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Comparative gene expression studies of candidate genes associated with defence response in ginger and mango ginger post inoculation with *Ralstonia solanacearum*

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ABSTRACT

Bacterial wilt of ginger (*Zingiber officinale* Rosc.) causes serious economic losses in all major ginger growing areas of the world. The Indian mango ginger (*Curcuma amada* Roxb.) is found to be resistant to the disease. With an aim to decipher the factors involved in mango ginger resistance, tissue-specific expression of nine candidate genes viz., Ethylene Response Factor (ERF), HMG-CoA synthase (HMGS), HMG-CoA reductase (HMGR), ABC transporter, WRKY8 transcription factor, β-(1, 3)-glucanase, Callose synthase, Heat Shock Protein (HSP) and Mlo14 were carried out at different time intervals in leaf and rhizome tissues post pathogen inoculation using real-time PCR. The transcripts varied in the level of up and down-regulation, with a marked difference in the intensity and time of response. Compared to leaf tissues, the rhizome tissues of both the plants showed increased expression of all the transcripts except β-(1, 3)-glucanase and Mlo14. In both the resistant and susceptible plants β-(1, 3)-glucanase showed higher expression in the leaf tissues, whereas HSP and Callose synthase showed higher expression in rhizome tissues of ginger and mango ginger respectively. Compared to ginger, the rhizome tissues of mango ginger showed peak level expression at earlier hours. The rhizome of mango ginger may play an important role in preventing the entry of the pathogen through the soil. Traversing all these barriers if the pathogen is delivered directly to the xylem tissues, they can multiply profusely and induce bacterial wilt in mango ginger.

1. Introduction

Ginger (*Zingiber officinale* Rosc.) is an economically important spice additionally valued for its medicinal properties. Bacterial wilt of ginger is one of the most prevalent and destructive diseases of ginger leading to severe yield losses in tropical and subtropical regions of the world [1]. It is caused by *Ralstonia solanacearum* (biovar 3), a soil borne pathogen that enters the vascular system of the plants through the natural openings or injured portions [2]. The Indian mango ginger (*Curcuma amada* Roxb.) that belongs to the same family was found to be resistant to bacterial wilt [3]. Understanding the defence response in mango ginger at molecular level could be a key to developing bacterial wilt resistance in ginger.

Plants are constantly exposed to a wide range of biotic and abiotic stresses, to overcome which they need to express specific genes in variable intensities depending on the need. The success of a plant to resist a pathogen principally depends on the well timed activation of its defence machinery. Upon invasion by pathogens, plants activate the signalling pathways that ultimately lead to the synthesis of defence related compounds. In order to understand the disease resistance mechanism in mango ginger, both the ginger and mango ginger plants were challenge inoculated with *R. solanacearum,* and the transcripts expressed as a result were sequenced and compared [4]. The study resulted in the identification of several genes whose expression was altered following pathogen invasion. In the present work nine genes were selected randomly for validation by qPCR in leaf and rhizome tissues *viz.*, The ERF transcription factors (ERFs), involved in resistance to pathogen attack through the activation of pathogenesis-related genes and therefore lead to increased resistance [5–7]; 3-Hydroxy-3-methylglutaryl-coenzyme A synthase (HMGS) and 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), that catalyse the committed step in isoprenoid biosynthesis, thus playing a vital role in defence against pathogen attack [8]; Callose synthases, the enzymes responsible for the synthesis of callose, a cell wall polymer that forms a barrier in response to pathogen infection [9]; ATP-binding cassette (ABC) proteins involved in transport of secondary metabolites that helps in defence against biotic stress [10]; WRKY transcription factors, which are the regulatory proteins predominantly involved in biotic and abiotic stress

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responses in plants participating in defence responses either as positive or negative regulators [11–13]; Heat Shock Proteins (HSPs) induced as a result of majority of biotic and abiotic stresses in plants and important for stress tolerance [14]; β-(1, 3)-glucanases, the pathogenesis-related (PR) proteins that are evoked in response to wounding or infection by pathogens ultimately leading to systemic acquired resistance [15]; Mlo proteins, that are highly conserved with seven transmembrane domains that confers durable and broad-spectrum resistance against powdery mildew in barley [16,17].

In the present work qPCR analysis was carried out to compare the expression of the selected transcripts from the leaf and rhizome tissues of ginger and mango ginger at different time intervals post inoculation with the bacterial wilt pathogen.

2. Materials and methods

2.1. Plant material

Disease free rhizomes of ginger 'IISR Varada' and mango ginger 'Amba' were procured from the experimental farm of ICAR-Indian Institute of Spices Research, Peruvannamuzhi, Kerala, India. The rhizomes were washed twice in sterile water and planted in autoclaved perlite. The plants were supplemented with Hoagland solution and maintained under greenhouse conditions.

2.2. Inoculum preparation and inoculation procedure

Bacterial inoculum was prepared by transferring virulent colonies of *R. solanacearum* strain GRsMep3 identified on CPG agar (Casein Peptone Glucose) into CPG broth and incubated at 28 °C for 16 h with constant shaking. The bacterial cells were pelleted by centrifugation. The pellet was suspended in sterile water and the absorbance was normalized to 0.1 at OD_{600}. The bacterial suspension (25 mL) was poured onto the base of ginger and mango ginger plants and observed for the symptom development.

2.3. RNA isolation and cDNAsynthesis

Total RNA was isolated from 100 mg of leaf and rhizome tissues of ginger and mango ginger [18] at 0, 1, 4, 8, 24, 48, 72, 96 and 120 h post inoculation (hpi) with *R. solanacearum*. About 1 µg of RNA samples were treated with TURBO DNA-free kit (Invitrogen, USA) for 30 min at 37 °C to remove the contaminating genomic DNA. After DNase treatment, first strand cDNA synthesis was carried out using 1 µL of RevertAid Reverse Transcriptase (200U/µL) (Thermo Scientific, USA) in 4 µL of 5X reaction buffer (Thermo Scientific, USA), 1 µL of Oligo $(dT)_{18}$ primer (Thermo Scientific, USA), 0.5 µL of RiboLock RNase Inhibitor (40U/µL) (Thermo Scientific, USA) and 2 µL of dNTP mix (Thermo Scientific, USA). The reaction mix was incubated for 60 min at 42 °C and terminated by heating at 70 °C for 5 min.

2.4. Quantitative real-time PCR

Real-time PCR analysis was performed using Quantifast SYBR Green Mastermix kit (Qiagen, USA) on the Rotor Gene Q Real-Time PCR system (Qiagen, USA). The primers specific to the sequences were designed using Primer-quest tool (Integrated DNA Technologies, Coralville, IA) (Table 1). The 20 µL reaction mixture contained 10 µL of QuantiFast SYBR Green PCR Mastermix, 1 µL of each primer (10 mM), 3 µL of the template (3 ng) and 5 µL of sterile distilled water. The thermal conditions were as follows: initial hold at 94 °C for 5 min followed by 40 cycles at 94 °C for 15 s and a final step at 60 °C for 45 s. Fold change of the transcripts was calculated relative to the control (0 hpi) using 2^{-ddCt} method [19] using *β*-actin as an internal control [20]. The expression level of the genes of interest was normalized to that of the constitutive actin gene by subtracting the CT value of the gene of interest from the CT value of actin (ΔCT). The fold differences were transformed by using a binary logarithm (log_2). A melt curve analysis was run from 62 to 99 °C. The specificity each primer pair was confirmed by single-peak melt curves.

Table 1
Primers designed to perform qPCR experiments with the shortlisted nine candidate genes in ginger (Zo) and mango ginger (Ca).

Primer sets		Sequence (5′ - 3′)
CaERF	Forward (F)	GTTGCATCTTCCATCAGAGT
	Reverse (R)	ACCAATCAAGCAATCCATAAAC
ZoERF	Forward (F)	ACCACCCTATCAGGATACAT
	Reverse (R)	TTCGCTTCTTCTCGGTAAAG
CaHMGR	Forward (F)	ACCCAGCAAGGTTCTTAATC
	Reverse (R)	TGGTCTGTGAAGCAATTATCA
ZoHMGR	Forward (F)	TGGTCTGTGAAGCAATTATCA
	Reverse (R)	ACCCAGCAAGGTTCTTAATC
CaHMGS	Forward (F)	CATTGCCAGCTTCCCTATTT
	Reverse (R)	ACTCCATGTACCGTCGATATTA
ZoHMGS	Forward (F)	TAGATACGGAGCCAAGGATT
	Reverse (R)	GCATAATGTCGACGGTACAT
CaMLO	Forward (F)	ACAATGAGAAGGCAGTCAAG
	Reverse (R)	TTCCGAACTGACGAAAGAAG
ZoMLO	Forward (F)	GTGTATTGCCGCCTTATCTT
	Reverse (R)	CTTTGTAGTTCGATCCCATCTG
CaWRKY8	Forward (F)	CTTTACCTGCACAGCATCTC
	Reverse (R)	GTGGAGTTTGGCAGTTGTAG
ZoWRKY8	Forward (F)	TGTTTCCATCTCCTACGTCTG
	Reverse (R)	GTTGGACTTGACCTCATCCT
CaHSP	Forward (F)	CGGAGAACAAAGAGGACTACAC
	Reverse (R)	CGTAGGAGATCCGCCAATTT
ZoHSP	Forward (F)	AAAGAGGACCAGCTGGAATAC
	Reverse (R)	GGTGGTCTTCTCTGTCCATAAA
CaCALLOSE SYNTHASE	Forward (F)	CTTCGACTTCTTCAGGATGCTAT
	Reverse (R)	GCCCACTAAGCGACAAGTAA
ZoCALLOSE SYNTHASE	Forward (F)	GTCCTGAAACCTCTTTCTAGTG
	Reverse (R)	GCAAGGAAGAGAGGCAATAC
CaGLUCANASE	Forward (F)	TTGAACATGGCGAACAGG
	Reverse (R)	CAACGCGCAGACATACAA
ZoGLUCANASE	Forward (F)	CGACGTCGGAGAGAAACTA
	Reverse (R)	GAGGGAAGCAGTGGAAGA
CaABC	Forward (F)	TGAGGCCACATCTTCAGTAG
	Reverse (R)	CGAGCACTAGAACCAGATCA
ZoABC	Forward (F)	AGTACCTCCCAGATCGTAGA
	Reverse (R)	GAGATCGAGGCTGAGCATAA
Actin	Forward (F)	TAGGTGCCCAGAGGTTCTATT
	Reverse (R)	ACCGCTAAGCACCACATTAC

3. Results and discussion

The ginger and mango ginger plants respond differently to *R. solanacearum* (Fig. 1). In order to gain an insight into the differential response of the susceptible ginger cultivar 'IISR Varada' and resistant mango ginger cultivar 'Amba' upon inoculation with *R. solanacearum*, the expression of nine genes involved in plant defence responses were investigated in the leaf and rhizome tissues at 1, 4, 8, 24, 48, 72, 96 and 120 hpi using quantitative real-time PCR. The un inoculated plants were used as control (0 hpi). The qPCR study resulted in variable expression of the nine genes in a tissue specific manner.

The ERFs are known to integrate signals from both ethylene and jasmonic acid dependent pathways and play a major role in conferring disease resistance to cotton against *Xanthomonas campestris* pv. *malvacearum* [21]. TSRF1, a tomato ERF protein was found to be up-regulated upon infection with *R. solanacearum* and mediated defence signalling by the activation of pathogenesis related proteins [7]. Moreover the ERFs were found to interact directly with *pto* resistance gene in tomato [5]. They are also found to be a desired candidate gene for engineering multiple stress resistance in rice [22]. In ginger and mango ginger, the ERF proteins showed different levels of up-regulation after inoculation with the pathogen. In mango ginger leaf tissues, their expression peaked up at 24 hpi (2.05). Their expression was faster and

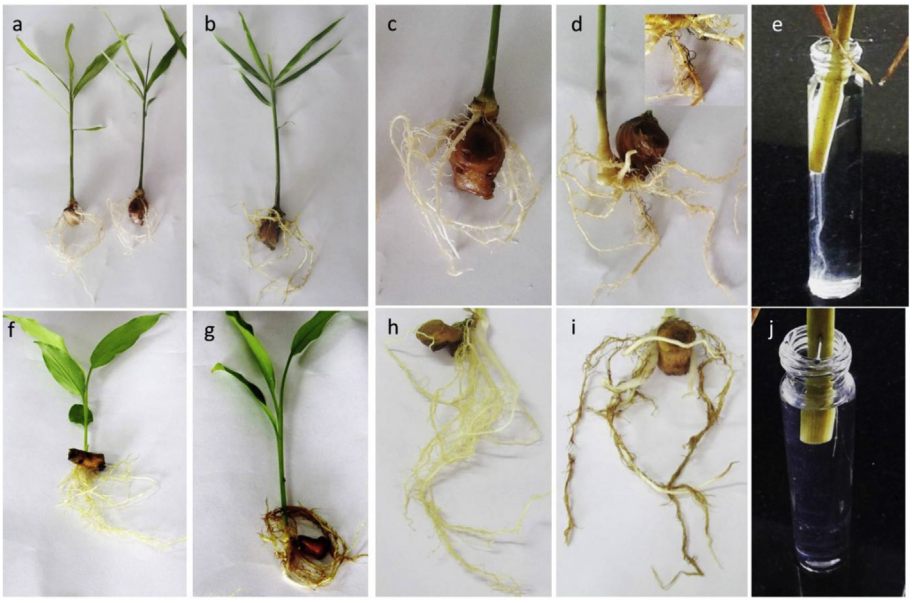

Fig. 1. Figures showing the symptoms of control and inoculated plants of ginger and mango ginger at 12 dpi (a) Control-ginger (b) Inoculated-ginger (c) Control-ginger roots (d) Inoculated-ginger roots (e) Milky white bacterial ooze from the infected pseudostem of ginger (f) Control-mango ginger (g) Inoculated-mango ginger (h) Control-mango ginger roots (i) Inoculated-mango ginger roots (j) The pseudostem of mango ginger with no bacterial ooze.

higher in rhizome tissues. In rhizome tissues, the expression peaked up at 8 hpi (2.89). In leaf tissues of ginger, ERF proteins showed the highest expression at 72 hpi (1.15), whereas in rhizome tissues the highest fold change was at 96 hpi (2.28) (Fig. 2-a). Thus compared to ginger the expression of ERF proteins peaked up earlier in mango ginger.

The expression of HMGR leads to the production of isoprenoid phytoalexins that are involved in defence response against plant pathogens [23]. In tomato the level of one of the isozymes of HMGR *hmg2* increased in response to pathogen or elicitors [24]. In the leaf tissues of mango ginger HMGR was down-regulated, whereas in rhizome tissues it increased in the first hour itself (2.33). In rhizome tissues of ginger the expression of HMGR was lower in the initial hours and the expression increased at 72 hpi (1.28) (Fig. 2-b). In leaf tissues of ginger HMGR expressed at 4 hpi (0.54) and 24 hpi (0.71). As HMGR is immediately expressed in the tissues located around the site of pathogen ingress, it can be used in engineering disease resistance in plants [24].

The fold change of HMGS in mango ginger leaf tissues peaked up at 24 hpi (1.73). In rhizome tissues it increased in the first hour itself (2.72). In rhizome tissues of ginger the expression of HMGS peaked up at 96 hpi (2.61), whereas in leaf tissues the expression increased at 1 hpi (0.65) (Fig. 2-c). Compared to ginger, the induction of HMGS was earlier in rhizome tissues of mango ginger. In *Arabidopsis* over expression of *Brassica juncea* HMGS led to the increase in the sterol content by over expressing the genes in sterol biosynthesis pathway, thereby leading to increased tolerance to stress [25].

The deposition of callose in between the cell wall and plasma membrane is an important defence response leading to an earlier containment of the pathogen [26]. In *Arabidopsis* early expression of callose synthases at increased levels resulted in high level of resistance to powdery mildew [27]. In mango ginger, the expression of callose synthase was lower in the leaf tissues in the initial hours and increased at 96 hpi (1.41). In rhizome tissues of mango ginger the expression increased 4 hpi onwards, with the highest expression at 8 hpi (4.14). In ginger, the expression was comparatively lower in leaf tissues. In rhizome tissues the expression increased at 48 hpi (1.5) (Fig. 2-d). *R. solanacearum* could induce bacterial wilt in mango ginger when the bacterium is delivered directly to xylem by piercing. As the activity of callose synthase was higher in mango ginger rhizome tissues in the initials hours itself, it might be one of the most critical point determining the outcome of infection.

ABC transporters are ATP driven pumps involved in detoxification process [28] and play an important role in defence response to pathogens [29]. The expression of ABC transporters in mango ginger increased at 8 hpi in leaf (1.38) and rhizome (2.29) tissues. In ginger the expression peaked up at 8 hpi (1.13) in leaf tissues and 96 hpi (2.41) in rhizome tissues (Fig. 2-e). Thus the expression levels of ABC transporters in mango ginger leaf and rhizome tissues peaked up at an early stage. In ginger leaf tissues also the expression peaked up at an early stage in leaf tissues but in rhizome tissues the expression was higher at the later stages.

β- (1, 3)-glucanases are hydrolytic enzymes known to be involved in resistance to pathogens and insects [30]. Our results showed that in mango ginger leaf tissues the expression of β-(1, 3)-glucanase increased at 8 hpi (5.48) and in rhizome tissues, the gene expressed at 1 hpi (1.68) itself and peaked up at 96hpi. In ginger the expression of β-(1, 3)-glucanase increased in leaf tissues at 24 hpi (5.58), whereas in rhizome tissues it was down-regulated when compared to control (Fig. 2-f). The expression of β - (1, 3)-glucanase in rhizome tissue plays an important role in preventing the entry of pathogen through the soil. As the expression in rhizome tissues of ginger was down-regulated, the pathogen might find it easy to infect ginger.

The WRKY8 TFs are found to be activated by MAPKs in *Nicotiana benthamiana*, thereby increasing the expression of defence related genes [31]. In *Arabidopsis* WRKY8 was found to be involved in resistance to *Tobacco mosaic virus* (TMV) by mediating the ABA and ethylene signalling pathways [32]. The expression of WRKY8 transcription factor increased in mango ginger leaf tissues in the initial hours itself, and the expression peaked up at 72 hpi (2.42). In rhizome tissues the highest expression was at 72 hpi (3.31). In ginger leaf tissues the expression was lower, whereas in rhizome tissues the expression increased in the initial hours itself, and the expression peaked up at 72 hpi (2.32) (Fig. 2-g). The expression analysis of WRKY8 transcription factor indicated that in mango ginger leaf and rhizome tissues the gene was markedly up- regulated. In ginger leaf tissues the expression was lower, whereas in rhizome tissues the expression increased in the initial hours itself, with maximal expression at 72 hpi.

The HSPs stabilize the structure of R-proteins [33] playing an important role in *R*-gene mediated defence responses. The expression of HSP in leaf tissues of mango ginger was very low compared to control, whereas in rhizome tissues it increased at 96 hpi (2.66). In ginger leaf tissues the expression of HSP increased at 24 hpi (1.34) and 72 hpi (1.37). In rhizome tissues the expression of HSP peaked up at 72 hpi (3.53) (Fig. 2-h). The transcript levels of the HSP were up-regulated in

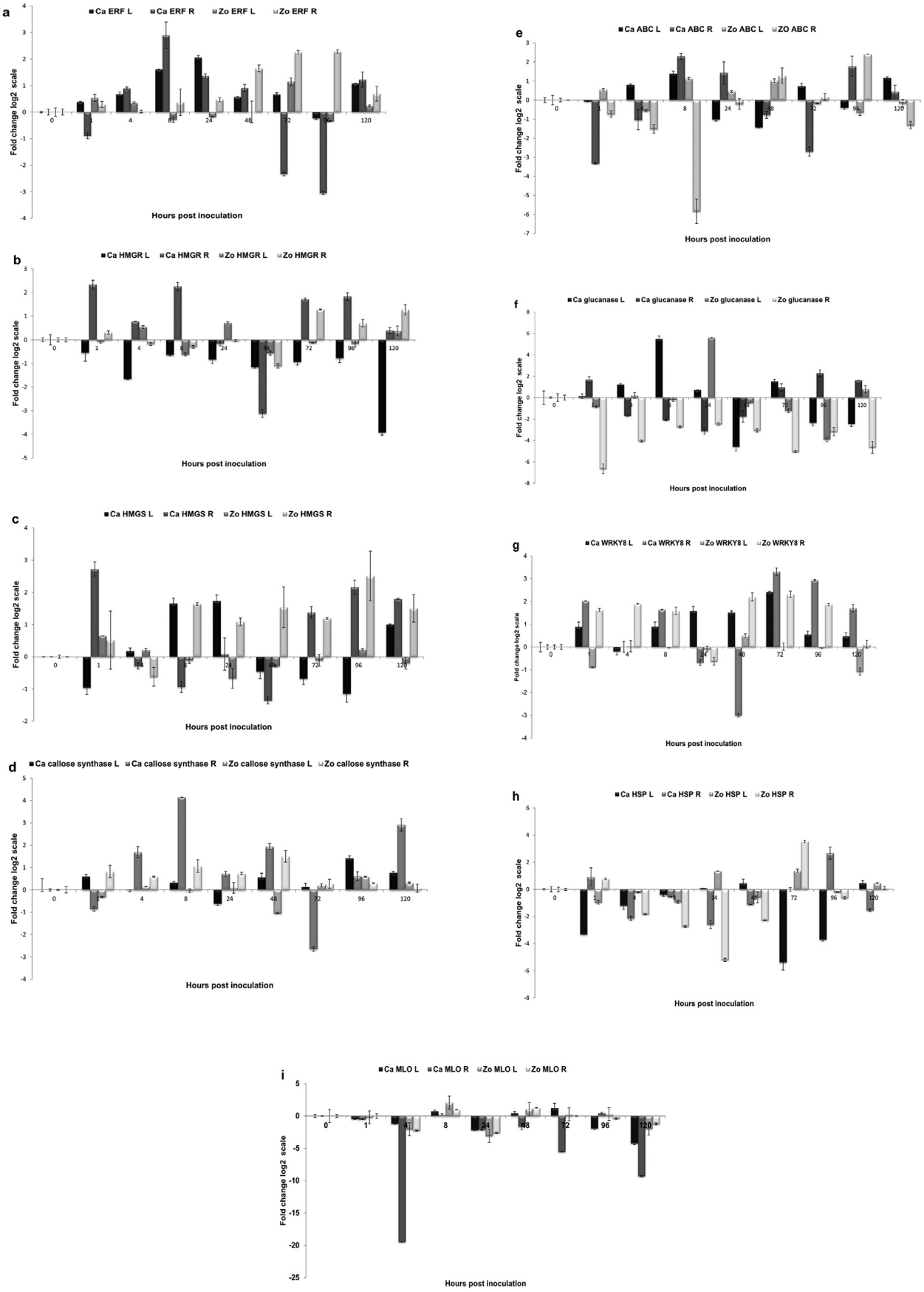

Fig. 2. Normalized relative gene expression levels of nine candidate genes namely (a) ERF (b) HMGR (c) HMGS (d) Callose synthase (e) ABC transporters (f) β-(1, 3)-glucanase (g) WRKY8 (h) HSP and (i) Mlo in ginger and mango ginger leaf and rhizome tissues at different time intervals post inoculation with *R. solanacearum*.

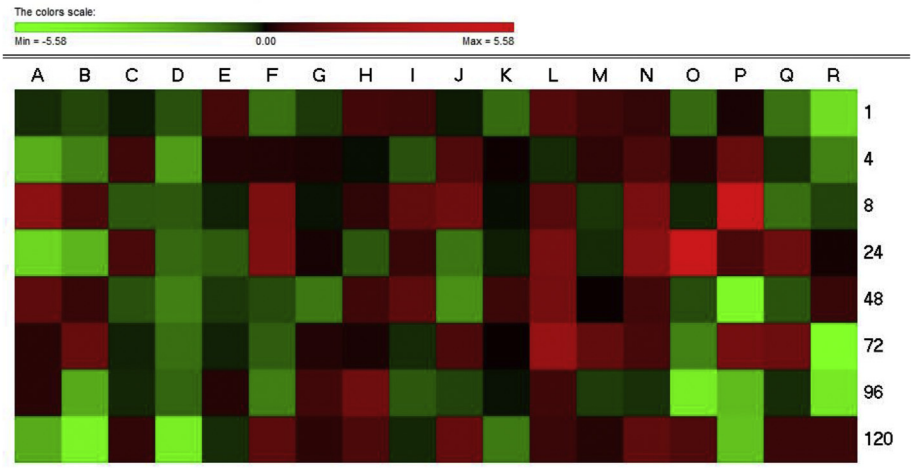

Fig. 3. Heatmap showing the expression levels of the nine transcripts in leaf tissues of ginger and mango ginger *viz*., (a) Zo-MLO (b) Ca-MLO (c) Zo-HMGR (d) Ca-HMGR (e) Zo-HMGS (f) Ca-HMGS (g) Zo-Callose synthase (h) Ca-Callose synthase (i) Zo-ABC (j) Ca-ABC (k) Zo-WRKY8 (l) Ca-WRKY8 (m) Zo-ERF (n) Ca-ERF (o) Zo-glucanase (p) Ca-glucanase (q) Zo-HSP (r) Ca-HSP at 1–120 hpi using PermutMatrix [35]. The color code indicates relative abundance, ranging from light green (low abundance) to red (high abundance). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ginger. In the leaf tissues of mango ginger, the activity was very low, whereas in rhizome tissues the expression was elevated at later phases. But in ginger leaf and rhizome tissues the expression of HSP peaked up significantly in both leaf and rhizome tissues.

Mlo resistance is mostly mediated by the formation of cell wall appositions, thereby restricting the entry of the pathogens [34]. In barley the ability of the powdery mildew pathogen, *Blumeria graminis* f. sp. *hordei* to induce disease largely depends on the presence of the recessive Mlo protein [17]. The expression of Mlo14 increased at 72 hpi (1.22) in mango ginger leaf tissues, whereas in rhizome tissues it was down-regulated. In ginger leaf tissue the expression of the Mlo transcript increased at 8 hpi (2.06) and in rhizome tissues the expression was peaked up at 48 hpi (1.26) (Fig. 2-i). The expression of the Mlo transcript was significantly up-regulated in ginger, with an early expression in the leaf tissues. In mango ginger, the expression of Mlo14 increased at late phase in leaf tissues, whereas in rhizome tissues the expression of the transcript was down-regulated. Thus along with HSPs, the Mlo transcripts were differentially up-regulated in ginger.

Thus the expression of the defence-related genes varied at different time periods after pathogen attack in a tissue-specific manner. The time period after pathogen inoculation could be divided into early (1–8 hpi), middle (24–48 hpi) and late (72–120 hpi) phases and the nine genes could be categorised into these three phases based on their expression patterns. The pathogen *R. solanacearum* induces bacterial wilt in 7–10 days after soil inoculation. So the early expression of the genes related to infection is very important in restricting the spread of the pathogen.

In mango ginger leaf tissues, the genes that were early expressed (1–8 hpi) included ERF, HMGS, callose synthase, ABC transporters, β-(1, 3)-glucanase, WRKY8 and Mlo. Out of these the WRKY8, ERF, callose synthase, and β-(1, 3)-glucanase started expressing from first hour itself. The callose synthase, β-(1, 3)-glucanase, ERF, HMGS, and WRKY8 were expressed in early, middle and late phases. The HMGR did not show upregulation in any of the time intervals in the tissues of mango ginger leaf. In the early hours, ERF, HMGS and β-(1, 3)-glucanase showed a fold change (log_2) greater than 1.5 compared to control. The transcript level was highest in β-(1, 3)-glucanase in the early phase, in ERF and HMGS the transcript level was highest in mid phase, whereas in callose synthase, ABC transporters, WRKY8, HSP and Mlo the expression peaked in late hours after pathogen inoculation.

In the leaf tissues of ginger ERF, HMGR, HMGS, callose synthases, ABC transporters, β-(1, 3)-glucanase and WRKY8 were expressed in earlier hours. However none of the transcripts showed a fold change greater than 1.5. The ERF, HMGR, callose synthases, β-(1, 3)-glucanase and WRKY8 expressed in all the three stages after pathogen inoculation. In HMGS and ABC transporters the peak level expression was in earlier hours. In HMGR, WRKY8 and β-(1, 3)-glucanase the expression peaked up in mid hours, whereas in ERF, callose synthase and HSP the expression peaked up in the late hours after pathogen inoculation. All the transcripts other than β-(1, 3)-glucanase showed a fold change less than 1.5. The differential gene expression of all the nine transcripts studied in the leaf tissues of ginger and mango ginger at different time intervals under the stress induced by the pathogen has been represented in the heat map (Fig. 3).

In the rhizome tissues of mango ginger the ABC transporter and ERFs got expressed in the early, mid and late hours after pathogen inoculation. All the nine transcripts got expressed in the early phase of pathogen inoculation. The ABC transporters, ERF, HMGR, HMGS and WRKY8 showed more than 1.5 fold increase in the initial hours. Out of these HMGR, HMGS and WRKY8 showed more than two fold increase in the first hour itself. In ERF, HMGR and HMGS the expression level peaked up in the earlier hours, whereas in the remaining six transcripts, the expression peaked up in the late hours after pathogen inoculation.

In the rhizome tissues of ginger ERF, HMGS, callose synthase and WRKY8 were expressed in all the three stages after pathogen inoculation. The ERF, HMGR, HMGS, callose synthase, WRKY8 and HSP were expressed in earlier hours. The HMGS and WRKY8 expressed a fold change above 1.5 in the earlier hours. The β-(1, 3)-glucanase was down regulated in the rhizome tissues. The expression of β-(1, 3)-glucanase in rhizome tissue plays an important role in preventing the entry of pathogen through the soil. As the expression in rhizome tissues of ginger was down-regulated, the pathogen might find it easy to infect ginger. The expression of WRKY8, HSP and Mlo peaked up in the earlier hours, the expression of callose synthase peaked up in the mid hours, whereas the expression of ERF, HMGR, HMGS, and ABC transporters were higher in late hours. All the transcripts other than HMGR and β-(1, 3)-glucanase showed a fold change greater than 1.5. The expression patterns of all the nine transcripts in the rhizome tissues of ginger and mango ginger has been represented in the form of a heatmap (Fig. 4).

In mango ginger, out of the nine candidate genes studied, β-(1, 3)-glucanase and callose synthase showed higher expression in the leaf and rhizome tissues respectively. Compared to leaf tissues the rhizome tissues showed increased expression of ERF, HMGR, HMGS, Callose synthase, WRKY8, HSP and ABC transporters. The expression of HMGR, HMGS and β-(1, 3)-glucanase genes peaked up in the first hour itself in the rhizome tissues. The rhizome of mango ginger may play an important role in preventing the entry of the pathogen through the soil. If

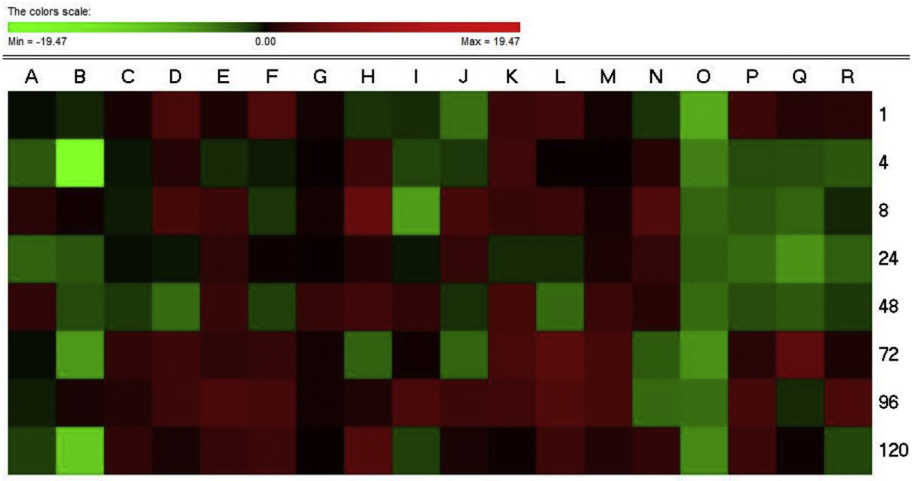

Fig. 4. Heatmap showing the expression levels of the nine transcripts in rhizome tissues *viz*., (a) Zo-MLO (b) Ca-MLO (c) Zo-HMGR (d) Ca-HMGR (e) Zo-HMGS (f) Ca-HMGS (g) Zo-Callose synthase (h) Ca-Callose synthase (i) Zo-ABC (j) Ca-ABC (k) Zo-WRKY8 (l) Ca-WRKY8 (m) Zo-ERF (n) Ca-ERF (o) Zo-glucanase (p) Ca-glucanase (q) Zo-HSP (r) Ca-HSP at 1–120 hpi using PermutMatrix [35]. The color code indicates relative abundance, ranging from light green (low abundance) to red (high abundance). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the pathogen is delivered directly to the xylem tissues, they can multiply profusely and induce bacterial wilt in mango ginger. In ginger, β-(1, 3)-glucanase and HSP showed higher expression in leaf and rhizome tissues respectively. ABC transporters and Mlo genes showed earlier expression in leaf tissues. ERF, HMGR, HMGS, Callose synthase, WRKY8, HSP and ABC transporters showed comparatively higher expression in the rhizome tissues. In rhizome tissues of ginger the peak level expression took place only in later hours, while in mango ginger the expression in rhizome tissues was comparatively earlier.

4. Conclusion

The molecular mechanism underlying the complexity of bacterial wilt resistance in mango ginger could involve a cross-talk between various signalling pathways leading to disease resistance. The pattern of the accumulation of the transcripts of the selected defence-response genes differed with respect to each other after pathogen exposure. These changes in the transcript levels may underlie the outcome of the host-pathogen interaction in mango ginger and ginger. All the nine genes under study were induced in both pathogens under inoculated and un inoculated conditions. The present results suggest that defence related genes that are induced rapidly in response to pathogen invasion might help the mango ginger to limit infection by *R. solanacearum*. The present data paves a way to understand the molecular basis of the defence response to *R. solanacearum* in mango ginger. The expression profiles of the nine candidate genes forms a base to build a road map to the mechanism involved in the complex defence process.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.pmpp.2018.03.007.

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