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Differential expression of PR genes in response to *Phytophthora capsici* inoculation in resistant and susceptible black pepper (*Piper nigrum* L.) lines

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Abstract Differential expression of three important genes encoding pathogenesis related proteins (PR proteins) viz. β-1,3-glucanase (*PR-2*), osmotin (*PR-5*) and cytosolic ascorbate peroxidase (cAPX*, PR-9*) were carried out in *Phytophthora* susceptible (Sreekara) and resistant (04-P24) black pepper lines compared to uninoculated plants using quantitative reverse transcription PCR (qRT-PCR). Upon *Phytophthora capsici* inoculation, expression of these three genes were either up-regulated or down-regulated. In the susceptible line, all three genes were expressed maximally on 1 day after inoculation (DAI) and thereafter the expression declined. In the resistant line, a steady increase in the expression pattern of genes was noticed during the course of infection. Highest expression levels of cAPX were noticed on 3 DAI and that of β-1, 3-glucanase and osmotin genes were maximum on 5 DAI. Soil inoculation of *P. capsici* affected the transcriptional activity of these genes in stem tissue also, indicating systemic defense response against the pathogen.

Highlights
• The study was carried out in *P. capsici* susceptible (Sreekara) and resistant (04-P24, shows root resistance to the pathogen) black pepper lines.
• Differential expression of 3 PR gene - β-1,3-glucanase (*PR–2*), osmotin (*PR-5*) and cAPX (*PR-9*)- in response to *P. capsici* inoculation was studied here and cAPX gene was found to play vital role in *P. capsici* resistance in black pepper.
• In our previous paper published in the journal PMPP (Vandana et al. 2014), we elucidated the role of peroxidase in root resistance to *P. capsici*.
The above result was confirmed in this study revealing the role of cAPX in defense response to *P. capsici*.

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Introduction

Once a pathogen establishes a successful contact with the plant, elicitors from the pathogen induce secondary defense responses like reinforcement of cell walls, production of phytoalexins and synthesis of PR proteins (Slusarenko et al. 2000). PR proteins are produced in plants and are induced as part of systemic acquired resistance (Sels et al. 2008). Beta-1,3-glucanase, osmotin and cytosolic ascorbate peroxidase (cAPX) are some of the important PR proteins in plants. Fungal elicitors released from pathogen cell walls induce β-1,3-glucanase in various parts of plant which cleaves the β-1,3-glucans on the cell wall of fungi and oomycetes. The

role of β-1,3-glucanase in defense response was elucidated in the interactions of different pathosystems, such as wheat – *Fusarium graminearum* (Li et al. 2001), in peach - *Monilinia fructicola* (Zemanek et al. 2002), in taro - *Phytophthora colocasiae* (Misra et al. 2008) and in black pepper – *P. capsici* (Vandana et al. 2014). Osmotin is a differentially expressed, stress responsive antifungal protein belonging to the PR-5 family. It is a developmentally regulated protein that protects the cells from invading fungal pathogens by causing inhibition of hyphal growth and spore germination (Louis and Roy 2010). Osmotin inhibits the germination and growth of *Phytophthora infestans* (Woloshuk et al. 1991). In vitro, it inhibits spore germination and hyphal growth of many phytopathogenic fungi including *F. oxysporum, Alternaria solani, Botrytis cinerea, Verticillium dahliae, Phytophthora nicotianae* and *Cercospora beticola* with delayed disease symptoms (Woloshuk et al. 1991; Huynh et al. 1992; Malehorn et al. 1994; Abad et al. 1996; Thompson et al. 2006). PR-9 is a specific type of peroxidase that helps in cell wall reinforcement by catalyzing lignification (Passardi et al. 2005) and enhances resistance against multiple pathogens (Passardi et al. 2004). Peroxidase activity is associated with disease resistance in plants and increases in host plants following pathogen infection. Higher peroxidase activity was noticed in tomato in response to *Rizoctonia solani* (Taheri and Tarighi 2011), in sugarcane upon *Colletotrichum falcatum* invasion (Ramesh Sundar et al. 1998), in wheat infected with *F. graminearum* (Mohammadi and Kazemi 2002), in taro upon inoculation with *P. colocasia* (Misra et al. 2008), in chilli - *P. capsici* interaction, chilli – *Xanthomonas campestris,* chilli - *Glomus intraradices* interactions (Hu-zhe et al. 2005; Wang et al. 2013), in black pepper upon inoculation with *P. capsici* (Vandana et al. 2014) and in apricot upon inoculation with *Hendersonula toruloidea and Phiaoacremonium aleophillium* (Al-Morad 2013).

In India and the world over, foot rot caused by *Phytophthora capsici* is one of the most serious threats in black pepper (*Piper nigrum* L.) cultivation. Crop loss due to this disease has been identified as a major constraint in black pepper production. The disease can be brought under check by the applications of chemical fungicides when diagnosed at the early stage of infection. But in most cases, the symptoms of the disease appear unnoticed until it reaches the advanced stages of infection, when the disease becomes beyond the level of control. Biological control of *P. capsici* using antagonists is also reported. *Trichoderma* has been reported antagonistic to *P. capsici* (Anandaraj and Sarma 1994; Anandaraj and Peter 1996). Aravind (2009) observed the biocontrol efficiency of black pepper endophytic bacteria *Pseudomonas aeruginosa*, *P. putida* and *Bacillus megaterium* against *Phytophthora* foot rot in black pepper. Biocontrol potential of selected black pepper endophytic fungi was reported by Sreeja et al. (2016). Recently, actinomycetes were also exploited for the biological control of foot rot disease (Bhai et al. 2016). Developing disease resistant varieties is one of the best available solutions for managing the disease. Several attempts have been made to identify resistant sources against the disease. After series of screening tests, an open pollinated (OP) progeny 04-P24 from a moderately resistant line IISR Shakthi was identified as resistant to *Phytophthora* root infection by all means of screening (Bhai et al. 2010). The mechanism of resistance in this OP progeny is being studied using biochemical and molecular means.

The role of biochemical defense parameters such as membrane conductivity, ortho dihydroxy (OD) phenols, lignin and peroxidase in 04-P24 root resistance to *P. capsici* has already been studied in our previous experiments (Vandana et al. 2014). The objective of the present study is to elucidate the role of PR proteins in defense by studying the differential expression of three important PR genes namely β-1,3-glucanase (*PR-2*), osmotin (*PR-5*) and cAPX (*PR-9*) in *Phytophthora* resistant black pepper line 04-P24 in comparison with its susceptible counterpart Sreekara upon inoculation with *P. capsici*.

Materials and methods

Plant material and pathogen inoculation

P. capsici susceptible (Sreekara) and resistant (04-P24, OP progeny of IISR Shakthi) lines of black pepper (*P. nigrum* L.) were used in this study. The plants were multiplied using serpentine propagation method (Thankamani 2008) using sterilized porting mixture composed by soil: sand: farm yard manure (2:2:1). Single node cuttings were grown and maintained in sterile porting mixture in polythene bags of size 20 × 10 cm under green house conditions. Plants of 4–5 leaf stage were selected for studying the gene expression profile. The *P. capsici* isolate 06–04 maintained in

National Repository of *Phytophthora*, ICAR-IISR, Kozhikode, Kerala, India was used for inoculation. The isolate was sub-cultured and maintained in carrot agar (CA) medium (Griffin 1977).

For inoculation, *P. capsici* was grown on CA for 72 h at 24 ± 1 °C. Inoculum plugs of 5 mm size were cut from the periphery of the actively growing culture and kept for sporulation under continuous fluorescent light for 48 h at 24 ± 1 °C. Five sporulating discs were used for soil inoculation of each plant (Bhai et al. 2010). These sporulating discs were placed on the root surface of each intact plant grown in the polythene bags after digging the soil near the root system without injuring the root and then the inoculated root system was covered with soil. Uninoculated plants served as control. Three biological replications were maintained.

Sampling

From the inoculated plants, root and stem samples were drawn from 1 to 5 days after inoculation (DAI) at 24 h interval and used for gene expression study. During each sampling, plants were uprooted and observed for symptom development on the root and stem using a microscope. Analysis was carried out in triplicates on three biological replications.

RNA isolation and cDNA synthesis

The roots and stems of *P. capsici* inoculated Sreekara and 04-P24 were collected at 1, 2, 3, 4 and 5 DAI and total RNA was isolated using Trizol® reagent (Invitrogen, USA) according to the manufacturer instructions. The purity of total RNA was assessed using the A260/280 and A260/230 ratios determined in biophotometer (Eppendorf, Germany). RNA quality was assessed by electrophoresis in 1% agarose gel. To remove contaminating genomic DNA in RNA preparations, 1 μg of total RNA was treated with one unit of RNase-free DNase I (Fermentas, USA) at 37 °C for 30 min.

First strand cDNA synthesis was done using Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

1 μg of DNase-treated total RNA was converted into cDNA using oligo (dT) 18 primer (Fermentas, USA) and Revertaid reverse transcriptase (Fermentas, USA). The reaction mixture containing 1 μl each of oligo (dT) 18 primer, dNTP mix (10 mM) and 1 μg RNA (DNase treated) was incubated at 65 °C for 5 min. To this, 4 μl RT buffer, 0.5 μl Ribolock RNase inhibitor (Fermentas, USA) and 1 μl Revertaid reverse transcriptase were added and made up the volume to 20 μl using DEPC treated water (Fermentas, USA). RNA was reverse transcribed to cDNA at 42 °C for 60 min and at the end of the reaction, the enzyme denaturation was done at 72 °C for 10 min. The product (cDNA) was used for PCR amplification.

Primer design

Gene-specific primers of cAPX and β-1,3-glucanase were designed from the EST sequence available in the GenBank database, Genbank ID: HQ207685.1 and Genbank ID: AY683478.1 respectively (Table 1). All primers were designed using Primer3Plus software (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and tested for their specificity by running PCR using cDNAs as templates and the products were run on agarose gels in which they gave single bands for each gene - β-1,3-glucanase (~ 200 bp), osmotin (~ 200 bp) and cAPX (~ 160 bp). On the basis of these analyses, the primers were judged to be specific for its target cDNA. Primers used for amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Umadevi et al. 2015) and osmotin (Johnson George et al. 2012) are also listed in the Table 1.

qRT-PCR specific primers for β-1,3-glucanase, osmotin and cAPX were first tested with regular PCR using cDNA as a template in order to determine gene-specific amplification. The PCR fragments were amplified and confirmed by running on an agarose gel in which they gave single bands - β-1,3-glucanase and osmotin around 200 bp and cAPX around 160 bp length. The primers were then used in qRT-PCR analyses to determine the gene expression profiles of these genes in root and stem of *Phytophthora* susceptible and resistant black pepper lines both under uninoculated and inoculated conditions. Black pepper GAPDH gene was used as a constitutive control in all gene expression experiments. Expression levels of these genes were measured relative to GAPDH expression.

PCR efficiencies of primers were determined with standard curves generated from 5-fold dilution series of purified PCR fragments as templates. Amplification efficiencies for each gene and coefficients of determination (r^2) were calculated (Table 1). The specificity of PCR amplification of each primer pair was confirmed by sequencing of PCR products and by melt curve

Table 1 qRT-PCR primers used in the study of differential expression of PR genes

Target gene	Primer sequence (5′–3′)	Annealing Temperature (°C)	Efficiency	r^2
GAPDH	F = ATGAAGGATTGGCGAGGTGG R = AGGCCATTCCAGTGAGCTTC	68.3 65.6	1.03 (103%)	0.99
β-1,3-glucanase	F = CCGGCCTACAGAATGACATC R = GGGTAGACGTTGGCTAGGAG	64.4 62.6	0.99 (99%)	0.99
Osmotin	F = ACCGTGTTTAAGACCGACCA R = ACCATTTCATGGGCAAAAGA	64.3 64.1	0.99 (99%)	0.99
cAPX	F = GAACTCAGGCCGTCCTCAAC R = GACCTTCTCGCCGGAATTCA	66.1 68.5	0.99 (99%)	0.97

F: forward primer

R: reverse primer

analysis during qRT-PCR. PCR efficiency of all genes used in this study – including target and reference genes – was also tested by generating standard curves. All the target genes showed efficiency above 98% (99% for β-1,3-glucanase, osmotin and cAPX genes and 103% for GAPDH) and the r^2 values varied from 0.97 to 0.99 (Table 1). The dissociation curves showed single and sharp peaks for all samples amplified, confirming that only one specific gene has been amplified. No peaks were generated for Non Template Control (NTC), samples which lacked DNA template.

Gene expression analysis by qRT-PCR

qRT-PCR analysis was performed using gene-specific primers for each cDNA (Table 1). qRT-PCR components and program are given in Tables 2 and 3. GAPDH was used as reference gene for black pepper (Umadevi et al. 2015). The comparative CT method (2^{-DDCT}) was used to analyze the relative expression of the target genes according to Livak and Schmittgen (2001). All qRT-PCR experiments were run with cDNA synthesized from three biological replicates. Uninoculated plants served as controls. Each sample was run in three technical replicates along with no RT and non template controls, on a 72-well plate.

The qRT-PCR products for each gene of interest were run on 1% agarose gel, purified using GenElute Gel Extraction kit (Sigma, USA) following manufacturer's procedure, the amplicons were sequenced and compared with the sequence for the target gene using BLASTN.

Statistical analysis

Data were analyzed for the significant differences by analysis of variance (ANOVA) with the statistical package SAS software (Version 9.3) and difference between means of treatments was determined using the least significant difference (LSD) test, $p < 0.05$.

Results

The root and stem samples were collected until 5 DAI at 24 h interval since gene expression reaction is initiated during earlier hours of infection. No symptom develop-

Table 2 qRT-PCR components

Reagent	Reagent stock conc.	Required conc.	Volume for one reaction (20 μl)
QuantiFast SYBR green PCR Master Mix			10 μl
PCR forward primer	100 μM	10 μM	1 μl
PCR reverse primer	100 μM	10 μM	1 μl
Nuclease free water			3 μl
cDNA		10×	5 μl

Table 3 qRT-PCR program

Cycle	Cycle point
Hold: 94 °C, 5 min	
Cycling: 35 repeats	Step 1: 94 °C, hold 30 s Step 2: 60 °C, hold 30 s, (acquiring to cycling A)
Melt: 62–99 °C, hold 1 s on the 1st step, hold 5 s on next step.	

ment was noticed on root of resistant plants whereas in susceptible plants, at 5 DAI, the feeder roots started showing foot rot symptom at the root tip upwards (Fig. 1).

Gene expression analysis by qRT-PCR

qRT-PCR specific primers for β-1,3-glucanase, osmotin and cAPX were tested with regular PCR using cDNA as a template in order to determine gene-specific amplification. The PCR fragments were amplified and confirmed by running on an agarose gel in which they gave single bands (Fig. 2).

β-1,3-glucanase gene expression in root and stem in response to *P. capsici* inoculation

In both root and stem of the susceptible and resistant lines, change in expression levels of β-1,3-glucanase gene was noticed compared to the uninoculated plants. In the roots of susceptible line, the mRNA transcriptional level was 4.6 fold higher on 1 DAI and 4.0 fold higher on 2 DAI under pathogen inoculated conditions. There onwards the expression level dropped until 5 DAI. In the resistant line, β-1,3-glucanase expression was low from 1 DAI up to 4 DAI although a two-fold increase in expression was observed at 4 DAI. β −1,3-glucanase expression was highest at 5 DAI with a 7.9-fold increase observed in line 04-P24. Moreover the gene expression was lower than that of uninoculated plants on 2 and 3 DAI (Fig. 3a).

In stems of the susceptible line, a higher level of transcription of the gene was noticed on 1 (~2.5 fold), 4 (~3.4 fold) and 5 DAI (~2.2 fold). In the resistant line, a higher level of β-1,3-glucanase was noticed only on 2 DAI (~5.3 fold) and on the remaining days, the gene expression level remained lower than that of the uninoculated plants (Fig. 3b).

Osmotin gene expression in root and stem in response to *P. capsici* inoculation

Highest relative expression of the osmotin gene in roots could be seen on 1 DAI in susceptible line and on 5 DAI in the resistant line – 3.5 and 7.5 folds respectively. In the susceptible line, osmotin gene transcription decreased from 1 to 5 DAI giving lowest level of expression on 5 DAI (lower than the expression level noticed in uninoculated plants). In the resistant line, osmotin gene expression showed 2.5 and 7.3 folds higher expression on 4 and 5 DAI respectively (Fig. 4a).

In the stem of susceptible line, a gradual increase in expression was noticed from 1 to 4 DAI and thereafter there is a sudden fall. A 3.6 fold higher expression of osmotin gene was found on 4 DAI. In the resistant line, higher expression level was noticed (~2.8 fold) at 2 DAI only (Fig. 4b).

Fig. 1 Root of black pepper lines 04-P24 (**a**) and Sreekara (**b**) showing development of foot rot symptom upon *P. capsici* inoculation, on 5 DAI. Rot symptoms were not developed on the root of 04-P24

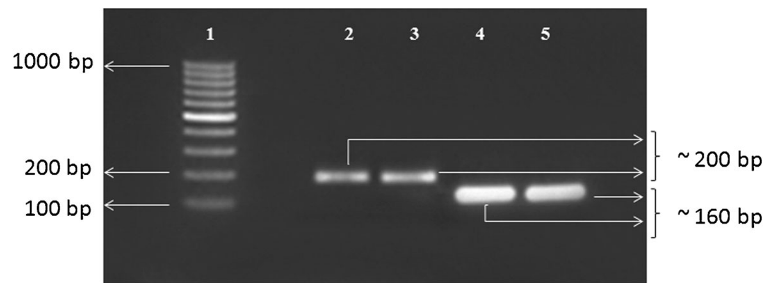


Fig. 2 Agarose gel showing DNA bands, DNA ladder -1Kb (lane 1), β-1,3-glucanase (lane 2), osmotin (lane 3), GAPDH (lane 4) and cAPX (lane 5)

cAPX gene expression in root and stem in response to *P. capsici* inoculation

cAPX gene expression showed drastic differences in susceptible and resistant plants. In roots of susceptible line, cAPX gene expression increased 12.2 fold on 1 DAI and thereafter a gradual decrease was noticed except on 4 DAI (~5.5 fold). However in the resistant line, cAPX mRNA level increased gradually until 3 DAI in which highest expression was seen on 2 and 3 DAI and then the expression suddenly declined (Fig. 5a).

In the stem, gene expression was very much higher in the susceptible line on 3 DAI where expression was ~5.5 fold higher in pathogen inoculated roots. On all other days, cAPX gene expression remained lower than that of control plants. In the resistant line, throughout the experiment the gene transcription level was repressed when compared to uninoculated control plants (Fig. 5b).

Discussion

The gene expression study clearly revealed the differential expression pattern of the three genes both in the root and in the stem of black pepper inoculated with *P. capsici*. However in all cases, the resistant line

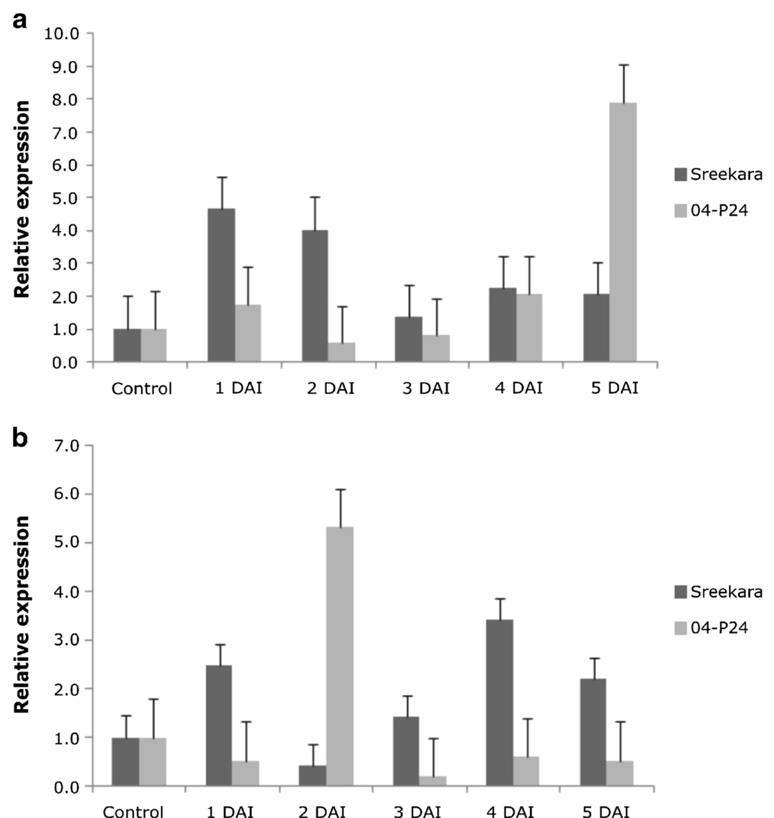
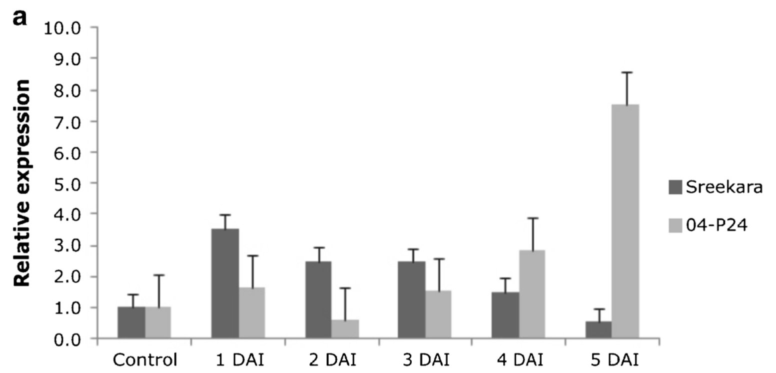


Fig. 3 Differential expression of β-1,3-glucanase gene in root (**a**) and stem (**b**) of uninoculated and *P. capsici* inoculated black pepper plants (*P. capsici* susceptible Sreekara and resistant 04-P24). Control: uninoculated plants and 1 to 5 DAI: inoculated plants from 1st to 5th day after inoculation. LSD ($p < 0.05$) for interaction between 'treatment' and 'days after inoculation' are 0.398 in root and 0.325 in stem



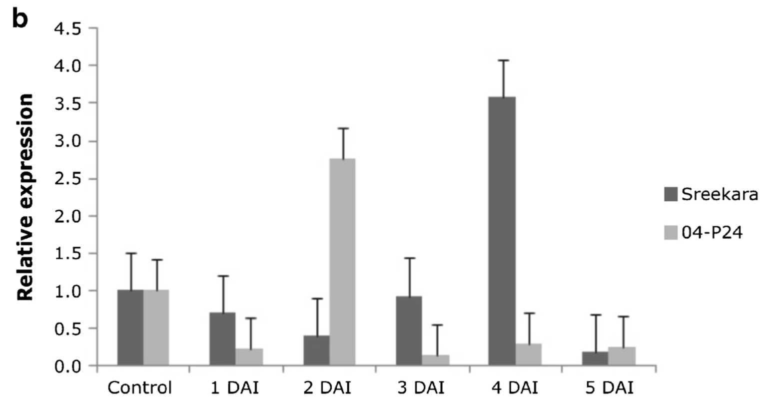
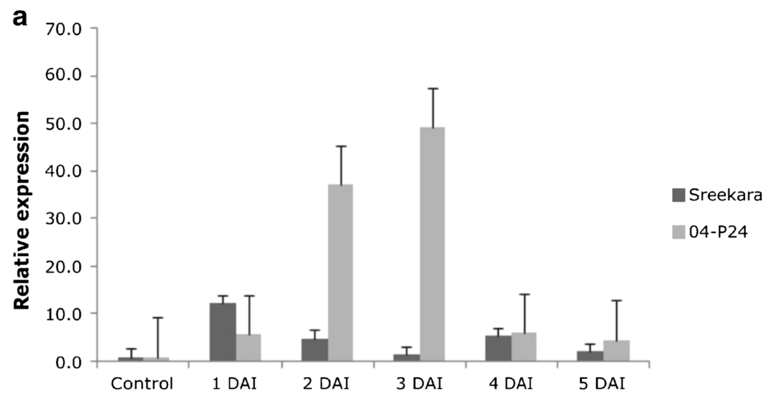


Fig. 4 Differential expression of osmotin gene in root (**a**) and stem (**b**) of uninoculated and *P. capsici* inoculated black pepper plants (*P. capsici* susceptible Sreekara and resistant 04-P24). Control: uninoculated plants and 1 to 5 DAI: inoculated plants from 1st to 5th day after inoculation. LSD ($p < 0.05$) for interaction between 'treatment' and 'days after inoculation' are 0.435 in root and 0.327 in stem



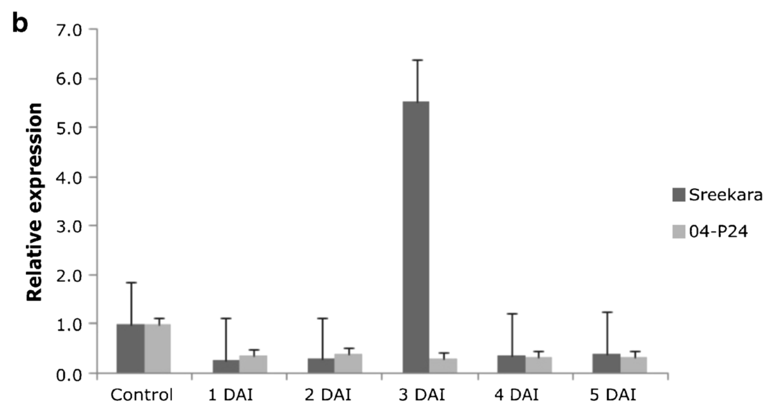


Fig. 5 Differential expression of cAPX gene in root (**a**) and stem (**b**) of uninoculated and *P. capsici* inoculated black pepper plants (*P. capsici* susceptible Sreekara and resistant 04-P24). Control: uninoculated plants, and 1 to 5 DAI: inoculated plants from 1st to 5th day after inoculation. LSD ($p < 0.05$) for interaction between 'treatment' and 'days after inoculation' are 0.509 in root and 0.211 in stem

showed higher expression of all the three genes in the root (Figs. 3a, 4a & 5a) rather than in the stem. This can be attributed to the root resistance shown by the resistant line (Bhai et al. 2010). Among the three genes, cAPX was the most up-regulated gene in both the lines with higher expression in the resistant line on 2 (37 fold) and 3 DAI (49 fold). Though cAPX was up-regulated in the early stage of infection in the susceptible line, there was gradual decline afterwards when pathogen overcomes the defense responses. The change in relative gene expression in the stem tissues (Figs. 3b, 4b & 5b) indicated the systemic induction of defense response in the plant system after soil inoculation. This finding is in agreement with the fact that plants activate the defense responses after the recognition of race-specific or race nonspecific pathogen derived elicitors leading to hypersensitive response (HR) (van Loon et al. 1994; Kushalappa et al. 2016) and systemic acquired resistance (SAR) (Hammond-Kosack and Parker 2003; Luna et al. 2012).

In the case of the susceptible line, the expression of genes in the stem tissue upon root inoculation was comparatively higher on the later stages but the defense response was not reaching up to the level found in the root system to resist the pathogen invasion from the soil. But in the resistant line, the expression was higher in the root tissue where it forms the defense barrier that inhibits the invasion of pathogen to other healthy tissue from the site of entry. This might be the reason for the lower expression level of genes in the stem tissue of resistant lines. Hence in the resistant line, the pathogen is confined to the site of entry on the root tissue and its further spreading is inhibited by HR reaction. This finding was confirmed by microscopic analysis in our previous study (Vandana et al. 2014). Microscopy was used for observation of HR response in tobacco in response to *P. syringae* (Krzymowska et al. 2007). Sometimes HR reaction involves single or a very few cells so that its manifestation is unnoticeable (Greenberg 1997; Mur et al. 2008). In our previous study (Vandana et al. 2014), we confirmed this by microscopic analysis of inoculated roots of both Sreekara and 04-P24. Neither hyphae nor sporangia of *P. capsici* could be detected inside the root tissue of 04-P24. So it is expected that such a response takes place at the site of attempted entry of *P. capsici* and hence further spread of the pathogen is prevented. Similar response was reported in *Capsicum annuum* upon *P. nicotiana* inoculation by Stamler et al. (2015). In *P. capsici* resistant black pepper line, symptom development was not observed on the root tissue upon pathogen inoculation since the root does not take up infection. But on leaf inoculation (data not shown), a small lesion was formed in the resistant line during early period of infection, but the size of the lesion did not increase during the later stages of interaction, indicating HR response at the site of pathogen entry into the tissue. In pepper, as suggested by Stamler et al. (2015), basal HR response was enhanced upon inoculation with non pathogenic *P. nicotiana*. Plant roots are able to induce HR against soil-borne oomycetes such as *Phytophthora sojae* (Dorrance and Schmitthenner 2000). Kosslak et al. (1996) observed the onset of HR in roots of soybean upon compatible and incompatible interactions with *Phytophthora sojae.* HR induced in potato upon infection by *P. infestans,* causes rapid death of the infected cells (Coffey and Wilson 1983). According to Naton et al. (1996), rapid cell death was the major defense response in parsley cell cultures infected with *P. infestans*.

The over expression of PR genes in relation with enhanced tolerance to pathogen infection has been described in several plants (Jung et al. 2005; Chassot et al. 2007). In plants, β −1,3-glucanase (PR-2) is strongly induced in response to infections by fungal, bacterial or viral pathogens (Senthilraja et al. 2013; Liu et al. 2013; Ebadzad and Cravador 2014). The antifungal and anti-oomycete activities of β-1,3-glucanase were reported by many researchers (Roy-Barman et al. 2006; Tripathi et al. 2013; Ebadzad and Cravador 2014). Vijesh Kumar et al. (2016) observed higher relative expression of β-1,3-glucanase gene in *Piper colubrinum*, which is resistant to foot rot disease, upon inoculation with *P. capsici*. In our study also, during black pepper – *P. capsici* interaction increased accumulation of β-1,3-glucanase transcripts was found in root tissue of both *P. capsici* susceptible and resistant black pepper lines compared to the uninoculated plants. Highest expression of the gene was noticed on 1 DAI in the root of *P. capsici* susceptible line, and then the expression started decreasing. The down-regulation of gene expression in later days in susceptible plants indicates the enhancement of infection by suppressing the defense response by *P. capsici*. This result is in accordance with the findings of Louis et al. (2016). They observed the down-regulation of transcription of a PR gene *StNPR1*

during *Cochliobolus lunatus* infection in *Solanum tuberosum* L. In the resistant line, the highest expression was observed on 5 DAI and the expression was approximately 1.7 fold higher than the highest level of expression observed in the susceptible line (Fig. 3a).

The increased expression of osmotin gene was observed in both susceptible and resistant lines. In the case of susceptible line, higher levels of osmotin expression occurred at the earlier stages of infection and there was a gradual increase thereafter while the reverse occurred in case of resistant line, where delayed higher expression of osmotin occurred at the later stage of infection. This is supported by Liu et al. (1994) who showed that in vitro over expression of PR-5 proteins in potato delayed symptom development of late blight disease caused by *P. infestans*. Tobacco osmotin was reported to cause lysis of spores and growth inhibition of *P. infestans* (Abad et al. 1996).

In vitro, osmotin inhibits spore germination and hyphal growth *P. nicotianae* and *P. infestans* with delayed disease symptoms (Thompson et al. 2006; El-Komy et al. 2010; Rivero et al. 2012). *P. capsici* and *P. infestans* induce osmotin transcription in pepper (Jung and Hwang 2000) and in potato (El-Komy et al. 2010), respectively. In black pepper, higher expression of osmotin gene upon interaction with *P. capsici* and *F. oxysporum* was reported (Mani et al. 2012). Vijesh Kumar et al. (2016) noticed increased level of osmotin gene expression in leaves of *P. colubrinum* inoculated with *P. capsici*. In this study, during black pepper - *P. capsici* interaction, osmotin gene transcription was found up-regulated in both *P. capsici* susceptible and resistant black pepper lines compared to the uninoculated plants. Highest expression of osmotin gene was noticed on 1 DAI in the root of *P. capsici* susceptible black pepper line. In the resistant line, the highest expression was observed on 5 DAI and the expression was twice the highest expression level of susceptible line (Fig. 4a).

Peroxidase activity is associated with disease resistance in plants and increases in host plants following pathogen infection (Zheng et al. 2004, 2005). Wang et al. (2013, 2015) studied the role of lignin forming anionic peroxidase in defense response of pepper to *P. capsici*. Enzymes like peroxidases, cinnamyl alcohol dehydrogenases and phenylalanine ammonia lyases participate in the phenylpropanoid metabolism (Baxter and Stewart 2013). Therefore, the up-regulation of the expression of cAPX upon infection by *P. capsici* in black pepper could be the manifestation of its participation in this metabolism as a response to the attack by the oomycete. In our previous study on biochemical defense responses of black pepper to *P. capsici*, peroxidase activity was found significantly high in the resistant line compared to susceptible line after pathogen inoculation (Vandana et al. 2014) which is well corroborated with the present observation that there is up-regulation of the expression cAPX upon infection by *P. capsici*. In the roots of *P. capsici* susceptible black pepper line, highest expression level of cAPX gene was noticed on 1 DAI and then the expression started declining suggesting the pathogen overrides the defense response. Whereas in the resistant line, the highest expression was observed on 3 DAI which was around 4 times more than that of susceptible line's highest expression (Fig. 5a). Anju et al. (2013) observed a progressive increase in APX activity in *P. capsici* inoculated black pepper variety IISR Shakthi and the inoculated plants remained healthy without showing the symptoms of foot rot indicating the role of APX in inducing tolerance during pathogenesis. Sarowar et al. (2005) demonstrated that *Capsicum annuum* ascorbate peroxidase-like 1 gene (CAPOA1) overexpressed in tobacco plants exhibited enhanced resistance to the oomycete pathogen, *P. nicotianae* and showed increased tolerance to oxidative stress.

Among the three genes studied in this experiment, cAPX gene expressed in higher level may play a significant role in root resistance of 04-P24 to *P. capsici*. This study indicates that the cAPX gene may be involved in the root resistance to *P. capsici* in the OP progeny 04-P24 identified as resistant to *Phytophthora* root infection at ICAR-IISR, Kozhikode (Bhai et al. 2010).

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Compliance with ethical standards

Ethical statement This research article is not submitted elsewhere for publication and this manuscript complies to the Ethical Rules applicable for this journal.

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