtlhaqpviqgkgdapiantilaqsrg

PtPRR73              MNQQGQVQHHRHCVHNMPLTIRNDLSLKNMAAAGPRFGSSNMLSTPMEGNAGNYSMNGSNGQNESCIALNPRGINLESNSGAAGKDENPGTGDESGSRSG  700
Phytozome_PtPRR73    MNQQGQVQHHRHCVHNMPLTIRNDLSLKNMAAAGPRFGSSNMLSTPMEGNAGNYSMNGSNGQNESCIALNPRGINLESNSGAAGKDENPGTGDESGSRSG  700
Consensus            mnqqgqvqhhrhcvhnmpltirndlslknmaaagprfgssnmlstpmegnagnysmngsngqnescialnprginlesnsgaagkdenpgtgdesgsrsg

PtPRR73              GGQNCFALREAALNKFRQKRKERCFEKKVRYQSRKKLAEHRPRVRGQFVRQVPFEHKDEDAQS                                      763
Phytozome_PtPRR73    GGQNCFALREAALNKFRQKRKERCFEKKVRYQSRKKLAEHRPRVRGQFVRQVPFEHKDEDAQS                                      763
Consensus            ggqncfalreaalnkfrqkrkercfekkvryqsrkklaehrprvrgqfvrqvpfehkdedaqs
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PtPRR91a             MGKVVLSSSSEEAGGMVVELETEKKDIGSSEVVRWEKFLPKMVLRVLLVEADDSTRQIIVALLRKCGYRVSAVPDGLMAWETLKERPHSIDLILTEVELP  100
Phytozome_PtPRR91a   MGKVVLSSSSEEAGGMVVELETEKKDIGSSEVVRWEKFLPKMVLRVLLVEADDSTRQIIVALLRKCGYRVSAVPDGLMAWETLKERPHSIDLILTEVELP  100
Consensus            mgkvvlssssеeaggmvveletekkdigssevvrwekflpkmvlrvllveaddstrqiivallrkcgyrvsavpdglmawetlkerphsidliltevelp

PtPRR91a             LISGYAFLALVMEHDVCKNIPVIMMSSHDSISVVLKCMLKGSADFLVKPVRKNELRNLWQHVWRRQTQTAGKIPRNSNRVEASSENNAASSDFATSLQKN  200
Phytozome_PtPRR91a   LISGYAFLALVMEHDVCKNIPVIMMSSHDSISVVLKCMLKGSADFLVKPVRKNELRNLWQHVWRRQTQTAGKIPRNSNRVEASSENNAASSDFATSLQKN  200
Consensus            lisgyaflalvmehdvcknipvimmsshdsisvvlkcmlkgsadflvkpvrknelrnlwqhvwrrqtqtagkiprnsnrveassennaassdfatslqkn

PtPRR91a             KDCSEKGSDAQSSCTTPCLEAESAHMQNIQGLSYLKYRSASNLSDADNEKYEDYAKLNKSPVNPESKTGVFVAERSNRTRPDREPYHGAYNPTASRLVEE  300
Phytozome_PtPRR91a   KDCSEKGSDAQSSCTTPCLEAESAHMQNIQGLSYLKYRSASNLSDADNEKYEDYAKLNKSPVNPESKTGVFVAERSNRTRPDREPYHGAYNPTASRLVEE  300
Consensus            kdcsekgsdaqsscttpcleaesahmqniqglsylkyrsasnlsdadnekyedyaklnkspvnpesktgvfvaersnrtrpdrepyhgaynptasrlvee

PtPRR91a             HACAKSAIHDENSRPENDREHANSSFGHDDVLAETSSGAIDLIGSFNNQPKHTYAYSSLHDATNKFEFPPLLELSLRRLYPSSSKNQGLDERHALNHSNS  400
Phytozome_PtPRR91a   HACAKSAIHDENSRPENDREHANSSFGHDDVLAETSSGAIDLIGSFNNQPKHTYAYSSLHDATNKFEFPPLLELSLRRLYPSSSKNQGLDERHALNHSNS  400
Consensus            hacaksaihdensrpendrehanssfghddvlaetssgaidligsfnnqpkhtyayssl hdatnkfefppllelslrrlypssskngglderhalnhsns

PtPRR91a             SAFSLYNSKTLQSLFPTSASNGSDSKEEASKSPDPSSNQLAQNVGTLSQIHDASLSGNQEIITTPVIGQSGKVELAHPSPQLGLIPVLGTRLDNISTGCG  500
Phytozome_PtPRR91a   SAFSLYNSKTLQSLFPTSASNGSDSKEEASKSPDPSSNQLAQNVGTLSQIHDASLSGNQEIITTPVIGQSGKVELAHPSPQLGLIPVLGTRLDNISTGCG  500
Consensus            safslynsktlqslfptsasngsdskeeaskspdpssnqlaqnvgtlsqihdaslsgnqeiittpvigqsgkvelahpspqlglipvlgtrldnistgcg

PtPRR91a             HVFSPLCYTQSNAAWNPNLAGRQQSPFPTTASVHSNPEVLDSKQNHKCYVDQNDLQQNNREPVDEMRHDSPAAGQSTSSSLCNRVANNNSSSAYESFGSG  600
Phytozome_PtPRR91a   HVFSPLCYTQSNAAWNPNLAGRQQSPFPTTASVHSNPEVLDSKQNHKCYVDQNDLQQNNREPVDEMRHDSPAAGQSTSSSLCNRVANNNSSSAYESFGSG  600
Consensus            hvfsplcytqsnaawnpnlagrqqspfpttasvhsnpevldskqnhkcyvdqndlqqnnrepvdemrhdspaagqstssslcnrvannnsssayesfgsg

PtPRR91a             NDVNASSVGTAEKSMAQENLNNGGNFNHDGFGGSDSYRSSQREAALTKFRLKRKDRCYEKRVRYQSRKRLAEQRPRVKGQFVRQAQNDCPVANG          694
Phytozome_PtPRR91a   NDVNASSVGTAEKSMAQENLNNGGNFNHDGFGGSDSYRSSQREAALTKFRLKRKDRCYEKRVRYQSRKRLAEQRPRVKGQFVRQAQNDCPVANG          694
Consensus            ndvnassvgtaeksmaqenlnnggnfnhdgfggsdsyrssqreaaltkfrlkrkdrcyekrvryqsrkrlaeqrprvkgqfvrqaqndcpvang
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PtPRR91b                MGEVVVSSSSEEVEGMAVELETEKKDIGSSEVVRWEKFLPRMVLSVLLVEADDSTRQIIAALLRKCSYRVAAVPDGLMAWETLKGGPHNIDLILTEVELP  100
Phytozome PtPRR91b      MGEVVVSSSSEEVEGMAVELETEKKDIGSSEVVRWEKFLPRMVLSVLLVEADDSTRQIIAALLRKCSYRVAAVPDGLMAWETLKGGPHNIDLILTEVELP  100
Consensus               mgevvvssssseevegmaveletekkdigssevvrwekflprmvlsvllveaddstrqiiaallrkcsyrvaavpdglmawetlkggphnidliltevelp

PtPRR91b                LISGYALLTLVTEHAVCKNIPVIMMSSQDSISMVLKCMLKGAADFLIKPVRKNELRNLWQHVWRRQTLSAGQIPQNLHKVEASSEINAASNGSSDSVMSS  200
Phytozome PtPRR91b      LISGYALLTLVTEHAVCKNIPVIMMSSQDSISMVLKCMLKGAADFLIKPVRKNELRNLWQHVWRRQTLSAGQIPQNLHKVEASSEINAASNGSSDSVMSS  200
Consensus               lisgyalltlvtehavcknipvimmssqdsismvlkcmlkgaadflikpvrknelrnlwqhvwrrqtlsagqipqnlhkveasseinaasngssdsvmss

PtPRR91b                RKNKDCSEKGCDAQSSCTTPCLEAESAHMQNMQGLSQMKYRSASNLSNTDREEFEECAKLDKSPVTPENKTGVFVPERPNRMESDGEPCSGAYNPTSLRL  300
Phytozome PtPRR91b      RKNKDCSEKGCDAQSSCTTPCLEAESAHMQNMQGLSQMKYRSASNLSNTDREEFEECAKLDKSPVTPENKTGVFVPERPNRMESDGEPCSGAYNPTSLRL  300
Consensus               rknkdcsekgcdaqssctpcleaesahmqnmqglsqmkyrsasnlsntdreefeecakldkspvtpenktgvfvperpnrmesdgepcsgaynptslrl

PtPRR91b                LEEHACAKSAIQDENSRPENDRGLANSSFGCDDVPFESSSGAIDLIGTLNNGPKTTYVHSSLHYGTNKFEFAPQLELSLKRLYPSSSKNQGVDERHALNH  400
Phytozome PtPRR91b      LEEHACAKSAIQDENSRPENDRGLANSSFGCDDVPFESSSGAIDLIGTLNNGPKTTYVHSSLHYGTNKFEFAPQLELSLKRLYPSSSKNQGVDERHALNH  400
Consensus               leehacaksaiqdensrpendrglanssfgcddvpfesssgaidligtlnngpkttyvhsslhygtnkfefapqlelslkrlypssskngqgvderhalnh

PtPRR91b                SHASAFSWKKQGCWDSGRDGIGGSDFRRYNSKTLQPPFPASASNGSDSKEEASKSPELSSNQHAQNINSISQRHGATLSGNQDMTIPIIGQSGKAELAYP  500
Phytozome PtPRR91b      SHASAFSWKKQGCWDSGRDGIGGSDFRRYNSKTLQPPFPASASNGSDSKEEASKSPELSSNQHAQNINSISQRHGATLSGNQDMTIPIIGQSGKAELAYP  500
Consensus               shasafswkkqgcwdsgrdgiggsdfrrynsktlqppfpasasngsdskeeaskspelssnqhaqninsisqrhgatlsgnqdmtipiigqsgkaelayp

PtPRR91b                SPRHGLIPVRRGMLDNISTEYGHDFSPLYYTQSSAAWSPKLAGWQQSPYPLSTSIHSNPDIHDSEKNHRCSDETTYNSVDQNDHQQNNKGPADEVRHDSP  600
Phytozome PtPRR91b      SPRHGLIPVRRGMLDNISTEYGHDFSPLYYTQSSAAWSPKLAGWQQSPYPLSTSIHSNPDIHDSEKNHRCSDETTYNSVDQNDHQQNNKGPADEVRHDSP  600
Consensus               sprhglipvrrgmldnisteyghdfsplyytqssaawspklagwqqspyplstsihsnpdihdseknhrcsdettynsvdqndhqqnnkgpadevrhdsp

PtPRR91b                AAGQSTGGLCNGVINHNKSSAYESFGSRDDGNAKEKAMAQDNLNDGDNFNRDGFRGIDSLRSSQREAALTKFRLKRKDRCYEKKVRYQSRKRLAEQRPRV  700
Phytozome PtPRR91b      AAGQSTGGLCNGVINHNKSSAYESFGSRDDGNAKEKAMAQDNLNDGDNFNRDGFRGIDSLRSSQREAALTKFRLKRKDRCYEKKVRYQSRKRLAEQRPRV  700
Consensus               aagqstgglcngvinhnkssayesfgsrddgnakekamaqdnlndgdnfnrdgfrgidslrssqreaaltkfrlkrkdrcyekkvryqsrkrlaeqrprv

PtPRR91b                KGQFVRQVQNDSPIANG                                                                                  717
Phytozome PtPRR91b      KGQFVRQVQNDSPIANG                                                                                  717
Consensus               kgqfvrqvqndspiang
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Data 5 The gene-specific primers of *PtPRR* genes for quantitative RT-PCR analysis

Gene	**Primer sequence**
PtPRR1	F: 5'-AGATGAGATCAAGCTGCGAGTG-3'
	R: 5'-ACCACAGACCTAGTCAACAGATTAG-3'
PtPRR37	F: 5'-TGGAATTGAGATCGGTTGGATTATG-3'
	R: 5'-CCCATTACTGGAGAAGGCTACC-3'
PtPRR5a	F: 5'-CCCTCACCTGCGGAAACTG-3'
	R: 5'-GTTATATCTACAAATCGGCTCAAGC-3'
PtPRR5b	F: 5'-TGTGAAGTCTAGAGGGAACAATATG-3'
	R: 5'-CTATATTTTGAGACGTGAAGATGGC-3'
PtPRR73	F: 5'-CCTTGGGAATTTTGTGCTTGAG-3'
	R: 5'-CGTGGCAGTAACATGTATATTAGTC-3'
PtPRR9la	F: 5'-TCTGAATCCTCTTCTACTCTGG-3'
	R: 5'-TCCACTTTCTCCTAATTAATTGATC-3'
PtPRR9lb	F: 5'-CCATGAAATTCATCATCACACG-3'
	R: 5'-GACCCAAAAATTACAGTTAGAACC-3'
ELF4A	F: 5'-ACACAGTCTCAGCTACTCATGGAGA-3'
	R: 5'- ATTTATGACAAGGGACACTTGCTG-3'