International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 6, Issue 1, Feb 2016, 69-78

REAL–TIME QUANTITATIVE RT-PCR OF SOME DEFENSE RESPONSE GENES IN PIPER COLUBRINUM CHALLENGE INOCULATED WITH PHYTOPHTHORA CAPSICI

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ABSTRACT

Foot rot caused by Phytophthora capsici is a major soil borne disease in black pepper, known as the king of spices. Piper colubrinum a distant relative of black pepper is known to be resistant to foot rot disease. The induction of PR proteins and defense response genes during pathogen stress is known to be an important strategy for plants to control the spread of infection during the initial stages of attack by the pathogen. Here we investigated the role of PR proteins viz osmotin, β-1,3-glucanase, defensins and thaumatin like protein, already proven in many plants to be an important factor in the control of various pathogens, through real time PCR. Two strains of pathogen maintained at National repository of Phytophthora, IISR were used for inoculation of the plant. Plants inoculated with 05-06 strain showed high level of expression for the genes under study but the plants inoculated with the other strain 98-93 showed subdued expression when compared to plants inoculated with the strain 05-06. These results show the importance of these genes and the effect of two different strains on resistant reaction of P. Colubrinum against Phytophthora capsici.

KEYWORDS: Osmotin; β-1,3-Glucanase; Defensin; Thaumatin-Like Protein; Real Time PCR; P. Colubrinum; P.Capsici

Received: Dec 01, 2015; **Accepted:** Dec 28, 2015; **Published:** Jan 05, 2016; **Paper Id.:** IJASRFEB201610

Original Article

INTRODUCTION

Black pepper, (Piper nigrum L.), the king of spices is an important commercial crop which fetches considerable export earnings to the country. Phytophthora foot rot has been identified as a major production constraint not only in India but also in other parts of the world. Foot rot is caused by Phytophthora capsici, a soil borne oomycete pathogen. Piper colubrinum a wild species of piper is known to be resistant to foot rot disease. The present investigation focuses mainly on the expression analysis of osmotin, β-1,3-glucanase, defensin and thaumatin like protein genes in challenge inoculated Piper colubrinum plants with P.capsici through quantitative RT- PCR.

Plants have different adaptations to counter the pathogen attack (Agrios 1997). The plants synthesize pathogenesis related proteins as a viable strategy (Van Loon et al. 1999) and other defense related or defense response protein. When challenged with a pathogen plants activate numerous host defence responses, which involve numerous biochemical changes within the host (Jones and Dangle, 2006). This defense mechanisms up to some extent include physical strengthening of the cell wall through lignification, suberization, and callose deposition; production of phytoalexins which are secondary metabolites, toxic to bacteria and fungi; and synthesis of pathogenesis-related (PR) proteins such as β-1,3-glucanases,osmotin, chitinases, defensin, thaumatin like

proteins (Bowles 1990).

The PR proteins are primarily expressed during stress related condition and predominantly during pathogen stress (Antoniw and Pierpoint 1978; Van Loon et al. 1994). The discovery of PR proteins was first done in tobacco plants which were infected with tobacco mosaic virus (van Loon and van Kammen). PR proteins are currently grouped into seventeen families based upon their properties Based on their primary structures, immunologic relationships, and enzymatic properties, PR proteins are currently grouped into seventeen families (PR-1 to PR-17) (Van Loon 1999; Görlach et al. 1996; Okushima et al. 2000; Christensen et al. 2002).The are responsible for the creation of systemic acquired resistance (SAR) during invasion by plant pathogens. Osmotin was originally isolated from salt-adapted tobacco cells (Singh et al. 1987). Additionally, several closely related proteins, usually referred to as osmotins and osmotin-like proteins, have been characterized in many plant species (King et al. 1988; Woloshuk et al. 1991; Anzlovar 2002).

Osmotin is grouped in to the PR-5 family (van Loon and van Strien, 1999). The PR-5 proteins from a variety of plant sources have been shown to inhibit fungal growth in vitro (Vigers et al. 1992; Salzman et al. 1998). They caused lysis of fungal spores, inhibition of hyphal growth and/or reduction of spore germination (Woloshuk et al. 1991; Abad, et al. 1996; Koiwa et al. 1997). Osmotin is known to cause sporangial lysis of Phytophthora infestans (Woloshik et al. 1991) by a oomycete membrane permeabilizing mechanism. Abad et al. (1996) reported that tobacco osmotin caused membrane leakage and dissipated the pH gradient across the cell wall/membrane of sensitive fungal species. In addition, transgenic plants constitutively expressing PR-5 proteins have been shown to exhibit enhanced disease resistance (reviewed in Velazhahan et al. 1999; Velazhahan and Muthukrishnan 2003). In this respect, PR-5 proteins must have an important role in plant defense against pathogens. β-1,3-glucanase belongs to PR-2 class and are able to catalyze endo-type hydrolytic cleavage of the 1,3-β-D-glucosidic linkages in β-1,3-glucans. β-1,3-Glucans are the major components of the cell walls of oomycetes (Wessels and Sietsma 1981). It mainly solublize elicitor active glucan molecules from the fungal cell wall (Mauch and Staechelin 1989). This is well documented for interactions between soybean and the β-glucan elicitor from the pathogenic oomycete Phytophthora megasperma f. sp. glycinea (Albersheim and Valent 1974). Cysteine rich proteins closely related to mammalian proteins are called as plant defensins (Thomma et al. 2002; Zasloff 2002; Lay and Anderson 2005). They are expressed in most, if not all, plants. Defensins bind specifically to the plasma membrane of fungi (Thevissen et al. 1997) and permeabilize them resulting in cell growth arrest (Thevissen et al. 1999). Receptor mediated signals are either transmitted through MAP kinases or directly to unidentified molecular factors eventually affecting the downstream processes. It is not clearly known if the interaction of plant defensins with pathogen cell wall components and/or plasma membrane components (other than sphingolipids) is required for entry into the pathogen cells. It remains to be determined if plant defensins have specific organelle and/or other subcellular targets inside the fungal cell. (Kaur et al 2011). Thaumatin-like proteins are having similiarity to the sweet protein thaumatin from the West African plant Thaumatococcus daniell (Iyengar et al. 1979). TLP are involved in plasma membrane permeabilization of sensitive pathogen, before this it binds to β-(1,3)-glucans (Abad et al. 1996) which usually requires direct insertion of the protein into fungal membranes to form transmembrane pores (Roberts and Selitrennikoff 1990).

MATERIALS AND METHODS

Piper colubrinum plants maintained in green house were used for this study. Young leaves were used for pathogen inoculation. Phytophthora capsici grown on carrot agar medium was used for inoculation. Isolate 05-06 and 98-93 maintained at the National Repository for Phytophthora at the Indian Institute of Spices Research were used for the present

study. Out of the two isolate, 98-93 isolate have been proven to be highly virulent (Vinitha et al unpublished results). 72 hour old culture was used for inoculation. The mycelial discs measuring 10 mm was cut out from the culture plates and was used for inoculation. The disc were kept below the surface of the leaves along with wet cotton and held together with cellophane tape. A total of nine treatments (different time intervals) were used for RNA extraction. These time intervals include 1, 2, 4, 8, 16, 24, 48, and 72 hours. An un inoculated leaf sample served as a control. Primer designing **(Table 1)** were done using Primer 3 software using sequence information obtained as a result of transcriptome sequencing done in Piper colubrinum challenge inoculated with P.capsici, by Indian Institute of Spices Research (Genotypic Technologies, Bengaluru).

Table 1: List of Primers Used for this Study

SL NO	Gene	Primer Sequences		Amplicon Length
1	OSMOTIN	5`-CTCCAACCTCGACTTCTTCG-3`	F	195
		5-`GCCGGCGTTGCAACAATACT-3`	R	
2	B-(1,3)-GLUCANASE	5`-CGATGGGTACGTACGTGAACAGTG-3`	F	190
		5`-CCGGCCTACAAAATGACATC-3`	R	
3	DEFENSIN	5`-GTTTCAACTTCCCTCCCACA-3`	F	164
		5`-ACTGAGCCACAGGTTCAAGG-3`	R	
4	THAUMATIN LIKE PROTEIN	5`GTACAAAACCCGGACCAGAA-3`	F	198
		5`-GTGGAGCGAGGTCAGAAAAG-3`	R	

RNA Extraction

Total RNA was extracted from the inoculated and control samples using kits manufactured by Sigma. Leaves homogenized in liquid nitrogen were used as a starting material. After the isolation the RNA quality was confirmed with the help of an agarose gel and simultaneously the concentration of the RNA was measured by spectrophotometer. The RNA was also given a DNase (Ambion) treatment to remove any contaminating DNA in the sample.

cDNA Synthesis

About two micrograms of RNA were used for cDNA synthesis. And cDNA synthesis was done according to standard procedure prescribed by the manufacturer.

Real Time PCR

Realtime PCR was done with 100ng cDNA. Quantifast SYBR green (Qiagen) was used and the PCR conditions and the concentration of the reagents were according to the manufacturer's instruction. Threshold cycle (C_T) value was determined mainly by using the software version 2.0.2 Rotorgene-Q and normalization of the data was done by actin gene.

RESULTS

The expression levels of osmotin, β-1,3 glucanase, defensin, thaumatin like protein known to play an effective role in plant defense, were compared by real time PCR technique in inoculated plants with respect to uninoculated control plants, during the interaction between Piper colubrinum and Phytophthora capsici at different time points. RNA samples were extracted from inoculated leaves at six time points after challenge inoculation with P.capsici strain (05-06) and another strain 98-93 which is known to be more virulent than 05-06. The time points chosen in this experiments were 1, 2, 4, 8, 16, 24, 48, 72 and 96 hours; and in case of defensin, 1 and 2 hour treatments were included as it showed high level of

expression in the preliminary experiments conducted. Prior to experimentation, the specificity of designed primers was checked with normal PCR and the products were run on agarose gels and it was found that the primers were working well with single distinct band produced in case of each primer.

Real time PCR experiments showed that accumulation of osmotin, β-1,3-glucanase defensin, thaumatin like protein RNA's were significantly higher in inoculated plants than that of the control plants. Plants inoculated with 05-06 strain showed higher level of expression of these defense genes when compared to the strain 98-93, except in the case of β-1,3-glucanase. Osmotin gene showed a peak expression at 72 hours post inoculation (hpi) and was down regulated at 96 hpi. In case of 98-93 higher expression was reached at 48 hpi **(Figure 1).** β-1,3- glucanase gene also showed an early induction at 16 hpi and reached its peak expression at 72 hpi and was showing a low level of expression at 96 hpi. In case of plants inoculated with 98-93 the peak expression was observed at 48 hours and was down regulated at 96 hpi **(Figure 2)**. Defensin gene showed an early induction and high expression at 1 hpi in both 05-06 and 98-93 strain inoculated plants **(Figure 3)**. Thaumatin like protein showed peak expression at 72 hpi in case of 05-06 and there was no significant expression in the case of treatments inoculated with 98-93 **(Figure 4).**

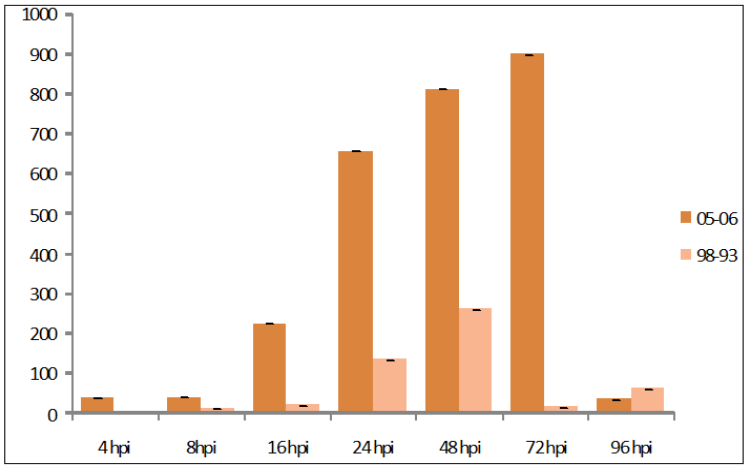

Figure 1: Graph for Relative Expression of Osmotin Gene

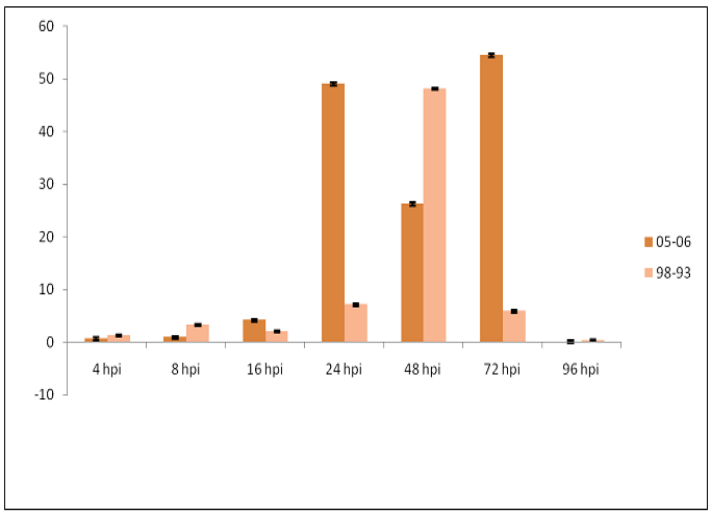

Figure 2: Graph for Relative Expression of β-1,3 Glucanase Gene

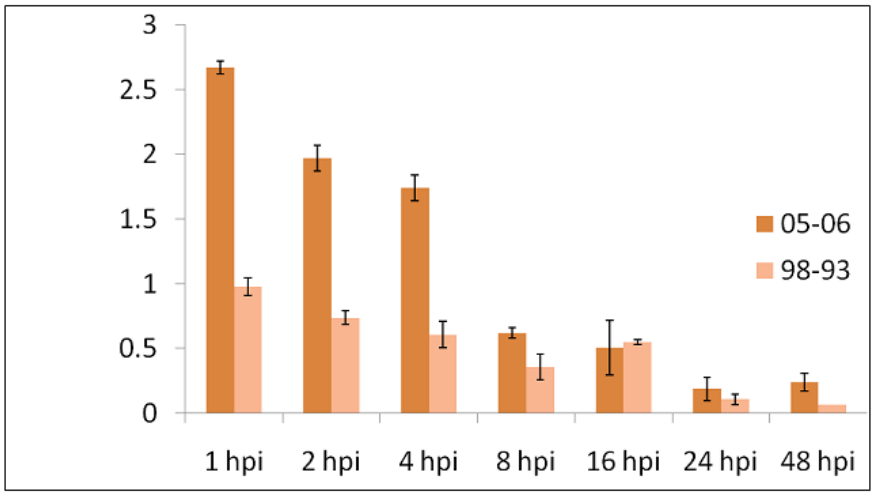

Figure 3: Graph for Relative Expression of Defensin Gene

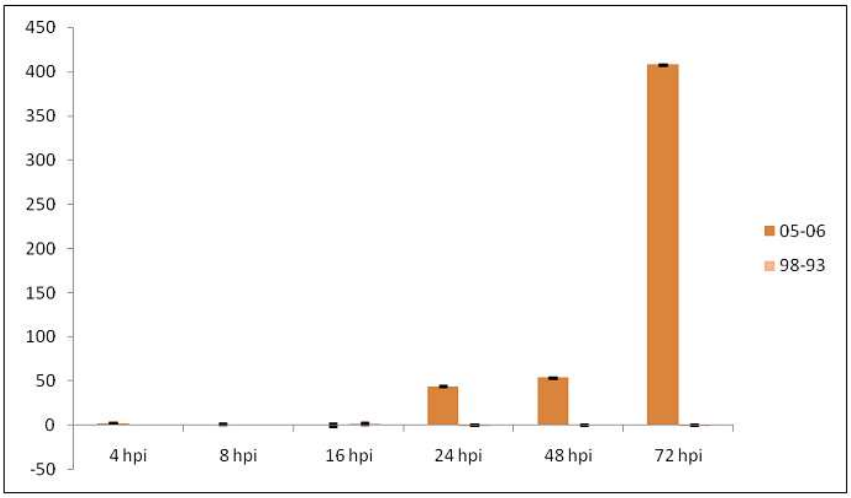

Figure 4: Graph for Relative Expression of Thaumatin like Protein Gene

DISCUSSIONS

Induction of genes encoding proteins with anti-oomycete activity is one of the strategies of the plant defense mechanism especially in resistant plants. Primary mRNA amounts, the rate, and the level of induction of these genes are three important factors to protect plants against pathogenic attacks. In this study we investigated the expression of some of the PR protein genes and other defense responsive genes in Piper colubrinum challenge inoculated with two Phytophthora capsici strains. An interesting finding in this research is that plants inoculated with the strain 05-06 showed higher level of expression for all the genes when compared to the strain 98-93, except in the case of β-1,3 glucanase gene. The low activity of defense genes in specific cases might be due to suppression of host defense genes by 98-93 strain found to be more virulent than the strain 05-06 (Vinitha et al unpublished results). The activity of effectors of the virulent pathogen might be one of the cause for this low expression as reviewed by (Brett Tyler 2009), where RxLR-DEER effectors from oomycetes caused suppression of defense responsive genes. P.infestans RXLR effector PexRD2 has evolved to interact with a specific host MAPKKK to perturb plant immunity–related signaling (King et al. 2014). Phytophthora sojae effectors have the peculiar ability to suppress RNA silencing in plants which eventually leads to enhanced plant susceptibility to Phytophthora infection (Qiao et al. 2013).

Pathogenesis Related proteins has been found systemically and locally in an infected plant (Van Loon et al. 2006). Among these osmotin which belongs to PR 5 group and β-1,3 glucanase belongs to PR 2 group are very vital for plant defense. Osmotins are thought to create transmembrane pores (Woloshik et al. 1991). Osmotin inhibits the germination and growth of an invading hyphae or the development of a functional haustorium in Phytophthora infestans (Woloshik et al. 1991) and β-1,3-glucanase attack β-1,3 glucans; components of the cell wall in fungi as well as in oomycetes. Here in this experiment significantly higher expression level of osmotin gene was observed (nearly 899 times compared to the control). Similiarly in Realtime PCR experiment in Piper colubrinum inoculated with the pathogen Phytophthora capsici showed induction of osmotin transcripts by more than 100 fold (Dicto and Manjula, unpublished results). β-1,3-glucanase showed early high level of expression at 24 hpi and peak expression was observed at 72 hpi. Glucanase have been reported to be strongly expressed at 24 hpi in pepper (Capsicum) cultivars inoculated with Phytophthora capsici (Silvar et al. 2007). Nazeem et al (2008) reported the role of β -1, 3 glucanase in the defense mechanism in foot rot tolerant variety of black pepper 'Kalluvally'. Significant induction of β-1,3-glucanase genes against Colletotrichum was observed in strawberry plants at 24 hpi and 48 hpi (Shi et al 2005). Defensin which is another important PR protein gene (PR 12) was expressed very early after challenge inoculation of plants with both the pathogen strains and expressed at higher levels at 1 hpi. This shows that defensin protein is an important component in disease resistant reaction probably required for membrane permeablization of pathogen at very early stage as defensin is known to cause membrane permeabilization. Defensins are known to have an important role in the first line of defense against pathogens (Terras et al. 1995). Defensin gene expression was observed to occur rapidly and strongly induced during early hours of infection of cowpea with cowpea severe mosaic virus (Padovan et al. 2009). Real time PCR analysis of defensin in transgenic lines of potato showed relative expression level ranging from 1 to 15 folds and was very effective against Phytophthora infestans infection (Portieles et al. 2010). Thaumatin like protein also showed late expression peak at 72 hpi. In Solanum tuberosum plants inoculated with Phytophthora infestans thaumatin like protein showed high level of expression at 72 hpi (Restrepo et al. 2005).

CONCLUSIONS

Real time PCR analysis of the above mentioned defense responsive genes, which showed high level of expression compared to un-inoculated plant, gives us some insight on the importance of these genes in defense reaction of Piper colubrinum against the oomycete pathogen Phytophthora capsici.

ACKNOWLEDGEMENTS

The authors acknowledge the generous grant provided by the Indian Council of Agricultural Research (ICAR) and Director, ICAR-Indian Institute of Spices Research (IISR) for providing the facilities for conducting the research.

The authors declare that they have no conflict of interest.

REFERENCES

1. *Abad LR, D'Urzo, MP, Liu D, Narasimhan ML, Reuveni M, Zhu JK, Niu X, Singh NK, Hasegawa PM, and Bressan RA (1996) Antifungal activity of tobacco osmotin has specificity and involves plasma membrane permeabilization. Plant Sci. 118:11–23*
2. *Agrios GN (1997) Plant Pathology. Academic Publishers, San Diego.*
3. *Albersheim P and Valent BS (1974) Host-pathogen interactions VII. Plant pathogens secrete proteins which inhibit enzymes of the host capable of attacking the pathogen. Plant Physiol. 53: 684-687.*

4. *Antoniw JF and Pierpoint WS (1978) The purification and properties of one of the 'b" proteins from virus-infected tobacco plants. J GenViro1. 39: 343-350.*
5. *Anzlovar S (1997) Permeabilizing activity of 25-kDa protein from flax seeds (Linum usitatissimum L.) on liposome membranes. M. Sc. Thesis. University of Ljubljana.*
6. *Bowles J D (1990) Defense-related proteins in higher plants. Annu. Rev. Biochem.59:873-907.*
7. *Boller T (1993) Antimicrobial functions of the plant hydrolases chitinase and ß-1,3-glucanase. In: Fritig B, Legrand M, eds. Developments In Plant Pathology, pp391-400.*
8. *Boller T (1995) Chemoperception of microbial signals in plant cells. Annu Rev Plant Physiol Plant Mol Biol. 46:189-214*
9. *Brett Tyler M (2009) Entering and breaking: virulence effector proteins of oomycete plant pathogens. Cellular Microbiology.11:13-20.*
10. *Christensen AB, Cho BH, Naesby M, Gregersen PL, Brandt J, Madriz-Ordenana,K, Collinge DB and Thordal-Christensen H (2002) The molecular characterization of two barley proteins establishes the novel PR-17 family of pathogenesis-related proteins.Mol.Plant Pathol. 3: 135-144.*
11. *Coca MA, Damsz B, Yun DJ, Hasegawa PM, Bressan RA and Narasimhan ML. 2000. Heterotrimeric G-proteins of a filamentous fungus regulate cell wall composition and susceptibility to a plant PR-5 protein. Plant J. 22: 61-69.*
12. *Görlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel KH, Oostendorp M, Staub T, Ward E, Kessmann H and Ryals J (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell. 8: 629-643.*
13. *Ibeas JI, Yun DJ, Damsz B, Narasimhan ML, Uesono Y, Ribas JC, Lee H, Hasegawa PM, Bressan RA and Pardo JM (2001) Resistance to the plant PR-5 protein osmotin in the model fungus Saccharomyces cerevisiae is mediated by the regulatory effects of SSD1 on cell wall composition. Plant J. 25:271-280.*
14. *Iyengar RB, Smith P, van der Ouderaa F, van der Wel H, van Brouwershaven J, Ravestein P, Richters G and van Wassenaar PD (1979) The complete amino-acid sequence of the sweet protein thaumatin I. Eur. J. Biochem. 96: 193-204.*
15. *Jones J D G and Dangl J L (2006) The plant immune system. Nature. 444:323-329.*
16. *Kaur J, Uma Shankar S, Dilip S (2011) Can plant defensins be used to engineer durable commercially useful fungal resistance in crop plants?. Fungal Biology Reviews. 25: 125-138.*
17. *King G, Turner VA, Hussey CE, Wurtele E (1988) Isolation and characterization of a tomato cDNA clone which codes for a salt-induced protein. Plant Mol. Biol. 401-412.*
18. *King SRF, McLellan H, Boevink P C, Armstrong M R, Bukharova T, Sukarta O, Win J, Kamoun S, Birch P RJand Banfield M J (2014) Phytophthora infestans RXLR Effector PexRD2 Interacts with Host MAPKKK to Suppress Plant Immune Signaling. The Plant Cell 1-15*
19. *Koiwa H, Kato H, Nakatsu T, Odu J, Yamada Y and Sato F (1997) Purification and characterization of tobacco pathogenesis-related protein PR-5d, an antifungal thaumatin-like protein. Plant Cell Physiol. 38:783-791.*
20. *Lay FT and Anderson MA (2005) Defensins - components of the innate immune system in plants. Curr. Protein Pept. Sci. 6:85-101.*
21. *NazeemPA,AchuthanCR,Babu,T.D,Parab GV,Girija,D,Keshavachandran R,Samiyappan R (2008) Expression of pathogenesis related proteins in black pepper (Piper nigrum L.) in relation to Phytophthora foot rot disease. J. Trop. Agr. 46: 45–51.*

22. Okushima Y, Koizumi N, Kusano T and Sano H (2000) Secreted proteins of tobacco cultured BY2 cells: identification of a new member of pathogenesis-related proteins. Plant Mol. Biol. 42: 479- 488.

23. Restrepo S, Myers KL, del Pozo O, Martin GB, Hart AL, Buell CR, Fry WE.and Smart CD(2005) Gene profiling of a compatible interactions between Phytophthora infestans and Solanum tuberosum suggest a role for carbonic anhydrase. MPMI. 18: 913-922.

24. Padovan L, Segat L, Tossi1 A, Calsa T, Kido E A, Brandao L, Guimaraes R L, Pandolfi V, Clara Pestana-Calsa M, Belarmino L C, Benko-Iseppon A M, Crovella S.Characterization of a new defensin from cowpea (Vigna unguiculata (L.) Walp.) (2009) Protein and Peptide Letters. 1-10

25. Portieles R,Camilo A, Ernesto G, Araiz G, Raisa R, Osmany C, Yunior L, Mayra R, Juan C, Merardo P, Gil E, Carlos B, Luis T, Bart PHJ and Orlando BH (2010) Nm Def 02, a novel antimicrobial gene isolated from Nicotiana megalosiphon confers high level pathogen resitance under green house and field conditions. Plant Biotechnology Journal. 8: 678–690.

26. Qiao Y et al (2013) Oomycete pathogens encode RNA silencing suppressors. Nature Genet. 45: 330–333

27. Roberts WK and Selitrennikoff CP (1990) Zeamatin, an antifungal protein from maize with membrane-permeabilizing activity. J Gen Microbiol. 1771–1778.

28. Salzman RA, Tikhonova I, Bordelon BP, Hasegawa PM and Bressan RA (1998) Coordinate accumulation of antifungal proteins and hexoses constitutes a developmentally controlled defense response during fruit ripening in grape. Plant Physiol. 117: 465-472.

29. Sela-Buurlage MB, Ponstein AS, Bres-Vloemans SA, Melchers LS, van den Elzen, PJM, Cornelissen BJC (1993) Only specific tobacco (Nicotiana tabacum) chitinases and ß-1,3-glucanases exhibit antifungal activity. Plant Physiol.101: 857-863.

30. Shi Y (2005)Isolation, Characterization, and Expression Analysis of β-1, 3-glucanase genes from strawberry plants. Ph.D thesis, Louisiana State University.

31. Singh N, Bracker CA, Hasegawa PM, Handa A, Hermodson MA, Pfankoch E, Regnier, FE and Bressan RA (1987) Characterization of osmotin. Plant Physiol. 85: 529-536.

32. Silvar C, Fuencisla M, Jose D(2008) Differential activation of defense-related genes in susceptible and resistant pepper cultivars infected with Phytophthora capsici. Journal of Plant Physiology. 65: 1120—1124.

33. Terras FRG, Eggermont K, Kovaleva V, Raikhel NV, Osborn RW, Kester A, Rees SB, Torrekens S, Van Leuven F, Vanderleyden J, Cammue BPA and Broekaert WF (1995) Small cysteine-rich antifungal proteins from radish: their role in host defense. Plant Cell. 7: 573-588

34. Thevissen K, Terras FR, Broekaert WF(1999) Permeabilization of fungal membranes by plant defensins inhibits fungal growth. Appl. Environ. Microbiol. 65: 5451-5458.

35. Thevissen K, Osborn RW, Acland DP, Broekaert WF(1997) Specific binding sites for an antifungal plant defensin from Dahlia (Dahlia merckii) on fungal cells are required for antifungal activity. Mol. Plant Microbe Interact. 13: 54-61.

36. Thomma BPHJ, Cammue BPA, Thevissen K (2002) Plant defensins. Planta. 216: 193-200.

37. Van Loon LC and Van Kammen A (1970) Polyacrylamide disc electrophoresis of the soluble leaf proteins from Nicotiana tabacum var. "Smsun" and "Samsun NN" II. Changes in protein constitution after infection with tobacco mosaic virus. Virology. 40: 199-206

38. Van Loon LC, Pierpoint WS, Boller T and Conejero V (1994) Recommendation for naming plant pathogenesis-related proteins. Plant Molecular Biology Reporter. 12: 245-264.

39. *Van Loon LC and Van Strien EA (1999) The families of pathogenesis- related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiological and Molecular Plant Pathology. 55: 85-97.*
40. *Van Loon LC (1999) Occurrence and properties of plant pathogenesis-related proteins. In "Pathogenesis-related proteins in plants", eds. Datta, S.K. and Mathukrishnan, S., CRC Press, Boca Raton. p1-19.*
41. *Vanitha SC, Niranjana SR and Umesha S (2009) Role of phenylalanine ammonia lyase and polyphenol oxidase in host resistance to bacterial wilt of tomato. J.Phytopathol. 157: 552–557.*
42. *Van Loon LC, Rep M and Pieterse CMJ (2006) Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 44: 135-162.*
43. *Velazhahan R, Datta SK and Muthukrishnan S (1999) The PR-5 family: Thaumatin-like proteins in plants. In S. K. Datta and S. Muthukrishnan (Eds), Pathogenesis-related proteins in plants, CRC press, Boca Raton. pp107-129..*
44. *Velazhahan R and Muthukrishnan S. (2003) Transgenic tobacco plants constitutively overexpressing a rice thaumatin-like protein (PR-5) show enhanced resistance to Alternaria. Biol. Plant. 47: 347-54.*
45. *Vigers AJ, Wiedemann S, Roberts, WK, Legrand M, Selitrennikoff CP, Fritig B (1992) Thaumatin-like pathogenesis-related proteins are antifungal. Plant Sci. 83: 155-161.*
46. *Wang X, El Hadrami A, Adam LR and Daayf F (2008) Differential activation and suppression of potato defence responses by Phytophthora infestans isolates representing US-1 and US-8 genotypes. Plant Pathology. 57: 1026–1037*
47. *Wang X, Zhu X, Tooley P and Zhang X (2013) Cloning and functional analysis of three genes encoding polygalacturonase-inhibiting proteins from Capsicum annuum and transgenic CaPGIP1 in tobacco in relation to increased resistance to two fungal pathogens. Plant Mol Biol. 81: 379–400.*
48. *Wessels JGH and Sietsma JH (1981) Fungal cell walls: a survey. In: Tanner W, Loewus FA, eds. Encyclopedia of Plant Physiology. Plant Carbohydrates II. Berlin:Springer Verlag. 13: 352-394.*
49. *Woloshuk CP, Meulenhoff JS, Sela-Buurlage M, Van den Elzen PJ and Cornelissen BJ (1991) Pathogen-induced proteins with inhibitory activity toward Phytophthora infestans. Plant Cell. 3: 619-628.*
50. *Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature. 415: 389-39*