Exploring expression patterns of PR-1, PR-2, PR-3, and PR-12 like genes in *Arabidopsis thaliana* upon *Alternaria brassicae* inoculation

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Abstract

In this study, we systematically examined the expression patterns of pathogenesis-related genes in model plant *Arabidopsis thaliana* after *Alternaria brassicae* inoculation using reverse transcription polymerase chain reaction (RT-PCR). Based on the results, none of the PR-1 and PR-2 like genes were induced significantly in the unwounded local or distal leaves upon *A. brassicae* challenge. However, only *At2g14580* of the PR-1 like gene showed a significant expression in wounded leaf after *Alternaria* challenge but not in control; confirming its expression in response to *A. brassicae* was aided by the wounding. Among PR-3 like genes, *At2g43590* showed local early expression and other PR-3 like genes showed significant distal expression after *A. brassicae* infection only in unwounded but not in wounded leaf samples. Although all the three PR-12 like genes were induced in local tissues, *At2g26020* was the only gene showed significant induction locally as well as systemically after pathogen infection in the both with and without wounding experiments. Therefore, among the PR-1, PR-2, PR-3 and PR-12 like genes studied, *At2g26020* can be the most promising candidate for the further line of research, viz, molecular characterization of its promoter to develop pathogen-inducible promoter in response to *Alternaria* and to develop fungus-resistant transgenics in *Brassica juncea*.

Keywords PR like genes · *Alternaria brassicae* · RT-PCR · Pathogen-inducible promoter

Introduction

The fungal pathogens are the most devastating among all the plant pathogens in the global agricultural sector. *Alternaria* leaf spot is one of the major fungal pathogens affecting the productivity of Indian mustard which is caused by the fungal pathogen *Alternaria brassicae*. The severity of occurrence of the disease decides the extent of losses and the reported yield losses in India ranged from 35 to 46% (Mishra et al. 2010) and up to 70% across the globe; one of the reasons

for the attributed losses might be due to the unavailability of hosts with genetic resistance to *Alternaria* (Vishwanath and Kolte 1997).

Husbandry strategies such as cultural practices and agrochemical application could decrease the fungal spread. Plant breeders are facing the difficulties to generate fungus-resistant varieties because of strong cross-incompatibility, polygenic background, and the differences in ploidy levels between cultivated and wild species of *Brassicaceae* family (Katiyar and Chopra 1990). Identification of stress-responsive defense gene(s) and molecular characterization of promoter regions of these genes are the crucial inputs for the development of transgenics for disease resistance. The transgenic approach is one of the potential upcoming technologies holding its promise to create biotic stress tolerant crop plants in the present scenario of changing climate (Grover and Pental 2003). Therefore, identification of suitable defense-related genes in response to *A. brassicae* is necessary.

Pathogenesis-related proteins are those which are produced as a result of plant–pathogen interactions during pathogenicity and are cysteine-rich, low molecular weight,

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and anti-microbial in their mode of action. These PR gene products not only impede pathogen progress but also helps in growth and metabolism of the host plant (Mehrotra and Aggarwal 2003). PR proteins accumulate locally in the infected and surrounding tissues and also in remote uninfected tissues (distal regions), systemically. Production of PR proteins in the uninfected plant's parts can prevent the affected plants from further infection (Delaney 1997).

PR proteins (Van Loon and Van Strien 1999) with an ability to inhibit fungal growth include PR-1 (Jayaraj and Punja 2007), PR-3 also called as chitinases (Swegle et al. 1992), PR-2 also termed as β -1, 3-glucanases, and PR-12 also denoted as plant defensins (Cammue et al. 1995). The resistance ability of plants in response to necrotrophic and biotrophic fungal pathogens is driven by the production of different sets of PR genes (Grover 2012). For instance, PR-3 (Chitinase) and PR-12 (Plant defensin) are known to impart resistance in response to necrotrophs, and are regulated by jasmonic acid (JA) and ethylene (ET) to activate induced systemic resistance (ISR). Whereas PR-1 and PR-2 (Glucanase) induction in plants provides them resistance against biotrophic fungi and are regulated by salicylic acid (SA) to activate systemic acquired resistance (SAR) (Ali et al. 2017a, b; Shah and Zeier 2013).

The *Arabidopsis*–*Alternaria brassicicola* pathosystem has been regarded as a model genetic system for incompatible plant–fungal interactions. *A. brassicicola* is the necrotrophic fungus; it feeds on dead cells and the symptoms produce in the form of lesions only at the place of inoculation with no further spread, indicates activation of incompatible reaction by plant-defense responses (Van Loon and Van Strien 1999; Mukherjee et al. 2009). The *A. brassicae* is more severe counterpart than *A. brassicicola* and can infect *Arabidopsis*. The defense genes induced under such conditions in response to *A. brassicae* would be excellent candidates to impart fungal resistance by genetic transformation and their over expression in the crop plants suffering from *Alternaria* blight (Grover and Gowthaman 2003).

Therefore, functional term search through Aramemnon plant membrane database was carried out with PR-1, Glucanase, Chitinase, and plant defensin in *Arabidopsis thaliana*. The microarray data of *Arabidopsis* from genevestigator pertaining to gene expression studies of these genes revealed that they respond to various biotic and abiotic stresses. Based upon this in silico information, three genes each from PR-1, glucanases, chitinases, and plant defensins encoding like genes were chosen to study the comparative expression of PR genes in *A. thaliana* upon *A. brassicae* challenge.

This investigation enlightens that, the genes encoding for putative pathogenesis-related proteins have an immense value for the fungus stress management. Chitinase (PR-3 like genes) and plant defensin (PR-12 like genes) encoding

like genes were induced both locally as well as systemically after *A. brassicae* infection indicating their role in induced systemic resistance (ISR), PAMP triggered immunity (PTI), and/or effector-triggered immunity (ETI) for broad-spectrum resistance. Plant defensin encoding like gene *At2g26020* was induced locally and systemically with high significance after *A. brassicae* inoculation with and without wounding experiments. Therefore, *At2g26020* was identified as a candidate gene for future functional validation.

Materials and methods

In silico analysis for the identification of biotic stress inducible PR genes

The PR genes encoding for PR-1, PR-2 (Glucanases), PR-3 (Chitinases), and PR-12 (Plant defensins) were searched from Aramemnon plant membrane database. Genevestigator microarray transcriptomic data of PR genes for biotic stresses especially fungal pathogens were studied to short list the genes for further RT-PCR. Based on microarray experimental data available in the genevestigator database, three genes from each category of PR genes, as mentioned in the Table 1, were utilized for gene expression analysis in response to *A. brassicae*. The protein properties like protein length, molecular weight, isoelectric point, and functional domains present in the PR proteins under study are presented in Table 2.

Designing and synthesis of PR and house keeping gene specific primers for RT-PCR

PCR primers were designed using the sequence information obtained from Aramemnon database by applying clone manager and primer-3 software's. α -tubulin the house keeping gene which was used in this study for normalization whose primers were designed from cDNA sequences of *Arabidopsis*. All the designed primers were synthesized and supplied by Sigma-Aldrich Company. The gene accession numbers of all the genes used in this study are listed in Table 1.

Alternaria isolation

Alternaria brassicae was isolated from the infected leaves of *B. juncea* variety Varuna with clear symptoms of infection brought from IARI, New Delhi, mustard field with I.D. No. 81651; has been given by Indian-type culture collection, IARI, New Delhi (Nayanakantha et al. 2016; Ali et al. 2017c). The culture was grown on PDA and then sub cultured on radish dextrose agar (RDA) medium to obtain pure culture (Thakur and Kolte 1985) in a growth chamber with incubation at 22 °C and 16/8-h light/dark periods. 25-day

Table 1 List of PR genes/house keeping gene primers used for RT-PCR

Gene name	Accession number	Sequence	A. T (°C)	bp
PR-1-like genes	<i>At2g14580</i>	Sense-5' TGCAGCCTACGCTCGGAACCTAC 3'	55	127
		Antisense-5' GTTCACGGCAGCAACACCAGAC 3'		
	<i>At4g33710</i>	Sense-5' CGATACGCCTGGAACCTATGC 3'	55	145
		Antisense-5' TCTCCCTGACCCACAATCTC 3'		
	<i>At4g07820</i>	Sense-5' CTCTGAAAGCCCAAGACC 3'	55	224
		Antisense-5' GGTGCTCACAACTCCTC 3'		
PR-2-like genes (Glucanase)	<i>At4g39000</i>	Sense-5' TCTCTTGACCATGCCCCAAC 3'	53	291
		Antisense-5' TGAACACCGCGCAACTTGAC 3'		
	<i>At2g32990</i>	Sense-5' CGATACGCCTGGAACCTATGC 3'	55	263
		Antisense-5' TCTCCCTGACCCACAATCTC 3'		
	<i>At4g09740</i>	Sense-5' TTCCGAAGCCGCAGGTGAAG 3'	58	179
		Antisense-5' AGCCTGAGTGTGAGCAGTAG 3'		
PR-3-like genes (Chitinase)	<i>At3g12500</i>	Sense-5' AATGATGCCGCTTGTCTTG 3'	60	522
		Antisense-5' TCCATAACCCGGTAATCTCCC 3'		
	<i>At3g47540</i>	Sense-5' GCGCTGCCGAATCAGTCAGTAG 3'	58	431
		Antisense-5' TTGGTTTCAGCACCGGCCTTACG 3'		
	<i>At2g43590</i>	Sense-5' GATGCCGATCAGGTCCTTG 3'	58	500
		Antisense-5' GTTCAGAACCGGCCTTACG 3'		
PR-12-like genes (Plant defensins)	<i>At2g26020</i>	Sense-5' TCTACGCTGCTCTTGTCTC 3'	55	183
		Antisense-5' GAGCTGGGAAGACATAGTTG 3'		
	<i>At5g44420</i>	Sense-5' TCTACGCTGCTCTTGTCTC 3'	57	165
		Antisense-5' GATCCATGTTTGGCTCCTTC 3'		
	<i>At5g44430</i>	Sense-5' TGGCTAAGTCTGCTACCATC 3'	55	213
		Antisense-5' GAGCTGGGAAGACATAGTTG 3'		
α -Tubulin	NM_100360.3	Sense 5'- CGTGCTGTCTTTGTTGATCTTG-3' Antisense 5'- TGAAGTGGATTCTTGGGTATGG-3'	53	613

bp RT-PCR product size in base pairs, A.T annealing temperature

post-inoculation mycelium was scraped and suspended in sterilized distilled water and centrifuged at 4000 rpm for 4 min. The supernatant with *Alternaria* spores was re-suspended in 0.02% tween-20 surfactant solution to prepare conidial suspension and the spore concentration of 5×10^3 conidia/ml was adjusted with the help of haemocytometer for pathogenicity tests with *A. thaliana* (Chandrashekar et al. 2015; Nayanakantha et al. 2016).

Plant material

Arabidopsis thaliana plants are grown to the 6–8 leaf stage for 30–35 days after sowing (DAS) before they were subjected for pathogenicity tests in vivo at National phytotron facility (NPF) IARI, New Delhi.

Pathogenicity treatments

Pots with 2–3 plants were kept in growth chamber (BOD) having set temperature of 21 °C, 16 h/8h light/dark cycle and light intensity of 12.5 $\mu\text{mol}/\text{m}^2/\text{s}$. After acclimatizing for 3-day plants were sprayed with autoclaved water and covered with transparent polythene bags to maintain higher humidity (Fig. 1d). Pathogenicity tests were conducted by placing 5 μl droplets (2–3 drops) of *A. brassicae* conidial suspension prepared with 0.02% tween 20 surfactant onto the upper surface of the third and fourth true leaves. Control plants were treated with 0.02% tween 20 single-distilled water (SDW) by placing 5 μl droplets on the leaves. After inoculation, plants were again covered with polythene bags to have internal higher humidity (Fig. 1d). They were placed in BOD at 22 °C under dark for 20 h and then provided with 16 h light (22 °C)/8-h-dark (22 °C) cycle. *Alternaria* infections were conducted with and without wounding treatments before infecting with the pathogen. Wounding treatment was given by gentle swabbing with fine-powdered glass beads to eliminate waxy

Table 2 Length (amino acid), molecular weight, isoelectric point, and functional domains of the PR proteins

Protein ID	Protein description	Protein length (aa)	Molecular weight (kDa)	Isoelectric point (pI)	Functional domain/s present
At2g14580	Putative PR-1-like protein	161	17.5	8.91	Cysteine-rich secretory protein family
At4g33710	Putative PR-1-like protein	166	19	9.86	
At4g07820	Putative PR-1-like protein	160	17.7	8.19	
At4g39000	Putative endo-1,4-beta glucanase (AtGH9B17)	493	54.7	7.15	Glycosyl hydrolase family 9
At2g32990	putative endo-1,4-beta glucanase (AtGH9B8)	525	59.1	5.64	
At4g09740	Putative endo-1,4-beta glucanase (AtGH9B14)	478	52.5	6.01	
At3g12500	Basic endochitinase, involved in ethylene/jasmonic acid-mediated signaling pathway (AtHCHIB/AtPR-3)	335	36.2	7.81	Chitin recognition protein and Chitinase class I
At3g47540	Putative class-IV chitinase-like protein	214	23.3	9.32	
At2g43590	Putative class-IV chitinase-like protein	264	28.4	8.43	
At2g26020	Putative plant defensin-like protein (AtPDF1.2b)	80	8.6	8.13	Gamma thionin family
At5g44420	Putative plant defensin-like protein (AtPDF1.2a/AtLCR77)	80	8.5	8.15	
At5g44430	Putative plant defensin-like protein (AtPDF1.2c)	80	8.6	8.15	

Source: Aramemnon plant membrane protein database (<http://aramemnon.botanik.uni-koeln.de>)

Fig. 1 Process of *A. brassicae* inoculation. **a** *A. brassicae* culture on root radish media. **b** Homogenised spore suspension ready for inoculation. **c** Experimental setup of pathogenicity tests. **d** Arabidopsis plants under humidity chamber. **e** Controls of without and with

wounded treatments. **f** *Alternaria* treatments of without wounding plants. **g** *Alternaria* treatments of with wounding plants. **h** Symptoms on *Alternaria* treated plants

layer to ease for the fungal infection (Chandrashekar et al. 2015; Nayanakantha et al. 2016).

Sample collection

Leaf samples were collected from control and *Alternaria* challenged plants for RNA isolation. Samples from pathogen inoculated plants were collected after 6-, 12-, 24-, 48-, 72-, and 96-h post-inoculation (hpi). Leaf samples were harvested from both local and distal leaves to study genes expression profiles both spatially and temporally. All the leaf samples harvested were immediately frozen in liquid nitrogen and preserved at -80°C for isolating total RNA.

RNA isolation for RT-PCR

Extraction of total RNA was performed for the harvested leaf samples from with and without wounding pathogenicity tests using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The isolated total RNA was run on formaldehyde agarose gel to check its integrity and further it was quantified with the help of NanoDrop 1,000 (NanoDrop Technologies, Inc., DE, USA). 1 μg of total RNA was taken for first-strand cDNA synthesis using Oligo (dT) primer (Thermo Scientific, USA) according to the manufacturer's instructions and cDNA was quantified as above-mentioned. The cDNAs were diluted to a working concentration of 100 ng/ μl for conducting RT-PCR with the chosen PR genes.

Thermal conditions followed for RT-PCR

Reverse transcriptase polymerase chain reaction was performed using a thermocycler (Applied Biosystems) in 25- μl final volume. PCR steps included an initial denaturation at 94°C for 4 min, followed by 30 cycles of 94°C for 30 s, 53 – 60°C (Annealing temperature varied for different genes as mentioned in Table 1) for 30 s, and 72°C for 1 min with a final extension at 72°C for 10 min.

Results and Discussion

Pathogenicity treatments in response to *A. brassicae* challenge

Without prior wounding

Arabidopsis plants were grown at National phytotron facility and they were inoculated with *A. brassicae* in vivo. Symptoms appeared after 3-day post-inoculation (dpi) only in the lower leaves but not in upper younger leaves. The spreading of symptom was almost limited at the site of inoculation

without any further increase in the diameter of the lesion. The leaf margin showed clear symptoms as compared with basal regions (Chandrashekar et al. 2015).

With prior wounding

The symptoms appeared as early as 24 hpi as compared to the without prior wounding. There were clear symptoms both at the leaf margin as well as in basal portions. The susceptibility of leaf margin over leaf base persisted in these experiments as like without wounding pathogenicity tests.

PR gene expression analysis in response to *A. brassicae* challenge

All the chosen PR proteins encoding like genes were analyzed for their expression patterns both locally as well as systemically in response to *A. brassicae* at different time intervals through RT-PCR. The results pertaining to their expression profiles analyzed under different experiments are discussed below.

Local and systemic expression analysis of PR-1, PR-2, PR-3, and PR-12 encoding like genes under without wounding experiments

PR-1 and PR-2 (β -1, 3 glucanase) genes

PR-1 encoding like genes, viz, *At2g14580*, *At4g07820*, and *At4g33710*, and PR-2 encoding like genes, viz, *At2g32990*, *At4g09740*, and *At4g39000*, were analyzed for the *Alternaria* induction from the *in vivo* inoculated *A. thaliana* plants across the time intervals, 6–96 hpi. Among PR-1 genes, *At4g07820* showed very little local expression at 6 hpi and systemic expression at 6, 48, and 96 hpi, but none of the PR-2 genes were induced locally and/or systemically (Fig. 2a, b). It indicates that PR-1 and PR-2 proteins encoding like genes might not be the candidate genes for necrotrophic fungal pathogens and their induction has been known to be mediated through systemic acquired resistance (SAR). SAR is mediated by salicylic acid (SA) where PR-1 and PR-2 genes are popularly known as marker genes for SA-dependent signaling pathways in *Arabidopsis* and tobacco (Durrant and Dong 2004). Narusaka et al. (2015) proved that increased transcript accumulation of PR1, PR2, and PR5 genes indicates the activation of SA pathway and increased transcript accumulation of PR3 and PR4 (endo-chitinases) genes indicates the activation of JA pathway. Upon pre-treatment of *B. juncea* with stimulators of SA and JA; there was an antagonistic relationship between SA and JA as there was downregulation of each other signature genes of SA and JA pathways (Ali et al. 2017d). Therefore, these results support the concept of SAR which is effective against biotrophic

Fig. 2 Time course transcript accumulation of PR-1 and glucanase (PR-2 like) genes in local and distal leaves upon *A. brassicae* inoculation without prior wounding of *Arabidopsis* plants in vivo. **a** PR-1 genes and **b** β -1, 3-glucanase genes (PR-2 genes). RT-PCR was per-

formed for each cDNA made from each RNA samples and α -Tubulin transcripts were used to normalize the sample and 30 PCR cycles were performed

pathogens (Pieterse et al. 2009). However, Ali et al. (2017c), reported that the *BjPR2* (*B. juncea* PR-2 gene) was induced rapidly in response to *A. brassicae*, salicylic acid application, and wounding, but not the jasmonic acid application in *B. juncea* which it accounts to an existence of cross-talks in their expressions. Therefore, *A. thaliana* PR-1 and PR-2-like genes studied after without wounded experiments might not be involved in the defense mechanisms against *A. brassicae*. Therefore, these PR-1 and PR-2 like genes cannot be called as candidates for further applications such as for the development of transgenics and or characterization of their promoter regions in response to fungal infections.

PR-3 (Chitinase) and PR-12 (Plant defensins) genes

PR-3 encoding like genes *Viz*; *At2g43590*, *At3g12500*, *At3g47540* and PR-12 encoding like genes *Viz*; *At2g26020*, *At5g44420*, and *At5g44430* were chosen for expression analysis in response to *Alternaria* challenge across different time intervals. The local expression analysis reveals that *At2g43590* of PR-3 displayed significant early induction after 6 hpi and also 24 hpi (Fig. 3a), but its expression seems to be seldom decreased after 24 hpi. However, *At3g47540* showed very little induction and *At3g12500* had no induction upon pathogen infection and all the three

chitinase genes showed very good induction in distal leaves. This indicates some kind of signal, not yet identified is definitely moving from the place of infection to the distal leaves. All the three genes in systemic induction experiments had a peculiar dip in expression at 72 hpi; it could be because of some diurnal effects. Since *A. brassicae* is the necrotrophic fungus and its resistance in plants is mediated through ISR, inducing other sets of defense genes such as plant defensins and chitinases (Thomma et al. 2002). Therefore, after comparing the results obtained from local and distal expression of PR-3 genes, *At2g43590* and *At3g47540* can be considered as the candidate genes for the development of transgenics for broad-spectrum resistance against necrotrophic fungi because of their local and systemic expression patterns. In addition, the promoter regions of these genes can be characterized to identify *cis*-regulatory elements specifically for necrotrophic fungi and, in turn, can be candidates for the identification of transcription factors interacting with these *cis*-elements. The defense pathways of necrotrophic fungi include signals produced and controlled by the jasmonic acid and ethylene, generally called as induced systemic resistance (ISR). The resistance through hormonal regulation is one of the effective means in the plant system for their survival. When the pathogen infects the plant, other parts of the plant get signal and it induces the products of PR genes one

Fig. 3 Time course transcript accumulation of PR-3 and PR-12 like genes in local and distal leaves upon *A. brassicae* inoculation without prior wounding of *Arabidopsis* plants in vivo. **a** Chitinases genes (PR-3 like genes) and **b** plant defensin genes (PR-12 like genes). RT-

PCR was performed for each cDNA made from each RNA samples and α -Tubulin transcripts were used to normalize the sample and 30 PCR cycles were performed

such gene is plant defensin. Among plant defensin genes, *At5g44430* exerted constitutive pattern of expression profile in the local infected leaves of without wounded samples, but expression was not significant in distal leaves. The other two genes *At5g44420* and *At2g26020* were induced significantly early after 6 hpi and continued to be induced highly up to 24 hpi and thereafter showed reduced levels of expression. Even though *At5g44420* and *At5g44430* induced systemically but not to the level of *At2g26020* (Fig. 3b). *At2g26020*, induction was increased as the time interval after *Alternaria* increased when compared with other two genes. It indicates that, even though *At5g44420* has been regarded as a marker gene in the jasmonic acid pathway, *At2g26020* showed better expression in distal regions (Thomma et al. 2000). *Bj*-defensin was induced significantly by *A. brassicae* challenge, jasmonic acid and wounding inoculation but not by salicylic acid (Rawat et al. 2017). Therefore *At2g26020* would increase its protein product in the distal regions of the plant in response to *Alternaria*. The accumulation of such products could give plant resistance for better survival under such pathological conditions. *At2g26020* would also be an excellent candidate for promoter characterization. The upstream region of this gene might be having significant *cis*-elements for pathogen inducibility. Characterization of such promoter

is essential for the development of fungus-resistant transgenics by utilizing plant-derived, pathogen-inducible promoter instead of using 35S promoter to drive the expression of the candidate genes.

Wounding aided influence of *Alternaria* challenge over the expression patterns of PR-1, PR-2, PR-3, and PR-12 encoding genes

Among PR-1 genes *At2g14580* induced after infection but not in the control samples of prior wounding, indicating that this gene expression has been aided by wounding (Fig. 4a). Since *Alternaria* did not induce this gene in the without wounding experiment, which means that wounding has aided the expression of this gene during infection process of *Alternaria*. Therefore PR-1 encoding gene that is *At2g14580* can be considered for defense signaling studies further to understand the biology of necrotrophs in combination with other stress signals that the plant come across. On the other hand, *At4g07820* showed their expression both in the treated as well as in the control samples indicating, these genes are wound inducible and *Alternaria* does not add to further induction (Fig. 4a). Like that of without wounding experiments, the glucanase

Fig. 4 Time course transcript accumulation of PR-1 and PR-2 like genes in *Alternaria* inoculated and corresponding control leaves of with wounded *Arabidopsis* plants in vivo. **a** PR-1 like genes. **b** β -1, 3-glucanase genes (PR-2 like genes). RT-PCR was performed for

each cDNA made from each RNA samples and α -Tubulin transcripts were used to normalize the sample and 30 PCR cycles were performed

Fig. 5 Transcript accumulation of PR-3- and PR-12-like genes at different time intervals from *Alternaria* inoculated and corresponding control leaves of with wounded *Arabidopsis* plants in vivo. **a** Chitinase genes (PR-3 like genes) and **b** Plant defensin genes (PR-12

like genes). RT-PCR was performed for each cDNA made from each RNA samples and α -Tubulin transcripts were used to normalize the sample and 30 PCR cycles were performed

(PR-2) genes could not induce in the control as well as in the treated leaves. To cancel the doubts of PCR functionality and other PCR-related problems, genomic DNA was used to keep positive control (Fig. 4b). As can be seen in Fig. 5a, PR 3 encoding like gene *At2g43590* was induced to the same level by wounding alone (control) or *Alternaria* infection after wounding suggesting that *Alternaria* does not induce this gene over and above wounding, whereas *At3g12500* was induced slightly at 72 hpi than wounding alone and the results of *At3g47540* are not significant. Plant defensin encoding like genes *At5g44420* and *At5g44430* were equally induced after wounding alone and also *Alternaria* infection after wounding. Again, it means that *Alternaria* does not induce these genes over and above wounding. However, *At2g26020* displayed additional induction in the *Alternaria* treated samples with prior wounding as compared to wounding alone (Fig. 5b).

Conclusion

The PR genes under this study showed differential expression patterns both spatially and temporally during the course of pathogenecity tests. Therefore, these genes can be explored for their utilization in the future molecular biology applications in crop improvement programmes. For instance, *At2g14580* of PR-1 is the only gene showed a significant gene induction out of PR-1 and PR-2 like genes studied in response to *Alternaria* challenge; since its expression was aided by prior wounding; it can be the potential candidate gene for the molecular characterization of pathogen-inducible promoters. Generation of such pathogen-inducible promoters would be highly effective under combined stresses such as when plants are infested with both insects and pathogens. Development of transgenics for fungal pathogens using such genes will be more promising under such circumstances for the effective management of fungal pathogens. PR-3 genes were induced systemically but not locally and they can be candidates for broad-spectrum resistance, *viz.*, induced systemic resistance. Plant defensin encoding genes *At2g26020*, *At5g44430*, and *At5g44420* were showed significant local expression; therefore, they can be utilized for the development of transgenics for PTI and/or ETI. However, for the isolation and molecular characterization of pathogen inducible promoter, *At2g26020* would be the excellent candidate, since it is induced locally as well as systemically as compared to *At5g44430* and *At5g44420*. Therefore *At2g26020* is an ideal and potential candidate gene to isolate pathogen-inducible promoter, which can be utilized further to drive the expression of biotic stress response genes in transgenic plants.

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Author contributions NC planned and conducted experimental work, SA helped in drafting the manuscript, and AG has designed the work and approved the final manuscript.

Compliance with ethical standards

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

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