Eur J Plant Pathol (2019) 154:607–620
https://doi.org/10.1007/s10658-019-01685-3

Proteomic analysis of the tripartite interaction between black pepper, *Trichoderma harzianum* and *Phytophthora capsici* provides insights into induced systemic resistance mediated by *Trichoderma* spp.

P. Umadevi · M. Anandaraj

Accepted: 21 January 2019 / Published online: 1 February 2019

Abstract *Trichoderma harzianum* (MTCC5179) is the biocontrol agent in the black pepper (*Piper nigrum*.L) production system against the destructive pathogen *Phytophthora capsici* which causes foot and root rot. We employed label-free quantitative proteomics to study the *T. harzianum* mediated induced systemic response in this system. We studied the defence response in leaves in *T. harziznum* primed plant roots which are also infected with *P. capsici*. The pattern of interactions was studied as black pepper × *T. harzianum* (two-way), black pepper × *P. capsici* (two-way) and black pepper × *T. harzianum* × *P. capsici* (three-way). The proteins induced only in the three-way interaction were identified as *Trichoderma* induced resistance proteins. Eighteen reactive oxygen species-related proteins and 22 defence-related proteins were identified as marker proteins. Apart from these groups, the ethylene synthesis, isoflavanoid pathway and lignin synthesis proteins were found to be enhanced. We report the early induced systemic resistance in leaves after *Trichoderma* priming at roots (72, and 96 h after interaction) against *Phytophthora capsici* after 12 and 24 h of infection at roots. The peptides/proteins from this study will serve as important marker peptides/proteins for the induced systemic resistance in plants by *Trichoderma*.

Keywords Proteomics · Peptides · ROS related proteins · Defense-related proteins · *Phytophthora capsici* · *T-ISR*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10658-019-01685-3) contains supplementary material, which is available to authorized users.

P. Umadevi · M. Anandaraj (✉)
ICAR- Indian Institute of Spices Research, Kozhikode, Kerala 673012, India
e-mail: arajiisr@gmail.com

Introduction

Black pepper (*Piper nigrum* L.), known as the king of spices, is a perennial crop native to India. This export-oriented important spice crop is grown in many tropical countries. Foot rot disease caused by *Phytophthora capsici*, an oomycete pathogen infects the vine both in the nursery and the field and contributes to major crop loss (Anandaraj 2000). The application of *T. harzianum* (MTCC 5179) resulted in enhanced growth in black pepper with increased numbers of nodes and biomass (Anandaraj and Sarma 2003). *T. harzianum* treatment alone imparted greater growth promotion and disease suppression than that of consortia of plant growth-promoting rhizobacteria (Sibi 2013). Based on these reports on its growth promotion (Sibi 2013; Anandaraj and Sarma 2003) and disease suppression (Rajan et al. 2002; Paul et al. 2005) *T. harzianum* has been included as a component of integrated disease management in black pepper cultivation in India. But studies towards understanding the actual molecular mechanisms has not been attempted. We recently reported: (i) the impact of probiotic application of *T. harzianum* on population and

functional dynamics of the microbial community in the rhizosphere of black pepper using Illumina HiSeq whole genome metagenome sequencing of rhizosphere soil (Umadevi et al. 2017a), and (ii) the endophytic interaction of this fungus in black pepper roots using anatomical studies (Umadevi et al. 2017b). At present there is no molecular information on the host (black pepper) response to *T. harzianum*. No study on induced systemic resistance (ISR) by this fungus towards the foot rot pathogen in black pepper has been attempted so far.

The defense response of black pepper primed with *T. harzianum* to *P. capsici* was found to be systemic on the leaves with the involvement of PR proteins exhibiting peroxidase activity (Ezziyyani et al. 2007). Khan et al. (2004) conducted split root experiments which demonstrated the induction of the systemic response in cucumber by *Trichoderma harzianum* 382 against *P. capsici*. Reduction of symptoms due to inhibition of disease related processes and upregulation of the defense mechanism after *Plasmapora viticola* in *Trichoderma* strain T39 –primed grape vine has been demonstrated (Perazzolli et al. 2012). Induced resistance by *Trichoderma* in a susceptible variety from their study was found to be partially mimicking the resistant variety of grapevine.

The nature and composition of ISR strongly depends on the tripartite combination of plant, biocontrol agent and pathogen (Duijff et al. 1998; Tjamos et al. 2005). Velazquez-Robledo et al. 2011, Segarra et al. (2012) and Karolev et al. (2008) reported the induction of ISR-like response with *T. asperellum* or *T. harzianum* T39 against *Botrytis cinerea* in tripartite interactions. Mathys et al. (2012) also found that the induction of ISR happened only after pathogen infection in the *Arabidopsis* - *Trichoderma* - *B. cinera* combination. The tripartite interaction (plant × *Trichoderma* × pathogen) has been less studied (Marra et al. 2006) in comparison to the two-way interaction (plant × *Trichoderma*) (Harman et al. 2004). Knowledge on the tripartite interaction at the molecular level is needed in order to enhance the applicability of bioconrol agents in agroecosystem and to examine the cross-talk involved in the beneficial association (Keswani et al. 2016). Tripartite interaction studies require experiments that investigate the changes in gene expression dynamics in each partner involved, singly and also in all possible combinations. Induced systemic resistance (ISR) is not only restricted to roots but also develops in aerial parts of the plants. ISR in plants is regulated by jasmonic acid (JA) and or ethylene (ET) synthesis, unlike in systemic acquired resistance (SAR) which is regulated by salicylic acid (SA) (Vallad and Goodman 2004). When compared to gene expression and transcriptomics approaches, the application of proteomics in this area of research is minimal. Gel-based proteomics was attempted by Marra et al. (2006) on *Trichoderma*-bean-*Botrytis* and *Trichoderma*–bean-*Rhizoctonia* interactions. The proteomics approach indicated that plant-specific pathogenesis-related (PR) proteins and other disease-related factors (i.e. potential resistance genes) regulated the three-way interaction. The presence of *Trichoderma,* modified quantitatively and qualitatively the plant response to pathogen attack. This was the only proteomics study on three-way interactions involving *Trichoderma*. Recently, we showed the application of label free proteomics in profiling the anti- microbial peptide (AMP) signature in black pepper upon *Phytophthora* infection (Umadevi et al. 2018). In the present study we applied label-free quantitative proteomics to study the tripartite interaction of black pepper, *Trichoderma* and *P. capsici* with the focus on profiling the *Trichoderma* induced systemic resistance (T-ISR), the expression dynamics of systemically induced resistance proteins that serve as a valuable marker in analyzing the induction of resistance. The activation of defense proteins is induced only upon infection by the pathogen. The present study aimed to understand the systemic defense induced in black pepper leaf when the roots are treated with *T. harziznum.* We elucidated: (i) the *Trichoderma* induced systemic resistance proteins in this tripartite interaction, and (ii) the pathway model for *Trichoderma* induced systemic resistance in black pepper against *P. capsici*.

Materials and methods

Black pepper- *Trichoderma* interaction

T. harzianum liquid culture preparation

Talcum powder formulation of *T. harzianum* MTCC 5179, obtained from the biocontrol laboratory, ICAR- Indian Institute of Spices Research, Kozhikode, Kerala was used for the pot culture study by mixing 3 g of formulation containing 10^6 colony forming units (CFU) with 3.5 kg of top soil. For the co-cultivation study (liquid culture of *T. harzianum*), a 72 h old culture on potato-

dextrose-agar (PDA) plates was cut into 5 mm^2 discs, and one such disc was inoculated in conical flasks containing 50 ml potato dextrose agar. After 10 days, 100 ml sterile double distilled water (ddH_2O) was added to the flasks, and spore mass was scraped out to be used as inoculum.

Plant material

Single node cuttings of black pepper variety 'Sreekara' were washed with tween-20 for 15 min, and again in running tap water for 30 min. The cuttings were treated in 0.2% copper oxychloride for 15 min, followed rinsing in sterile ddH_2O twice. The cuttings were sterilized using 0.1% mercuric chloride for 5 min on a clean bench, then washed twice with sterile ddH_2O. The cut ends were quick dipped in 8000 ppm indole butyric acid (IBA) and planted in containers (7.5 × 7.5 × 10 cm, Himedia) having pre-sterilized perlite medium, fortified with sterile Hoagland's solution. The containers were maintained in a tissue culture room at 25 ± 1 °C, and 3000 lx for the production of saplings.

Trichoderma inoculation

Black pepper plantlets with 4–5 leaves were used for the interaction study. The plantlets grown under axenic condition as described above were inoculated with *T. harzianum* spore suspension (10^6spores/ml) in sterile containers and maintained at 24 ± 1 °C with intermittent shaking manually.

Defining the time frame

Leaf samples from three biological replicates were taken from the plants at 72 (T72) and 96 (T96) hai for proteomic analysis. Control plants were kept with sterile water.

Black pepper- *P. capsici* interaction

Zoospore inoculum preparation

The virulent *Phytophthora capsici* isolate 05–06 maintained in national repository of *Phytophthora*, ICAR-IISR, Kozhikode was sub-cultured in carrot agar medium (200 g Carrot; 16 g Agar; 1 L Water) and incubated at 24 ± 1 °C for 72 h. After 72 h mycelial discs (5 mm) were cut using cork borer, placed gently to float in Petri plates containing sterile distilled water (avoiding immersion of the disc in to the water) and incubated in laminar flow chamber at 24 ± 1 °C for 72 h. After the incubation period the plates were taken and kept at 4 °C for few minutes in order to liberate zoospores. The zoospores 10^6 spores/ml was used to infect the axenic plants. The zoospore suspension was added to the plants in the containers and kept at 24 ± 1 °C for infection of the black pepper roots by *P.* capsici. The leaf samples from three biological replicates were taken at 12 (P12) and 24 (P24) hai along with the control sample.

Black pepper-*Trichoderma-P. capsici* inoculation

The *T. harzianum* primed plants from 72 and 96 hai were used for the tripartite interaction experiment. The primed plants were kept in the sterile containers and *P. capcisi* zoospores (10^6spores/ml) were added. The plants were kept at 24 ± 1 °C in a sterile environment. The unprimed plants were used as the control. Leaf sampling was done from three biological replicates for the control, and after 12 (T72P12 & T96P12) and 24 (T72P24 & T96P24) hai for the proteomic analysis.

Proteomic analysis

Protein extraction, protein fractionation and quantification

The leaf samples (three biological replicates) T72, T 96, P12, P24 and T72P12, T72P24, T96P12, T96P24 (sample description as given in Table 1) were used to extract the total leaf protein by following the protocol developed previously (Umadevi and Anandaraj 2015). The whole protein extract was fractionated using 50KDa Amicon filters. 500 ul of sample was loaded in the filters, centrifuged at 1000×g for 2 min. The concentrate was removed separately and the Lowry method (Lowry et al. 1951) for protein quantification was followed. LC-LTQ Orbitrap MS analysis and peptide identification was done following Umadevi et al. (2018)

Statistical analysis

For the proteomics analysis, proteins from three biological replicates were analyzed. Relative quantification using Hi3 analysis was done. Anova, with a significance level of $p < 0.05$, was used to determine a statistically significant fold-change expression of peptides.

Table 1 List of codes used to describe the samples taken for proteomic analysis at different time points of bipartite interaction (Black pepper – *P. capcisi* & black pepper –*Trichoderma*) and the tripartite interaction (Black pepper - *Trichoderma* - *Phytophthora capsici*)

Sample code	Description
T72	Samples taken after 12 h of interaction with *T.harziznum*
T96	Samples taken after 24 h of interaction with *T.harzianum*
P12	Samples taken after 12 h of interaction with *Phytophthora capsici*
P24	Samples taken after 24 h of interaction with *Phytophthora capsici*
T72P12	Samples taken from plants primed for 72 h with *T.harzaznum* and infected with *Phytophthora capsici* for 12 h
T72P24	Samples taken from plants primed for 72 h with *T.harzianum* and infected with *Phytophthora capsici* for 24 h
T96P12	Samples taken from plants primed for 96 h with *T.harzianum* and infected with *Phytophthora capsici* for 12 h
T96P24	Samples taken from plants primed for 96 h with *Trichoderma* and infected with *Phytophthora* for 12 h

Results and discussion

In general, in the tripartite interaction – the three parties being black pepper, *T. harzianum*, and *P. capsici* – the number of proteins up-regulated in the treatments T72P12, T72P24, T96P12, and T96P24 (Supplementary file S3) was much larger than that in the bipartite interaction involving black pepper and *T. harziznum* (in T72 and T 96; Supplementary file S1); the other bipartite interaction, namely that involving black pepper and *P. capsici* (P12 and P24; Supplementary file S2), showed a systemic response mediated by *T. harzianum* in the above ground part of the black pepper plant. The tripartite interaction T72P12 showed 78 up-regulated proteins, 36 down-regulated proteins, and 9 completely down-regulated proteins; the corresponding numbers in 72T24P were 86, 31, and 7; those in T96P12 were 60, 27, and 9; and those in T96P24 were 8, 19, and 19 (Supplementary file S3). The proteins are considered completely down-regulated when they show infinite fold-down regulation values in a differential expression than they do in control samples in Hi3 analysis. The term 'completely down-regulated' is comparable to the denotation in some 2DE-based reports of proteins found in the control but not in any treatment. Completely down-regulated proteins are those that are completely down-regulated when under stress but begin to show up in abundance under favourable conditions. All the proteins were functionally annotated as belonging to the following categories: biological process (BP), cellular component (CC), and molecular functions (MF) (Supplementary file S4).

The systemically altered defence readiness was demonstrated by Waller et al. (2005) in barley plants primed with *Piriformospora indica*: powdery mildew infection in barley leaves was reduced, and the levels of antioxidants and those of the components of the glutathione pool (GSH and oxidized glutathione) were higher. Glutathione reductase activity was also enhanced in leaves during the first 3 weeks of inoculation, and the degree of infection was closely correlated to the systemic antioxidant capacity induced by *P. indica*. A similar systemic defense response in leaves of pepper plants when the roots of which had been inoculated with *Trichoderma* was reported by Ahmed et al. (2000). Such bipartite, or two-way, interaction has been reported in many other plants as well. Contreras-Cornejo et al. (2011) reported that colonization of *Arabidopsis* roots by *T. virens* or *T. atroviride* reduced foliar symptoms and lowered the mortality caused by *Botrytis cinerea*. Root colonization (Shoresh and Harman 2008) by *Trichoderma harzianum* Rifai strain 22 (T22) induced changes in the proteome of shoots of 7-day-old seedlings of maize, although the infection was confined only to the roots. Brotman et al. (2012) reported quantitative differences in gene expression in *Arabidopsis* plants primed with strain T203 of *T. harzianum* and then inoculated with the bacterial pathogen and thus showing the priming activity of the beneficial fungus. Martinez-Medina et al. (2013) showed that Jasmonic acid-dependent defence underlies the systemic induction of defence by *Trichoderma* in tomato against *B. cinerea*. Yedidia et al. (2003) observed Pal1 activation – both local and systematic – after inoculation of roots with *Trichoderma*, and Shoresh et al. (2006) reported systemic expression of the mitogen-activated protein kinase gene in cucumber leaves that had been inoculated with *Trichoderma asperellum*.

Marra et al. (2006) examined the proteomics triggered by two tripartite interactions involving *Trichoderma*,

bean, and either *Botrytis* or *Rhizoctonia*. The protein profile of the interaction involving *Botrytis* as compared to that without *Botrysis* featured 57 new spots, 93 missing spots, 25 up-regulated proteins, and 62 down-regulated proteins. These numbers indicate that the differential proteins are the ones responsible for systemic resistance to the pathogen as a result of infection. The corresponding numbers in the interaction involving *Rhizoctonia solani* were 63, 116, 27, and 29, again pointing to the systemic response triggered by *Trichoderma*. However, that study by Marra et al. reported only the changed numbers but not the details of the proteins.

In the tripartite interaction examined in the present study, we observed a complex response from proteins that are part of the photosynthesis-related pathway. The response comprised proteins from three categories, namely those that were up-regulated, down-regulated, or completely down-regulated. Less et al. (2011), using transcriptomics, reported the down-regulation of photosynthesis-related genes in response to many forms of biotic stress in *Arabidopsis* including infection by the virulent pathogen *P. infestans*. The increase in the number of up-regulated proteins in the tripartite interaction suggests that proteins that are part of the assimilatory process are also induced (the induction is not confined to the defence-related proteins). The treatments T72P24 and T96P12 recorded more up-regulated proteins than T7212 and T96P24 did. Plant defence is a complex process and requires abundant supply of energy from primary metabolic processes, and the dynamics of regulation also extended to the genes involved in photorespiration, carbohydrate metabolism, and the synthesis of amino acids: these genes are negatively regulated when they are no longer needed (Bolton 2009).

The two types of interaction, bipartite and tripartite, were compared in terms of the presence or absence of proteins and the amount of expression. The proteins present and up-regulated in the tripartite interaction were taken as the proteins related to *Trichoderma* - induced systemic resistance (T-ISR) and referred to, now on, as T-ISR proteins. The pattern of expression and the amount of change are given in Table 2, and details of peptides are given in Table 3. Many T-ISR proteins from the ROS-scavenging proteins group, the defence-related proteins group, and other important proteins group serve as marker proteins of the tripartite interaction.

Reactive oxygen species (ROS) scavenging proteins related to T-ISR

Proteins that served as markers of T-ISR and belonged to the ROS-scavenging proteins group were identified on the basis of their differential expression. Monodehydro-ascorbate reductase is one of the key antioxidant enzymes responsible for scavenging ROS and its activity is known to be up-regulated in plants under different forms of stress. The expression of this protein in T72P12 as 23.45-fold indicating early involvement of T-ISR proteins in black pepper, and absent in all the other treatments of the tripartite interaction and also in both the bipartite interactions (black pepper with either *Trichoderma* or *P. capsici*).

Superoxide dismutases (MnSOD, CuSOD, ZnSOD, and FeSOD) belong to the group of important enzymes that form the first line of antioxidant defence. Increased SOD activity often appears to enhance a plant's tolerance to oxidative stress. We observed the up-regulation of SOD as a T-ISR protein in all the tripartite interactions and its down-regulation in black pepper infected only with the pathogen, a patterns that points to the important role for SOD in defence as a T-ISR protein.

Tobacco class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance (Dorey et al. 1998). In *Arabidopsis* cat2, a knockout mutant for the major leaf catalase results in elevated levels of H_2O_2 (Han et al. 2013). In maize, three biochemically distinct catalase enzymes have been reported, each with a temporal and spatial specificity in expression (Scandiolios et al. 1984) and with variable expression under different forms of environmental stress. This differential expression of catalase isozymes was also recorded in the present study. Antioxidant proteins in this important group were down-regulated in the bipartite interaction involving black pepper and *Phytophthora* and absent in that involving black pepper and *Trichoderma* whereas in the tripartite interactions, the enzymes were up-regulated, pointing to their role in T-ISR in black pepper. The proteins were up- or down-regulated as follows, depending on the treatment and the enzyme: catalase 2 isozyme was up-regulated in T72P12 and down-regulated in T72P24, and catalase 1 and catalase 3 isozymes were up-regulated in T72P24 and T96P12.

In infected plants, stress-inducible GSTs play a key role in suppressing the necrosis caused by the pathogen; they do so by detoxifying the organic

Table 2 Dynamics of T-ISR proteins identified by the proteomic analysis from the tripartite interaction

S.No	Name of the Protein	T72	T96	P12	P24	T72P12	T72P24	T96P12	T96P24
A.	**Defense Related Proteins**								
1	RPP13						32.48		
2	Germin like Protein	∞	∞	6.54	41.06	1.12	2.47	2.59	
3	Subtilisin like protease	∞	∞		6.17	2.18	2.12	2.2	
4	Carbonic anhydrase like protein	∞	∞	∞	1.49	1.18	2.43	2.4	573.79
5	Methionine synthase	∞	∞	∞	∞	4.24			
6	NADP malic enzyme		∞		2.91	2.13	2.83	2.73	∞
7	Malate dehydrogenase		∞		4.06	4.41	3.62	2.81	∞
8	Protein transport inhibitor 1			∞	∞		∞	2.07	
9	WD repeat containing proteins			∞	∞				
10	Aldolase type TIM					6.69		82.7	
11	Nucleoside diphosphate kinase 2					9.1	4.31	4.51	
12	Isocitrate dehydrogenase				9.3	6.41	5.14	1.76	
13	Succinate semialdehyde dehydrogenase (mitochondrial)					3.37	2.1		
14	NmrA like (-)ve transcriptional regulator family protein					7.44		1.7	
15	Isoflavone reductase					∞			
16	WIN 2 wound induced protein					7.1			
17	Translationally controlled tumor like protein						∞		
18	2-methylene furane 3 one reductase					1.83	3.35	1.23	
19	Porin								8.66
20	Cinnamoyl Co-A						2.42		
21	Leucine amino peptidase		∞		∞	1.13	2.48	2.42	
22	Fructose bis phosphate aldolase (cytoplasmic)			2.14	∞	35.83	1.35		∞
B.	**ROS Scavenging Proteins**								
1	Dehydrogenase family protein					6.5		5.56	
2	2-cys peroxiredoxin BAS1	∞			4.71	2.39	4.91	5.73	
3	Superoxide dismutase (Cu-Zn)		∞	∞	∞	3.96	16.80	2.33	1320.44
4	Superoxide dismutase (Fe-Mn)			∞	∞		5.93		
5	Catalase isozyme 1,2,3		Cat2- ∞	Cat2- ∞	Cat1 - ∞ Cat 3- ∞	Cat 2 -37.71	Cat1- 11.79 Cat 2- 2.72 Cat 3- ∞		
6	Pexoxisomal(S)-2-hydroxy-acid oxidase GLO1-like				184.37	1.93	2.12		
7	Peroxidase 12	∞	∞		∞	1.50	2.06	1.71,1.92	
8	Peroxidse 12 like precursor				∞	41.53			
9	Peroxidase 5-like					2.06	325.12	-	
10	Peroxidase 16					8.59		4.41	
11	Peroxidase 60								1.34
12	Glutathione S-transferase F13 like				1.13	1.84	2.14	3.24	2.01
13	Reactive intermediate Deaminase A chloroplastic					12			
14	Monodehydro ascorbate reductase,					23.45			
15	Peroxiredoxin Q					4.35			
16	Ascorbate peroxidase 2 cytosolic			∞	∞	1.10	36.54	110.39	
17	Ascorbate peroxidase 1 cytosolic	∞	∞				1.04	1.04	
18	Ascorbate peroxidase 6 cytosolic						2.29		

(Green Color – Up regulation; Orange Color – Down regulation; Infinite symbol in green box denotes high up regulation while in the orange box denotes the complete down regulation)

hydro peroxides of fatty acids produced as a result of the peroxidation of membranes (Dixon et al. 2002; Gullner and Komives 2001). Our results support this observation. The up-regulation of GSTs was noticed only in the bipartite interaction involving *Phytophthora* and but not in that involving *Trichoderma*, indicating that the GSTs were induced by the pathogen but not by the beneficial organism *Trichoderma*. In the tripartite interaction, the change was many times higher than that in the bipartite interaction involving *Phytophthora*, which suggests that the particular protein was a T-ISR protein.

Table 3 T-ISR marker peptides with the sequence, score, ion and mass

Protein name of peptide	Peptide sequence under quantitation	Peptide ion	Score	Mass
RPP13	AQELLMSLLK	1651	53.91	1144.6340
Germin like Protein	VTFLDDAQVK	3955	44.37	1134.5923
Subtilisin like protease	LADPFDYGGGLVNPNK	876	65.01	1675.8218
Carbonic anhydrase like protein	NPELYGELAK	611	39.02	1132.5760
Methionine synthase	YLFAGVVDGR	1525	52.51	1095.5709
NADP malic enzyme	SIQVIVVTDGER	1073	64.34	1314.7144
	VLIQFEDFANHNAFVLLAK	1514	34.30	2218.1445
Malate dehydrogenase	DDLFNINAGIVIC	2776	51.82	1317.6929
Protein transport inhibitor 1	KLEMLSIAFAGDGDLGLHHVISGCESLR	927	33.80	2983.4453
WD repeat containing proteins	VTSVASFFVSR	2892	62.06	1198.6349
Aldolase type TIM	GLVGEIISR	939	63.09	942.5501
Nucleoside diphosphate kinase 2	IIGATNPADSAPGTIR	807	58.25	1552.8217
Isocitrate dehydrogenase	DQYLNTEEFIDAVAEELK	3919	120.51	2126.0075
Succinate semialdehyde dehydrogenase (mitochondrial)	VETLLQDATSK	1512	58.64	1203.6349
NmrA like (−)ve transcriptional regulator family protein	FFPSEFGNDVDR	2059	51.01	1428.6317
Isoflavone reductase	YLPSEFGNDVDR	3843	30.90	1410.6416
WIN 2 wound induced protein	YGWTAFCGPVGPR	4112	55.70	1466.6764
Translationally controlled tumor like protein	VVDIVDVFR	3194	74.12	1060.5917
2-methylene furane 3 one reductase	VAAAALNPUDSK			
Porin	SLFTISGEVDTR	2783	61.75	1323.6673
Cinnamoyl Co-A	DVAEALILLYEK	4323	48.79	1375.7609
Leucine amino peptidase	EVFAASCVSGEK	871	43.56	1251.5983
Fructose bis phosphate aldolase (cytoplasmic)	TIEVNNTDAEGR	3101	49.12	1317.6165
Dehydrogenase Family protein	TAEQTPLSALYAAK	2137	40.25	1462.7674
2-cys peroxiredoxin BAS1	APDFEAEAVFDQEFINVK	1561	46.20	2067.9801
	GLFIIDKEGVIQHSTINNLAIGR	2701	59.83	2507.3898
	LNTEVLGVSIDSVFSHLAWVQTDR	1865	35.37	2685.3773
Superoxide dismutase (Cu-Zn)	AFVVAELEDDLGKGGHELSLTTGNAGGR	1613	70.04	2878.4228
Superoxide dismutase (Fe-Mn)	LVVETTANQDPLVTK	2789	72.16	1626.8832
Catalase1	EGNWDLVGNNFPVFFIR	683	67.41	2022.9972
Catalase 2	EGNWDLVGNNFPVFFIR	809	66.86	2022.9985
Catalase 3	DLYSISAGNYPEWK	979	37.36	1756.7952
Peoxisomal (S)-2-hydroxy-acid oxidase GLO1 like	AIALTVDTPLLGR	519	79.73	1338.7872
	VPVFLDGGVR	1048	62.58	1057.5919
Peroxidase 12	IVSCADITAIAAR	483	100.61	1359.7189
Peroxidase 12 like precursor	QGLFTSDQDLYTDC	3336	73.68	1657.7589
Peroxidase 5-like	GCDGSVLIDSTASVSEK	2764	41.33	1809.8604
Peroxidase 16	FSQTFVTAPATLR	2194	50.14	1437.7616
Peroxidase 60	GVVSCADIIAMAAC	163	75.66	1448.7446
Glutathione S tranferase F13 like	NPFGQIPVLDGDLTLFESR	977	55.60	2246.7268
	VLDVYEER	232	40.24	1021.5090
	VLDYYEGR	753	44.27	949.4865

Table 3 (continued)

Protein name of peptide	Peptide sequence under quantitation	Peptide ion	Score	Mass
Reactive intermediate Deaminase A chloroplastic	FVSDTIEEQTEQVLK	4049	48.41	1764.8408
Peroxiredoxin	LPFTLLSDEGNK	2578	70.05	1332.6933
	NGVVQLIYNNQFQPEK	3498	28.41	1889.9637
Ascorbate peroxidase 2 cytosolic	TGGPFGTIR	1213	43.09	904.4763
Ascorbate peroxidase 1 cytosolic	ALLSDPVFRPLVDK	2317	55.87	1568.8929
Ascorbate peroxidase 6 cytosolic	RDEDLLVLPTDAVLFEDPSFK	3648	51.95	2418.2370

Organic peroxides are part of the plant's response to pathogen (Mauch and Dudler 1993); if not reduced, the peroxides are converted to aldehyde derivatives, which are cytotoxic and can kill the cells (Dean et al. 2005). Proteins of the dehydrogenase family are stress-related proteins and important in detoxifying the aldehydes. In the present study, a protein of that family was present and up-regulated only in the tripartite interaction, suggesting that it too was a T-ISR protein. The protein was probably expressed soon after the infection (12 hai), because was up-regulated only in T72P12 and T92P12 and was absent in later stages of the infection (24 hai), namely T72P24 and T96P24.

Plant cells express many peroxidase isozymes in different organs and tissues, and different subsets of isozymes are translated for different forms of environmental stress. We observed peroxidase 5, 12, 16, and 60 and a peroxides-like precursor protein to be up-regulated only in the tripartite interaction, which makes this enzyme group a T-ISR protein in black pepper. The expression dynamics are different for each isozyme. In T72P12, all isoforms were up-regulated whereas in T72P24, only the isoforms 5 and 12 were up-regulated; in T96P12, isoforms 12, 16, and 60 were up-regulated; and in T96P24, peroxidase expression was absent.

Reactive intermediate deaminase A chloroplastic (RidA) is responsible for scavenging ROS (Lambrecht et al. 2013). By converting the reactive enamine or imines to harmless 2-oxoacids, RidA preempts damage to branched-chain amino transferase, thereby allowing the synthesis of isoleucine to proceed without interruption. (Niehaus et al. 2014). The present study showed RidA to be a T-ISR protein in black pepper, deployed against *P.capsici*, because the protein was expressed very early (the amount being 12 times that in T72P12.

2-Cys peroxiredoxin BAS1 is part of the antioxidative defence system of chloroplasts (Baier and Dietz 1997). This protein was found to be up-regulated only 24 hai during the bipartite interaction involving *P. capsici* but in the tripartite interaction, because of the presence of *T. harzianum* the protein was expressed as early as 12 hai in T72P12.

Ascorbate peroxidase exists as an isozyme with important roles in metabolizing H_2O_2 in plants. The activity of this enzyme generally increases along with that of other antioxidant enzymes, namely catalase and SOD, in response to various sources of stress in the environment (Shigeoka et al. 2002). In the present study, we observed three isoforms of ascorbate peroxidase, namely 1, 2, and 6. The expression of this protein was down-regulated in the bipartite interactions and up-regulated in the tripartite interaction, suggesting that this protein too is an important T-ISR protein in black pepper.

Defence-related proteins related to T-ISR

We also grouped the defence related proteins, the other important group of proteins involved in the tripartite interaction we studied. Cytosolic isocitrate dehydrogenase (cICDH) is the most abundant isoform in leaves, responsible for up to 90% of $NADP^+$-dependent activity in leaf extracts (Hodges 2002; Kruse et al. 1998). Mhamdi et al. (2010) demonstrated that this protein also plays a role in redox signalling, which is linked to the response to pathogens in *Arabidopsis*. In the present study, the protein was up-regulated not only in the bipartite interaction involving *Phytophthora* but also in the tripartite interaction; in the bipartite interaction, this protein was expressed only 24 hai whereas in the tripartite interaction, the up-regulation occurred early in the interaction (12 hai). The amount of change decreased with the increase in *T. harzianum* priming and also with

time after the infection. This pattern suggests that this protein is one of those proteins that are induced early upon the infection of black pepper by *P.capsici,*

Malic enzyme (ME) plays a role in defence-related deposition of lignin. Nicotinamide adenine dinucleotide phosphate - malic enzyme (NADP-ME) is involved in producing NADP hydrogen (NADPH) for the synthesis of activated oxygen species, which are produced to kill pathogens or to attack them (Casati et al. 1999). In the present study, NADP-ME was up-regulated only during the later stages (24 hai) in the bipartite interaction involving *P. capsica,* whereas, in the tripartite interaction, the enzyme was induced earlier (12 hai), pointing to its being one of those proteins that are part of T-ISR.

The nucleoside diphosphate protein kinase (NDPK) gene TAB2 of tomato was earlier considered a non-regulatory housekeeping enzyme. Overexpression of the wild type TAB2 also enhanced resistance to *Pseudomonas syringae* pv. Tomato, which is a virulent form of the pathogen. The phosphoproteomics approach to studying the defence pathway of tMEK2 led to the identification of TAB2 as a downstream protein of LeMPK3 and as an effective component of the tMEK2-mediated pathway in disease resistance (Xing et al. 2008). The present study identified this protein as a T-ISR protein with enhanced expression in the tripartite interaction but missing in both the bipartitie interactions (with *P.capsici* or *T.harzianum*), indicating that the protein is an important early-response protein in inducing systemic resistance (since the enzyme was not expressed in T96P24).

The transport inhibitor protein 1 (TIR1) gene encodes an F-box protein containing 16 degenerate leucine-rich repeats (LRRs) (Ruegger et al. 1998). Gray et al. (1999) showed that overexpression of TIR1 in transgenic plants enhanced the response to auxins, a part of that response being increased expression of the auxin-dependent gene. In the present study, this protein was absent in the biparrtite interaction involving *Trichoderma* but was down-regulated in that involving *Phytophthora* and also in the tripartite interactions, which suggests negative regulation of the auxin-dependent gene in black pepper.

Wound-induced (Win proteins Win 1 and Win 2) are defence-related proteins, and ethylene plays a role in regulating their transcription in the systemic response (Stanford et al. 1989). Precursors of WIN2 were highly expressed in response to inoculation by the fungus *Aspergillus flavus* (Dhakal et al. 2017), and Harris et al. (1997) reported rapid expression of a Win peptide in abscission tissue of tomato leaves to protect the exposed tissue surface from bacteria and fungi. In the present study, the WIN2 precursor in T72P12 was up-regulated 7.10 times suggesting that the peptide is an important signature of T-ISR and also participates in defending, through ethylene, black pepper against *P.capsici.*

Isoflavone reductase is an important enzyme in the synthesis of isoflavanoid phytoalexins (glyceollins) in plants. Cheng et al. (2015) demonstrated that over expression of GmIFR in transgenic soybean enhanced the resistance to *Phytophthora sojae* and also increased transcriptional levels of the genes involved in the phenyl propanoid pathway, namely PAL, 4CL, and CHS. The significantly lower expression levels of ROS in transgenic plants than those in non-transgenic plants after incubation with *P. sojae* showed that GmIFR may function as an antioxidant to reduce ROS in soybean. In the present study, the protein was infinitely up-regulated in T72P12, pointing to the role of the isofalvanoid pathway in defence against *Phytophthora*. The enzyme was expressed only in the tripartite interaction (in T72P12), which shows that it was be part of the T-ISR.

An Nmr-like negative transcriptional regulator family protein – NmrA is a repressor of genes involved in nitrogen metabolism (Stammers et al. 2001) – was up-regulated in the tripartite interaction (in T72P12). Leucine amino peptidase is an inducible component in the defence response: LapA RNAs, proteins, and their activity increase in response to infection by the oomycete pathogen *Phytophthora parasitica* (Pautot et al. 1993). This particular protein was expressed and up-regulated only in the tripartite interaction but was absent in both the bipartite interactions and therefore to be a T-ISR protein induced by the action of *Trichoderma* against *P. capsici.*

Germin-like proteins confer broad-spectrum disease resistance (Manosalva et al. 2009) on plants. This type of protein was down-regulated multiple times in the bipartite interactions (6.54 times in P12 and 41.06 times in P24) but reduced to some extent in the tripartite interaction, suggesting that its expression was improved by the action of *Trichoderma*. The amount of change in the down-regulation was as follows: in T72P12, 1.12 times; in T72P24, 2.47 times; and in T96P12, 2.59 times.

Subtilisin-like protease plays a role in recognizing a pathogen and immune-priming the plant against that pathogen (Figueiredo et al. 2014). In the present study,

this protein was down-regulated in the bipartite interaction involving *Trichoderma* and up-regulated in that involving the pathogen. This difference suggests that the protein is associated with infection and also primed by *Trichoderma* in black pepper. Further studies are needed to establish the role of subtilisin protein in this system.

Lu et al. (2012) identified eight family genes of fructose 1, 6 bisphosphate aldolase localized in cytoplasm and plastids. These proteins have a highly conserved TIM barrel domain and a C-terminal domain with variable N terminal domain. The expression pattern of each protein is different in response to various forms of abiotic stress. In the present study, this protein was up regulated only in the tripartite interaction, pointing to its identity as a T-ISR protein and its role in resistance to the pathogen. The up regulation of fructose bisophophate aldolase protein was recorded only in tripartite condition T72P12 (35.83 fold) and the aldolase TIM barrel family protein was recorded at T72P12 (6.69 fold) and T96P12 (82.70 fold).

The R protein RPP 13 is known to act via a novel signalling pathway independent of salicylic-acid-mediated pathway in Arabidopsis (Bittner–Eddy et al. 2000). In the present study, the putative orthologs of this protein were completely down-regulated only in the bipartite interaction with *Trichoderma* (T24) – but not with the pathogen – and in the tripartite interaction T72P24 (the extent of down-regulation was 32.48 times. The protein was absent in all the other treatments. *Trichoderma* thus had a positive effect on the expression of this protein as the down-regulation increased 32.48-fold. In an earlier study, the RPP 13 type R gene PnCNBS4 was highly expressed in a variety of black pepper resistant to *P. capsici* but down-regulated in the susceptible variety (Umadevi and Anandaraj 2017). The priming by *Trichoderma* promoted the expression of this RPP13 R gene in the susceptible variety in the present study, which suggests that T-ISR can improve the fitness of a susceptible genotype (Fig. 1).

Lignin and lignin-related compounds are induced following infection. Cinnamoyl CoA reductase (CCR) catalyses the first step of the monolignol-specific branch from the phenylpropanoid pathway and is considered a potential control point in regulating the overall carbon flux towards lignin production (Chabannes et al. 2001). The induction of H_2O_2 as one of the defence responses may stimulate the polymerization of monolignols in the infected regions. Kawasaki et al. (2006) found that OsRac1 (small GTPase) controls lignin synthesis by regulating both NADPH oxidase and OsCCR1 as part of the defence response in rice. In the present study, CCR was up-regulated only in the tripartite interaction T72P24, which shows that CCR is an important T-ISR protein and suggests that *Trichoderma* mediates lignin production in leaves as part of the ISR.

The gamma amino butyric acid (GABA) shunt is the metabolic pathway that bypasses two steps of the TCA cycle. This pathway is composed of succinate semi aldehyde dehydrogenase. Boucher et al. (2003) reported that the pathway is involved in preventing the accumulation of ROS and also cell death in *Arabidopsis*. The succinate semi aldehyde dehydrogenase protein is up regulated in tripartite interaction and is one of the T-ISR proteins in black pepper.

The proteins involved in ethylene biosynthesis were up-regulated in the tripartite interaction, which is a clear indication of the involvement of the ethylene pathway in ISR in our study: compared to that in the control, methionine synthase increased 4.24 times and 5-methyltetrahydropteroyltriglutamate - homocysteine methyl transferase increased 11.31 times in T72P12; in T72P24, the increase was nearly infinite. Hence *Trichoderma* also induces the ethylene-mediated defence pathway in black pepper against *Phytophthora*. Brotman et al. (2012) found that in Arabidopsis, T203 strongly induced the ET-responsive transcription factor ERF13 when inoculated with *Pseudomonas syringae*, pointing to the activation of ET signalling cascades by T203.

Another T-ISR protein that was upregulated only in the tripartite interaction in T72P12 and T72P24 was 2-methylene furan-3-one-reductase, which was absent in both the bipartite interactions. Translationally controlled tumor protein plays a negative role in inducing HR. The role of NbTCTP in such induction was investigated by silencing NbTCTP by *Agrobacterium*-mediated transient expression of HR-inducible elicitors including INF1 from *Phytophthora infestans* (Gupta et al. 2013). Induction of cell death by INF1 expression was significantly accelerated in NbTCTP-silenced plants compared to the control plants 24–48 hai. In the present study, this protein was up-regulated only in the tripartite interaction T72P24, which suggests that it reduces the reaction in black pepper and that *Trichoderma* mediates this form of resistance.

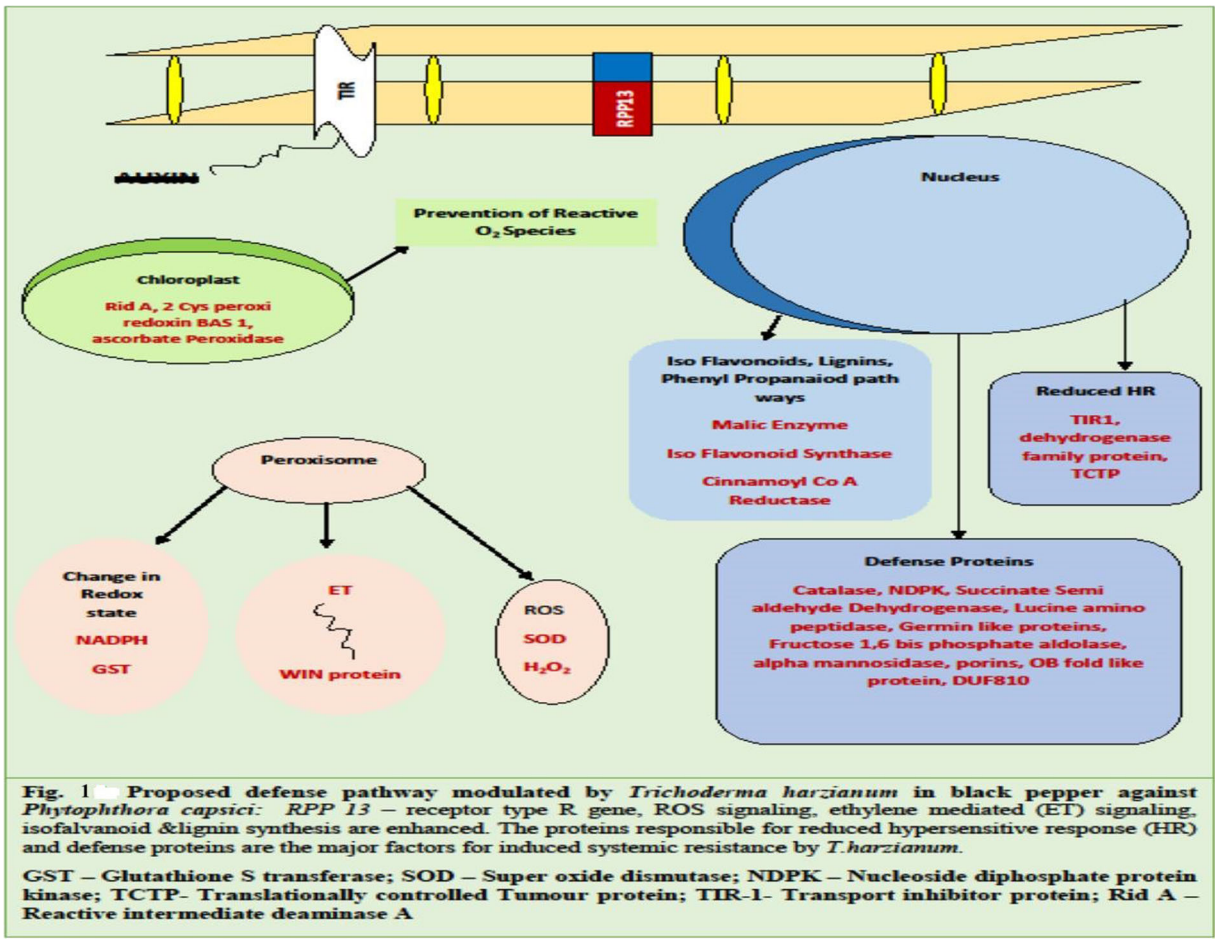

Fig. 1 The putative model depicting the modulation of defense response by *T. harzianum* in black pepper against *Phytophthora*

Other proteins as T-ISR proteins

Proteins that are important in T-ISR but are neither ROS-related proteins nor defence-related proteins are grouped as other proteins. These proteins are also potential markers of T-ISR in black pepper. Alpha mannosidase is involved in the turnover of complex N-glycans in plants. The protein was up-regulated in T72P24 (22.05 times) and T96P24 (infinitely). A hairpin-binding protein was up-regulated only in the tripartite interaction (T72P12 and T72P24) and down-regulated in the bipartite interactions. The tripartite interaction T72P24 up-regulated the mitochondrial outer membrane protein porin (8.66 times), the DUF 810 domain containing protein (infinitely), and the nucleic acid binding OB-fold like protein (7.18 times). Enhanced expression of the above proteins only in the tripartite interaction suggests that these proteins play a role in the ISR mediated by *Trichoderma* in black pepper.

Conclusion

Taken together, our observations of the expression dynamics of proteins in the tripartite interaction vis-à-vis the bipartite interaction indicates that *Trichoderma* induces systemic resistance in black pepper against *Phytophthora capsici.* The strong ROS-related activity suggests that ROS-mediated signalling is a major component of the defence, along with many other defense-related proteins in T-ISR. The isoflavanoid pathway, lignin synthesis and ethylene synthesis were enhanced in T-ISR. *Trichoderma*-induced systemic resistance is thus a major component of the defence readiness of black pepper against *Phytophthora capsici* and sets in

early (72–96 hai). The peptides identified in the present study will serve as important marker peptides of induced systemic resistance in plants in general and in black pepper in particular.

Acknowledgments The authors acknowledge with thanks the financial support from ICAR-Outreach programme on *Phytophthora, Fusarium* and *Ralstonia* diseases of horticultural and field crops (PhytoFuRa). The mass spectrometry performed by C-CAMP, Bangalore, is gratefully acknowledged.

Compliance with ethical standards

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

Involvement of human participants and /or animals The present research did not involve any experimentation on humans or animals.

Informed consent Authors are ready to provide any additional information of the present study to the readers.

References

Ahmed, A. S., Sanchez, C. P., & Candela, M. E. (2000). Evaluation of induction of systemic resistance in pepper plants (*Capsicum annum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. *European Journal of Plant Pathology, 106*, 817–824.

Anandaraj, M. (2000). Diseases of black pepper. In P. N. Ravindran (Ed.), *Black pepper (Piper nigrum L.)* (pp. 239–268). Harwood Academic Publisher.

Anandaraj, M., & Sarma, Y.R. (2003). The potential of PGPRs in disease management of spice crop. In 6th International PGPR workshop, Calicut, India,5–10 October 2003.

Baier, M., & Dietz, K. J. (1997). The plant 2-cys peroxiredoxin BAS1 is a nuclear encoded chloroplast protein: Its expressional regulation, phylogenetic origin, and implications for its specific physiological function in plants. *The Plant Journal, 12*, 179–190.

Bittner–Eddy, P. D., Crute, I. R., Holub, E. B., & Beynon, J. L. (2000). RPP13 is a simple locus in Arabidopsis thaliana for alleles that specify downy mildew resistance to different avirulence determinants in *Perenospora parasitica*. *The Plant Journal, 21*, 177–188.

Bolton, M. D. (2009). Primary metabolism and plant-defense-fuel for the fire. *Molecular Plant Microbe Interaction, 11*, 1196–1206.

Boucher, N., Fait, A., Bouchez, D., Moller, S. G., & Fromm, H. (2003). Mitochondrial SSADH of the GABA shunt is required to restrict levels of reactive oxygen intermediates in plants. *Proceedings of the National Academy of Sciences U.S.A, 100*, 6843–6848.

Brotman, Y., Lisec, J., Méret, M., Chet, I., Willmitzer, L., & Viterbo, A. (2012). Transcript and metabolite analysis of the *Trichoderma*-induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*. *Microbiology, 158*, 139–146.

Casati, P., Drincovich, M. F., Gerald, E., Edwards, G. E., & Andreo, C. S. (1999). Malate metabolism by NADP-malic enzyme in plant defense. *Photosynthesis Research, 61*, 99–105.

Chabannes, M., Barakate, A., Lapierre, C., Marita, J. M., Ralph, J., Pean, M., Danoun, S., Halpin, C., Grima-Pettenati, J., & Boudet, A. M. (2001). Strong decrease in lignin content without significant alteration of plant development is induced by simultaneous down regulation of cinnamoyl CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD) in tobacco plants. *The Plant Journal, 28*, 257–270.

Cheng, Q., Li, N., Dong, L., Zhang, D., Fan, S., Jiang, L., Wang, X., Xu, P., & Zhang, P. (2015). Overexpression of soybean Isoflavone reductase (*GmIFR*) enhances resistance to *Phytophthora sojae* in soybean. *Frontiers in Plant Science, 6*, 1024.

Contreras-Cornejo, H. A., Macias-rodriguez, L., Beltran–Pena, E., Herrera– Estrella, A., & Lopez-Bucio, J. (2011). *Trichoderma* –induced plant immunity likely involves both hormonal and camalexin dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungus *Botrytis cinerea*. *Plant Signaling Behaviuor, 6*, 1554–1563.

Dean, J. D., Goodwin, P. H., & Hsiang, T. (2005). Induction of glutathione S-transferase genes of *Nicotiana benthamiana* following infection by *Colletotrichum destructivum* and *C. orbiculare* and involvement of one in resistance. *Journal of Experimental Botany, 56*, 1525–1533.

Dhakal, R., Chai, C., Karan, R., Windham, G. L., Williams, W. P., & Subudhi, P. K. (2017). Expression profiling coupled with in-silico mapping identifies candidate genes for reducing aflatoxin accumulation in maize. *Frontiers in Plant Science, 8*, 503.

Dixon, D. P., Lapthorn, A., & Edwards, R. (2002). Plant glutathione transferases. *Genome Biology, 3*, 3004.1–3004.10.

Dorey, S., Baillieul, F., Saindrenan, P., Fritig, B., & Kauffmann, S. (1998). Spatial and temporal induction of cell death, defense genes, and accumulation of salicylic acid in tobacco leaves reacting hypersensitivity to a fungal glycoprotein elicitor. *Molecualr Plant Microbe Interaction, 11*, 1102–1109.

Duijff, B. J., Pouhair, D., Olivain, C., Alabouvette, C., & Lemanceau, P. (1998). Implication of systemic induced resistance in the suppression of fusarium wilt of tomato by *Pseudomonas fluorescens* WCS417r and by nonpathogenic *Fusarium oxysporum* Fo47. *European Journal of Plant Pathology, 104*, 903–910.

Ezziyyani, M., Requena, M. E., Egea-Gilabert, C., & Candela, M. E. (2007). Biological control of *Phytophthora* root rot of pepper using *Trichoderma harzianum* and *Streptomyces rochei* in combination. *Journal of Phytopathology, 155*, 342–349.

Figueiredo, A., Monteiro, F., & Sebastiana, M. (2014). Subtiltisin –like protease in plant pathogen recognition and immune priming- a perspective. *Frontiers in Plant Science, 5*, 739.

Gray, W. M., Carlos del Pozo, J., Walker, L., Hobbie, L., Risseeuw, E., Banks, T., Crosby, W. L., Ming Yang, M., Hong Ma, H., & Estelle, M. (1999). Identification of an SCF ubiquitin–ligase complex required for auxin response in *Arabidopsis thaliana*. *Genes and Development, 13*, 1678–1691.

Gullner, G., & Komives, T. (2001). The role of glutathione and glutathione-related enzymes in plant-pathogen interactions. In D. Grill, M. Tausz, & L. Kok (Eds.), *Significance of glutathione in plant adaptation to the environment* (pp. 207–239). Dordrecht: Kluwer Academic Publishers.

Gupta, M., Yoshioka, H., Ohnishi, K., Mizumoto, H., Yasufumi Hikichi, Y., & Akinori, K. (2013). A translationally controlled tumor protein negatively regulates the hypersensitive response in *Nicotiana benthamiana*. *Plant and Cell Physiology, 54*, 1403–1414.

Han, Y., Mhamdi, A., Chaouch, S., & Noctor, G. (2013). Regulation of basal and oxidative stress-triggered jasmonic acidrelated gene expression by glutathione. *Plant Cell & Environment, 36*, 1135–1146.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species-oppurtunistic, avirulent plant symbionts. *Nature Reviews Microbiology, 2*, 43–56.

Harris, N., Jane, E., Taylor, J. E., Jeremy, A., & Roberts, J. A. (1997). Characterization and expression of an mRNA encoding a wound-induced (win) protein from ethylene-treated tomato leaf abscission zone tissue. *Journal of Experimental Botany, 48*, 1223–1227.

Hodges, M. (2002). Enzyme redundancy and the importance of 2-oxoglutarate in plant ammonium assimilation. *Journal of Experimental Botany, 53*, 905–916.

Karolev, N., Rav David, D., & Elad, Y. (2008). The role of phytohormones in basal resisance and *Trichoderma*- induced resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. *Biological Control, 53*, 667–682.

Kawasaki, T., Koita, H., Nakatsubo, T., Hasegawa, K., Wakabayashi, K., Takahashi, H., Umemura, K., Umezawa, T., & Shimamoto, K. (2006). Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is an effector of small GTPase Rac in defense signaling in rice. *Proceedings of the National Academy of Sciences U.S.A, 103*, 230–235.

Keswani, C., Bisen, K., Singh, S. P., Sarma, B. K., & Singh, H. B. (2016). A proteomic approach to understand the Tripartite interataions between Plant-*Trichoderma*- Pathogen: Investigating the potential for efficient biological control. In K. R. Hakeem & M. S. Akhtar (Eds.), *Plant, Soil and Microbes* (pp. 79–93). Switzerland: Springer Publications.

Khan, J., Ooka, J. J., Miller, S. A., Madden, L. V., & Hoitink, H. A. J. (2004). Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophthora* crown rot and leaf blight. *Plant Disease, 88*, 280–286.

Kruse, A., Fieuw, S., Heineke, D., & Müller-Röber, B. (1998). Antisense inhibition of cytosolic NADP-dependent isocitrate dehydrogenase in transgenic potato plants. *Planta, 205*, 82–91.

Lambrecht, J. A., Schmitz, G. E., & Downs, D. M. (2013). RidA proteins prevent metabolic damage inflicted by PLP-dependent dehydratases in all domains of life. *mBio, 4*, 00033–00013.

Less, H., Angelovici, R., Tzin, V., & Galli, G. (2011). Coordinated gene networks regulating *Arabidopsis* plant metabolism in response to various stresses and nutritional cues. *Plant Cell, 23*, 1264–1271.

Lowry, O.H., Rosebrough, N. J., Farr, A. L. & Randal, R. J. (1951). Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry, 193*(1), 265–75.

Lu, W., Tang, X., Huo, Y., Xu, R., Qi, S., Huang, J., Zheng, C., & Wu, C. A. (2012). Identification and characterization of FBA genes in *Arabidopsis* reveal a gene family with diverse response to abiotic stresses. *Gene, 503*, 65–74.

Manosalva, P. M., Davidson, R. M., Liu, B., Zhu, X., Hulbert, S. H., Leung, H., & Leach, J. E. (2009). A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice. *Plant Physiology, 149*, 286–296.

Marra, R., Ambrosino, P., Carbone, V., Vinale, F., Woo, S. L., Ruocco, M., Ciliento, R., Lanzuise, S., Ferraioli, S., Soriente, I., Gigante, S., Turra, D., Fogliano, V., Scala, F., & Lorito, M. (2006). Study of the three-way interaction between *Trichoderma atroviride*, plant and fungal pathogens by using a proteomic approach. *Current Genetics, 50*, 307–321.

Martinez-Medina, A., Fernandez, I., Sanchez-Guzman, M. J., Jung, S. C., Pascual, J. A., & Pozo, M. J. (2013). Deciphering the hormonal signaling network behind the systemic resistance induced by *Trichoderma* in tomato. *Frontiers in Plant Sciences, 24*, 206.

Mathys, J., DeCremer, K., Timmermans, P., VanKerckhove, S., Lievens, B., & Vanhaecke, M. (2012). Genome-wide characterization of ISR induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against *Botrytis cinerea* infection. *Frontiers in Plant Sciences, 3*, 108.

Mauch, F., & Dudler, R. (1993). Differential induction of distinct glutathione-S-transferases of wheat by xenobiotics and by pathogen attack. *Plant Physiology, 102*, 1193–1201.

Mhamdi, A., Mauve, A. C., Gouia, H., Saindrenan, P., Hodges, M., & Noctor, G. (2010). Cytosolic NADP-dependent isocitrate dehydrogenase contributes to redox homeostasis and the regulation of pathogen responses in *Arabidopsis* leaves. *Plant Cell &Environment, 33*, 1112–1123.

Niehaus, T. D., Nguyen, T. N. D., Gidda, S. K., ElBadawi-Sidhu, M., Lambrecht, J. A., McCarty, D. R., Downs, D. M., Cooper, A. J. L., Fiehn, O., Mullen, R. T., & Hanson, A. D. (2014). *Arabidopsis* and maize RidA proteins preempt reactive enamine/imine damage to branched-chain amino acid biosynthesis in plastids. *Plant Cell, 26*, 3010–3022.

Paul, D., Saju, K. A., Jisha, P., Sarma, Y. R., Kumar, A., & Anandaraj, M. (2005). Mycolitic enzymes produced by *Pseudomonas fluorescens* and *Trichoderma* spp. against *Phytophthora capsici*, the foot rot pathogen of black pepper (*Piper nigrum* L.). *Annals of Microbiology, 55*, 129–133.

Pautot, V., Holzer, F. M., Reisch, B., & Walling, L. L. (1993). Leucine aminopeptidase: An inducible component of the defense response in *Lycopersicon esculentum* (tomato). *Proceedings of the National Academy of Sciences U.S.A, 90*, 9906–9910.

Perazzolli, M., Moretto, M., Fontana, P., Ferrarini, A., Velasco, R., & Moser, C. (2012). Downy mildew resistance induced by *Trichoderma harzianum* T39 insusceptible grape vines partially mimics transcriptional changes of resistant genotypes. *BMC Genomics, 13*, 660.

Rajan, P. P., Sarma, Y. R., & Anandaraj, M. (2002). Management of foot rot disease of black pepper with *Trichoderma spp*. *Indian Phytopathology, 55*, 34–38.

Ruegger, M., Dewey, E., Gray, W. M., Hobbie, L., Turner, J., & Estelle, M. (1998). The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast Grr1p. *Genes and Development, 12*, 198–207.

Scandiolios, J. G., Tsaftaris, J. M., Chandlee, T. M., & Skadsen, R. W. (1984). Expression of the developmentally regulated catalase (*Cat*) genes in maize. *Developmental Genetics, 4*, 2–293.

Segarra, G., Casanova, E., Bellido, D., Antonia Odena, M., Oliveira, E., & Trillas, I. (2012). Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. *Proteomics, 7*, 3943–3952.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., & Yoshimura, K. (2002). Regulation and function of ascorbate peroxidase isozymes. *Journal of Experimental Botany, 53*, 1305–1319.

Shoresh, M., & Harman, G. E. (2008). The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: A proteomic approach. *Plant Physiology, 147*, 2147–2163.

Shoresh, M., Gal-On, A., Leibman, D., & Chet, I. (2006). Characterization of a mitogen-activated protein kinase gene from cucumber required for *Trichoderma* -conferred plant resistance. *Plant Physiology, 142*, 1169–1179.

Sibi, M. C. (2013). *Development of biocontrol consortia for tissue cultured black pepper (Piper nigrum L.,) plants*. Mangalore University: Dissertation.

Stammers, D. K., Ren, J., Leslie, K., Nichols, C. E., Lamb, H. K., Cocklin, S., Dodds, A., & Hawkins, A. R. (2001). The structure of the negative transcriptional regulator NmrA reveals a structural superfamily which includes the short chain dehydrogenase/reductases. *TheEMBO Journal, 20*, 6619–6626.

Stanford, A., Bevan, M., & Northcote, D. (1989). Differential expression within a family of novel wound- induced genes in potato. *Molecualr and General Genetics, 215*, 200–208.

Tjamos, S. E., Flemetakis, E., Paplomatas, E. J., & Katinakis, P. (2005). Induction of resistance to *Verticillium dahliae* in *Arabidopsis thaliana* by the biocontrol agent K-165 and pathogenesis-related proteins gene expression. *Molecular Plant Microbe Interaction, 18*, 555–561.

Umadevi, P., & Anandaraj, M. (2015). An efficient protein extraction method for proteomic analysis of black pepper (*Piper nigrum* L.) and generation of protein map using nano LC-LTQ Orbitrap mass spectrometry. *Plant Omics, 8*, 500–507.

Umadevi, P., & Anandaraj, M. (2017). Genotype specific host resistance in blackpepper for *Phytophthora*. *Physiological and Molecular Plant Pathology, 100*, 237–241.

Umadevi, P., Anandaraj, M., & Benjamin, S. (2017a). Endophytic interactions of *Trichoderma harzianum* in a tropical perennial rhizo – Ecosystem. *Research Journal of Biotechnology, 12*, 22–30.

Umadevi, P., Anandaraj, M., Srivastav, V., & Benjamin, S. (2017b). *Trichoderma harzianum* MTCC 5179 impacts the population and functional dynamics of microbial community in the rhizosphere of black pepper (*Piper nigrum* L.). *Brazilian Journal of Micorbiology*. https://doi.org/10.1016/j.bjm.2017.05.011.

Umadevi, P., Soumya, M., Johnson, K. G., & Anandaraj, M. (2018). Proteomics assisted profiling of anti microbial peptide signatures from black pepper (*Piper nigrum* L). *Physiology and Molecular Biology of Plants, 24*, 379–387.

Vallad, G. E., & Goodman, R. M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science, 44*, 1920–1934.

Velazquez-Robledo, R., Contreras-Cornejo, H. A., Macias-Rodriguez, L., Hernandez-Morals, A., Aguirre, J., Casas-Flores, S., Lopez-Bucio, J., & Herrera- Estrella, A. (2011). Role of the 4- phosphopantetheinyl transferase of *Trichoderma virens* in secondary metabolism and induction of plant defense responses. *American Phytopathological Society, 24*, 1459–1471.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., & Fischer, M. (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences U.S.A., 102*, 13386–13391.

Xing, T., Rampitsch, C., Sun, S., Romanowski, A., Conroy, C., Stebbing, J. A., & Wang, X. (2008). TAB2, a nucleoside diphosphate protein kinase, is a component of the tMEK2 disease resistance pathway in tomato. *Physiological and Molecular Plant Pathology, 73*, 33–39.

Yedidia, I., Shoresh, M., Kerem, Z., Ben-hamou, N., Kapulnik, Y., & Chet, I. (2003). Concomitant induction of systemic resistance to *Pseudomonas syringae* pv. *lachrymans* in cucumber by *Trichoderma asperellum* (T-203) and accumulation of phytoalexins. *Applied and Environmental Microbiology, 69*, 7343–7373.