

Expression of Proteins Related to *Phytophthora capsici* Tolerance in Black Pepper (*Piper nigrum L.*)

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Abstract – Vietnam is the number one black pepper exporter in the world. However, pepper production in Vietnam is facing challenges from natural disasters and soil-borne diseases, especially *Phytophthora* foot rot. Pepper leaves from five black pepper cultivars commonly grown in Vietnam were inoculated with *Phytophthora capsici*. The activities of pathogenesis-related proteins such as chitinase, β -1, 3-glucanase and phenylalanine ammonia lyase (PAL) significantly increased in the infected leaves compared to the healthy leaves. An SDS-PAGE analysis revealed the expression of two polypeptides with molecular masses of approximately 30 and <10 kDa in the *P. capsici*-infected leaves after 2 and 3 days following inoculation, respectively. Furthermore, western blot analysis confirmed the presence of a 34 kDa band corresponding to β -1, 3-glucanase in protein extracts from the infected leaves. This study suggests the involvement of β -1, 3-glucanase and related enzymes in the defense response of black pepper to fungal pathogens.

Keywords – Black Pepper, Chitinase, β -1, 3-glucanase, *Phytophthora Capsici*.

I. INTRODUCTION

Vietnam is currently the world's largest producer and exporter of black pepper (*Piper nigrum L.*), supplying 50% of the world's black pepper [1]. However, black pepper production in Vietnam has been challenged by natural disasters and plant diseases. The most common and devastating disease affecting Vietnam's pepper areas is foot rot or quick death caused by *Phytophthora capsici* [2]. This fungal strain is one of the most important phytopathogens in the world because it causes significant decreases in, or total losses of pepper crops. In some cases, the mortality rate of pepper vines was 100%. *Phytophthora* foot rot is rapidly spread and difficult to control. Plants infected by this pathogen die within 2-3 weeks in the rain, and adjacent plants may be infected within one or two months [3].

It has been reported that plant defense responses against invading phytopathogens (e.g., bacteria, fungi, or viruses) include the accumulation of a number of specific proteins known as pathogenesis related (PR) proteins [4]. They are low molecular weight thermostable proteins (6–43 kDa) that are selectively extractable and stable at low pH ~ 3 [5]. However, little is known about the tolerance mechanisms of Vietnam's pepper cultivars during pathogen infection.

In this study, the induction of PR proteins of *P. capsici* was evaluated in five commonly grown Vietnamese pepper cultivars. The results provide useful insights into the tolerance mechanisms of pepper to fungal disease, which is important information for black pepper breeding strategies focused on *Phytophthora* resistance.

II. MATERIALS AND METHODS

A. Preparation of Inoculum, Plant Growth and Inoculation

Mycelium from a single colony of *P. capsici*, grown in carrot agar plates for 4 days, was inoculated into 15 mL of carrot broth medium and incubated at 27°C for 48 h in the dark. Sporangium formation was observed under a light microscope (10X). Plates with a high concentration of sporangia were incubated at 4 °C for 1 h, then illuminated with an incandescent bulb for 30 min. Zoospores were collected by filtration and suspended in sterile distilled water at a concentration of 10⁴ zoospores/mL [6]. The third and fourth black pepper leaves from *ex vitro* grown plants of the varieties Vinh Linh, Trau, Se, Se Xanh and Kuching were used in this study. The leaves were surface sterilized by washing in sterile water and soaking in 70% alcohol for 30 s and then again washed 2-3 times with sterile water. For inoculation, wounds were made on the adaxial midribs of the pepper leaves using a sterile needle and inoculated with 40 μ L of *P. capsici* zoospore suspension. The infected leaves were then incubated at 27 °C in the dark for 5 days. Wounded leaves inoculated with autoclaved distilled water were used as controls.

B. Total Protein Extraction

Total leaf protein was extracted according to a previous study as in [7]. In brief, 0.5 g of leaves were ground to a fine powder in liquid nitrogen using a mortar and pestle. An extraction buffer was added to the leaf powder at a ratio of 1:2 (w/v). The buffer consisted of Tris (pH 8.0), 0.001% PMSF, 0.05 M cysteine-HCL, 10% PVP, 5% ascorbic acid, and 0.001% β -mercaptoethanol. The mixture was then centrifuged at 12,000 rpm for 5 min to collect the supernatant. The resulting supernatant was combined with Tris-phenol at a 1:1 (v/v) ratio, briefly vortexed, and centrifuged at 12,000 rpm for 5 min prior to adding 0.1 M ammonium sulfate (1:1, v/v). The mixture was incubated overnight at –20°C and then centrifuged at 12,000 rpm for 5 min. The pellet was collected and washed twice with 80% acetone and 70% ethanol, and then resuspended in TE buffer containing 0.001% PMSF. Protein concentration was measured using the Lowry assay with BSA as a protein standard. The concentration of BSA ranged from 0.02 - 0.1 mg/mL. The absorbance at 660 nm was recorded [8].

C. Enzyme assays

Chitinase activity - Chitinase was extracted from black pepper leaves using the method described in [8]. The activity of chitinase was determined by quantitative estimation of N-acetylglucosamine produced from a reaction between chitinase and colloidal chitin as

mentioned in [9] with minor modifications. Each reaction consisted of 0.4 mL of enzyme solution and 0.4 mL of 1% colloidal chitin in 1.2 mL of acetate buffer. Reactions were incubated for 30 min at 30°C, stopped by adding 0.4 mL of 1% NaOH and boiled for 3 min. These mixtures were subsequently centrifuged at 4,000 rpm for 5 min. The amount of reducing sugars released in the supernatant was measured based on a method that uses dinitrosalicylic (DNS) acid reagent, and the absorbance at 540 nm was measured [10].

Glucanase activity - Glucanase extraction from black pepper leaves was performed using a method as in [7]. Assays for β -1, 3-glucanase activity were based on the release of reducing sugar (glucose) from glucan as described by [11]. For each assay, enzyme extract (0.3 mL) was incubated with 0.3 mL of 1% glucan in 0.9 mL of acetate buffer at 35°C for 30 min. The reaction was stopped by boiling for 3 min. The mixture was centrifuged at 5,000 rpm for 5 min. The amount of reducing sugar released was measured using the DNS method [10].

Phenylalanine ammonia lyase (PAL) activity - PAL extraction from black pepper leaves was performed following a method by Nazeem *et al.* [7]. PAL activity was measured using the method suggested by Sadasivam and Manikam [12] with slight modifications. Briefly, 1 mL of L-phenylalanine solution was added to a mixture containing 0.5 mL of borate buffer, 0.2 mL of enzyme solution and 1.3 mL of distilled water. The mixture was incubated for 1 h at room temperature. The reaction was stopped by adding 0.5 mL of trichloroacetic acid and the absorbance at 290 nm was measured.

SDS-PAGE and western blot analysis - For SDS-PAGE analysis, 50 μ g of total protein was mixed with loading buffer (1:1, v/v) and boiled for 5 min, then loaded in each well on a 15% SDS polyacrylamide gel. Electrophoresis was started at 40 V to move the protein through the stacking gel and into the separating gel, then run at 80 V. The gel was stained with Coomassie Brilliant Blue for 1 h to observe the protein profiles.

For western blotting, the protein that was separated on the SDS-PAGE gel was electro blotted on to a PVDF membrane (0.45 μ m) following the procedure of Sambrook *et al.* [13]. The membrane was probed with PR2-GLU I-class I β -1, 3-glucanase (1:500, v/v), followed by goat-rabbit IgG, HRP conjugate (1:25000, v/v). For detection, the membrane was incubated with ECL substrate and exposed to X-ray film.

D. Data Analysis

Experiments were performed in triplicate. The results are expressed as the mean \pm SD (error bars) of three independent experiments. Statistically significant differences ($p < 0.01$) were determined using Statistical Analysis Systems 9.1 (SAS 9.1).

III. RESULTS & DISCUSSION

Changes during the time-course in the activities of chitinase, β -1, 3-glucanase and PAL in black pepper leaves inoculated with *P. capsici* are shown in Figure 1. In general, the *P. capsici* inoculum increased the activities of these enzymes in the infected leaves compared to the healthy controls. The increase in enzyme activities differed between pepper varieties ($p < 0.01$). The highest chitinase activities (Fig. 1A) were recorded at: 3 days post-inoculation (dpi) in the cultivar Vinh Linh (1.56 ± 0.05 μ g NAG/mg protein/min), 5 dpi in Trau pepper (3.00 ± 0.10 μ g NAG/mg protein/min), 1 dpi in Se (0.81 ± 0.03 μ g NAG/mg protein/min), 4 dpi in Se Xanh (1.63 ± 0.09 μ g NAG/mg protein/min), and 5 dpi in Kuching (1.72 ± 0.04 μ g NAG/mg protein/min). A similar increasing trend was observed for β -1, 3-glucanase activity (Fig. 1B), except that the highest activity in the Se variety was reached at 3 dpi (2.98 ± 0.18 μ g glucose/mg protein/min).

Many previous studies have demonstrated that the accumulation of PR proteins such as chitinase and β -1, 3-glucanase in plants is a defense mechanism in response to pathogen attack [7], [14] – [16]. The expression of these proteins is an important factor contributing to phytopathogen tolerance in plants [17]. The results of this study suggest that the pathogen infection might induce the expression of chitinase and β -1, 3-glucanase. This is consistent with a previous report by Lee *et al.* [18] that showed an increase in chitinase and β -1, 3-glucanase activities in *P. capsici*-treated leaves after 6 days of infection. Moreover, the increased level of chitinase [19] and activity of β -1, 3 glucanase [7] in *P. capsici* inoculated *P. nigrum* leaves after 48 h and 3 days, respectively, was reported. A higher level of β -1, 3 glucanase activity in the tolerant ‘Kalluvally’ (1.70 μ mol/mg protein/10 min.) compared to the susceptible ‘Panniyur-1’ (0.262 μ mol/mg protein/10 min.) was recorded by Nazeem *et al.* [7], while the chitinase activity of black pepper was found to be 40% higher than that of *P. colubrinum* under the same conditions [19]. The resistant genotype *P. colubrinum* possessed high β -1, 3 glucanase activity (>1.5 μ mol/mg protein/10 min.), but it did not show any significant variation following infection [7].

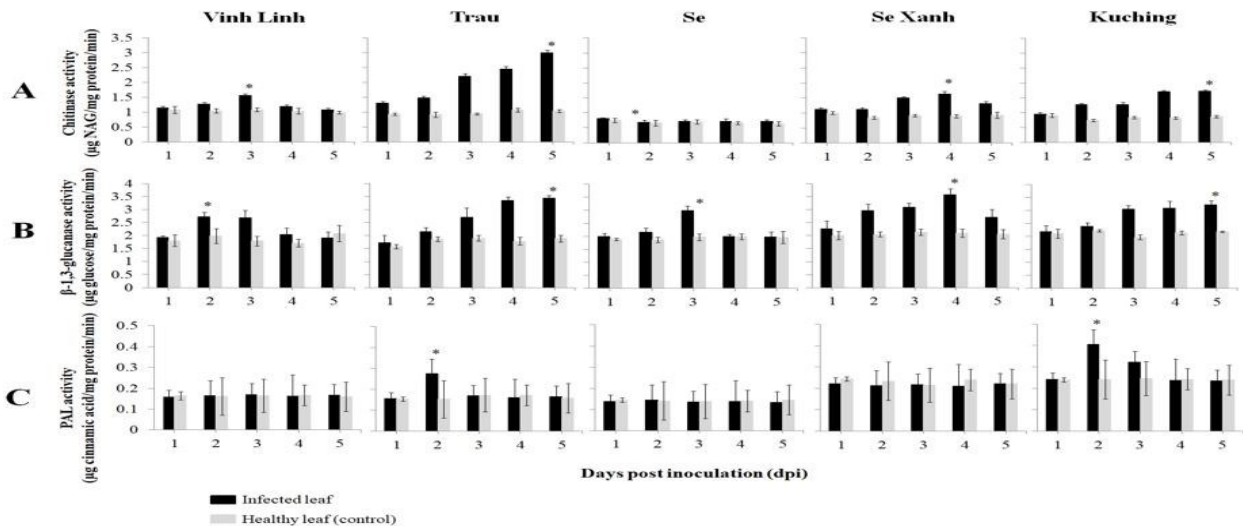


Fig. 1. Variations in activity of chitinase (A), β -1, 3-glucanase (B) and PAL (C) during *P. capsici* infection in the *P. nigrum* varieties Vinh Linh, Trau, Se, Se Xanh and Kuching. The asterisk denotes significant difference from the control ($p < 0.01$). NAG: N-acetylglucosamine.

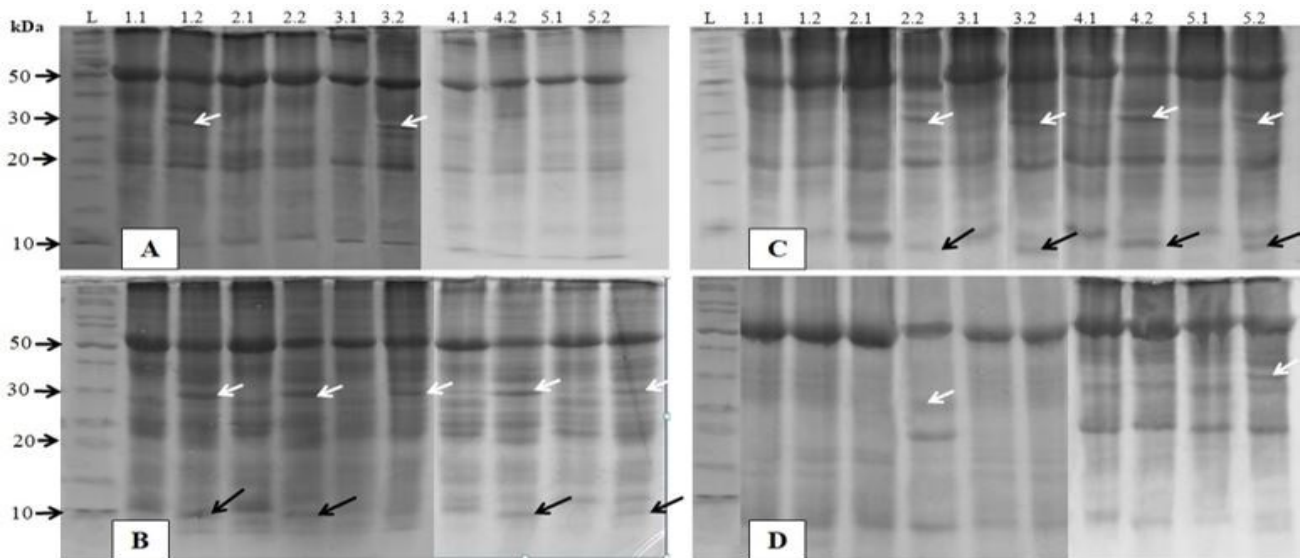


Fig. 2. SDS-PAGE analysis for the detection of *P. capsici* tolerance-related proteins in the *P. nigrum* cultivars (Lane 1.1) Vinh Linh-control, (Lane 1.2) Vinh Linh-infected, (Lane 2.1) Trau-control, (Lane 2.2) Trau-infected, (Lane 3.1) Se Xanh-control, (Lane 3.2) Se Xanh-infected, (Lane 4.1) Se-control, (Lane 4.2) Se-infected, (Lane 5.1) Kuching-control, (Lane 5.2) Kuching-infected after (A) 2, (B) 3, (C) 4 and (D) 5 dpi. L: ladder (Bio basic), white arrows: 30 kDa proteins, black arrows: <10 kDa proteins.

Chitinase and β -1, 3 glucanase are known to inhibit the growth of fungi by lysing fungal cell walls [20]. Antifungal activity of chitinases can be synergistically enhanced by β -1, 3-glucanase, both *in vitro* and *in vivo* [30]. β -1, 3-glucanases hydrolyzes the (1, 3)- β -D-glucosidic linkages in (1, 3)- β -glucans present in the fungal cell wall and chitinase reacts to a natural homopolymer of β -1, 4-linked N-acetyl-D-glucosamine (NAG) residues [21]. The differences in the expression of chitinase and β -1, 3-glucanase might be due to variations in *P. capsici* resistance among the pepper genotypes in terms of the timing and magnitude of the defense response [22].

PAL activity (Fig. 1C) did not change significantly after inoculation except in the Trau (0.275 ± 0.07 μ g cinnamic acid/mg protein/min) and Kuching (0.405 ± 0.07 μ g

cinnamic acid/mg protein/min) varieties. PAL is a key enzyme in the phenylpropanoid biosynthetic pathway that plays important roles in flavonoid production [23]. The activity of this enzyme is associated with the biosynthesis of defense-related metabolites, such as phytoalexins, phenols, lignins and salicylic acid [24]. Increases in PAL activity have been reported in plants under different types of stress [25]-[27]. However, in this study, the activity of PAL in infected leaves showed subtle change in the pepper varieties and significant increase in the Trau and Kuching varieties after 2 dpi. This result is consistent with the report by Zhang *et al.* [22] that there was no change in PAL activity in the *Capsicum annum* cultivars CM334 and PBC602 before or after inoculation, but a significant increase and peak in activity between 2 and 4 dpi. The

enhanced PAL activity might result from a hypersensitive response that occurs in many plants following wounding or infection [7], which causes the increased expression of related genes under pathogen stress, or different resistance mechanisms among pepper varieties. PAL involves in synthesis of salicylic acid which then activates expression of PR genes [28]. Delayed activity of chitinase and β -1, 3-glucanase up to the fifth day in Trau and Kuching may relate to the elevated activity of PAL at 2 dpi (Fig.1). The results of this study support the roles of chitinase, β -1, 3-glucanase and PAL in the suppression of *P. capsici* disease in *P. nigrum*. Previous studies have confirmed a significant role of β -1, 3-glucanase and PAL, but suggested less involvement from chitinase in the tolerance of black pepper to *Phytophthora* infection [7], [22]. It was also reported that chitinase and PAL are usually coexpressed with, and can induce β -1, 3-glucanase expression in pathogenesis-related responses [7], [29], [30]. This suggests that the interaction of PR proteins is important in defending against pathogen infection.

An SDS-PAGE analysis of total protein from *P. capsici* infected pepper leaves is shown in Figure 2. A protein of 30 kDa was observed in infected leaves of Vinh Linh and Se Xanh at 2 dpi (Fig. 2A). At 3 dpi, this protein was found in all infected varieties (Figure 2B) and was not found in the corresponding controls. However, by 4 dpi, the 30 kDa protein was not found in infected Vinh Linh (Figure 2C) and only Trau and Kuching peppers expressed this protein at 5 dpi (Figure 2D). In addition, a polypeptide <10 kDa was also observed in the infected pepper leaves at 3 and 4 dpi (Figure 2B and 2C). Western blot analysis (Figure 3) showed the presence of a 34 kDa protein in both infected and healthy leaves, which immunologically reacted to a PR2-GLU I-class I β -1,3-glucanase. The lowest expression of β -1, 3-glucanase was observed in the Se variety showing only a weak protein band on the membrane (Figure 3D). Relatively stable expression was found in the Vinh Linh (Figure 3A) and Se Xanh (Figure 3B) varieties, while the expression of β -1, 3-glucanase was clearly increased in Trau and Kuching (Figure 3C and 3E, respectively) after infection.

The expression of these proteins was consistent with the changes in enzyme activities, particularly β -1, 3-glucanase (Figure 1). At 2 dpi, there was a significant increase in β -1, 3-glucanase activity in Vinh Linh and Se Xanh varieties along with the presence of the 30 kDa protein, but the activity rapidly decreased to the control level at 4 and 5 dpi when the protein band was not observed in these varieties. Additionally, in Trau and Kuching varieties, increased β -1, 3-glucanase activity was accompanied by the presence of the 30 kDa protein from 3 to 5 dpi. The presence of the <10 kDa protein was found at 3 and 4 dpi when the activities of most enzymes were elevated compared to the controls. Consistent with these results, Nazeem et al. [7] detected a <10 kDa protein (8 kDa) in black pepper *P. capsici*-infected leaves after 3 days of infection. These results indicate the involvement of 30 kDa and <10 kDa proteins in the defense mechanisms induced by *P. capsici* inoculation. The Western blot analysis (Figure 3) also revealed that the expression of β -1, 3-glucanase was stronger in the infected

leaves compared to the healthy leaves, which again confirms the role of glucanase in response to pathogen infection in plants.

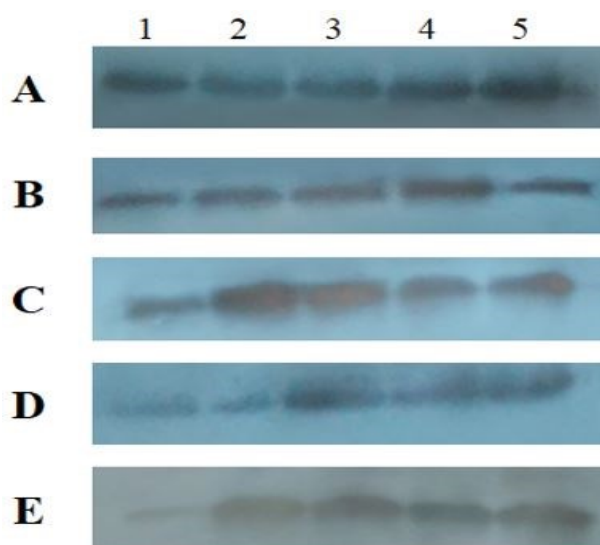


Fig. 3. Western blot analysis of β -1, 3 glucanase in the different pepper varieties (A) Vinh Linh, (B) Se Xanh, (C) Trau, (D) Se and (E) Kuching. Lane 1: healthy control, Lane 2, Lane 3, Lane 4 and Lane 5: 2, 3, 4, and 5 dpi of *P. capsici*, respectively.

In conclusion, the present study demonstrates the important roles of chitinase, β -1, 3-glucanase and PAL in the defense response of black pepper against foot rot disease caused by *Phytophthora*. The increases in the activities of these enzymes in pepper leaves upon *P. capsici* infection and the positive results in the SDS-PAGE and western blot analyses confirmed the involvement of β -1, 3-glucanase and related enzymes in black pepper's tolerance to pathogens. Molecular mechanisms leading to the expression of these genes in black pepper should be studied further to gain a better understanding of the cultivar-specific responses to *Phytophthora* species.

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REFERENCES

- [1] Vietnam Pepper Association. Report: "Pepper sector in 2014 and prediction in 2015", 2014. Available: <http://www.peppervietnam.com>.
- [2] N. T. Ton, T. N. T. Nam, L. D. Don, M.V. Tri, N. M. Hieu, and N. B. Phuong, "Study on the scientific, technological and marketing measures for the development of black pepper production serving to processing and export", *Final report of national research project*, KC.06.11.NN, MOST (in Vietnamese), 2005.
- [3] D. Manohara, and M. Rizal. "Pests and diseases on pepper in Indonesia and their management". Paper presented at the symposium on pests and diseases on pepper. Sarawak, Malaysia, 2002.

- [4] M. S. Castro, and W. Fontes, "Plant defense and antimicrobial peptides", *Protein and Peptide Letters* 12, 2005, pp. 13–18.
- [5] L. C. van Loon. "Occurrence and properties of plant pathogenesis-related proteins". In: *Pathogenesis-related proteins in plants*, S. K. Datta and S. Muthukrishnan, Eds. Florida: CRC Press LLC, 1999, pp. 1–19.
- [6] L. B. Charles, B. D. Bruton, M. M. Wall, and M. Rivas, "Phytophthora capsici zoospore infection of pepper fruit in various physical environments", *Acad. Sci.* 75, 1995, pp. 1–5.
- [7] P. A. Nazeem, C. R. Achuthan, T. D. Babu, G. V. Parab, D. Giriya, R. Keshavachandran, and R. Samiyappan, "Expression of pathogenesis related proteins in black pepper (*Piper nigrum* L.) in relation to *Phytophthora* foot rot disease", *J. Trop. Agr.* 46 (1-2), 2008, pp. 45–51.
- [8] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent", *J. Biol. Chem.* 193(1), 1951, pp. 265–275.
- [9] X. Zhu, Y. Zhou, and J. Feng, "Analysis of both chitinase and chitosanase produced by *Sphingomonas* sp. CJ-5", *J. of Zhejiang Univ. Sci. B* 8(11), 2007, pp. 831–838. doi: 10.1631/ jzus.2007. B0831
- [10] G.L. Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugar", *Anal. Chem.* 31(3), 1959, pp. 426–428. doi: 10.1021/ac60147a030
- [11] W. Hong, S. Hitoshi, and I. Kiyoshi, "Purification and characterization of β -1, 6- glucanase of *Streptomyces rochei* application in the study of yeast cell wall proteins", *Biosci. Biotechnol. Biochem.* 66, 2002, pp. 2515–2519.
- [12] S. Sadasivam, and A. Manikam, "Phenylalanine ammonia lyase". In: *Biochemical methods for agricultural science*. New Delhi: Wiley Eastern Ltd., 1992, pp.134–135.
- [13] J. Sambrook, E. F. Fritsch, and T. Maniatis, *Molecular Cloning: A laboratory Manual*, 2nd edn, Cold Spring Harbor Laboratory Press, NY, 1989, pp. 4.26–4.32.
- [14] Z. Liu, L. Shi, S. Yang, Y. Lin, Y. Weng, X. Li, A. Hussain, A. Noman, and S. He, "Functional and promoter analysis of *ChilV3*, a chitinase of pepper plant, in response to *Phytophthora capsici* infection", *Int. J. Mol. Sci.* 18(8), 2017, pp. 1661. doi: 10.3390/ijms18081661.
- [15] S. S. Pareek, I. Ravi, and V. Sharma, "Induction of β -1, 3- glucanase and chitinase in *Vigna aconitifolia* inoculated with *Macrophomina phaseolina*", *J. Plant Interact.* 9(1), 2014, pp. 434–439, doi: 10.1080/17429145.2013.849362.
- [16] I. Thanseem, A. Joseph, and A. Thulaseedharan, "Induction and differential expression of -1,3- glucanase mRNAs in tolerant and susceptible *Hevea* clones in response to infection by *Phytophthora meadii*", *Tree Physiology* 25, 2005, pp. 1361–1368.
- [17] D. L. Funnell, C. B. Lawrence, J. F. Pedersen, and C. L. Schardl, "Expression of the tobacco β -1, 3- glucanase, PR-2d, following induction of SAR with *Peronospora tabacina*", *Physiol. Mol. Plant Pathol.* 65, 2004, pp. 285–296. doi:10.1016/j.pmp.2005.02.010
- [18] H. J. Lee, K. H. Park, J. H. Shim, R. D. Park, Y. W. Kim, J. Y. Cho, et al., "Quantitative changes of plants defense enzymes in bio control of pepper (*Capsicum annuum* L.) late blight by antagonistic *Bacillus subtilis* HJ927", *J. Microbiol. Biotechnol.* 15(5), 2005, pp. 1073–1079.
- [19] Sandeep Varma, R., Johnson George, K., Balaji, S., & Parthasarathy, V. A. (2009). Differential induction of chitinase in *Piper colubrinum* in response to inoculation with *Phytophthora capsici*, the cause of foot rot in black pepper. *Saudi Journal of Biological Sciences*, 16(1), 11-16. doi: 10.1016/j.sjbs.2009.07.002
- [20] A. B. Zemanek, T. S. Ko, K. Thimmapuram, A. Freddi, F. A. Hammerschlag, and S. S. Korban. (2002) "Changes in β -1,3- glucanase mRNA levels in peach in response to treatment with pathogen culture filtrates, wounding and other elicitors", *J. Pl. Physiol.* 159, , pp. 877–889.
- [21] A. Kasprzewska (2003). Plant chitinases - regulation and function. *Cell Mol. Biol. Lett.* 8, pp. 809–824.
- [22] Y. L. Zhang, D. W. Li, Z. H. Gong, J. E. Wang, Y. X. Yin, and J. J. Ji, "Genetic determinants of the defense response of resistant and susceptible pepper (*Capsicum annuum*) cultivars infected with *Phytophthora capsici* (Oomycetes; Pythiaceae)", *Genet. Mol. Res.* 12(3), 2013, pp. 3605–3621.
- [23] H. M. El-Shora (2002). Properties of phenylalanine ammonia lyase from marrow cotyledons. *Plant Sci.* 162, pp. 1–7.
- [24] M.B. Mauch, and A.J. Slusarenko (1996). Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of *Arabidopsis* to *Peronospora parasitica*. *Plant Cell* 8(2), pp. 203–212.
- [25] R. A. Dixon, "The phytoalexin response: Elicitation, signalling and the control of host gene expression", *Biol. Rev.* 61, 1986, pp. 239–291.
- [26] K. Hahlbrock, and D. Scheel, "Physiology and molecular biology of phenylpropanoid metabolism", *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 1989, pp. 347–369.
- [27] E. Koç, and A. Sülün üstün, "Phenylalanine ammonia lyase activity in stem of pepper (*Capsicum annuum* L.) infected by *Phytophthora capsici* L.", *GUJ. Sci.* 25(2), 2012, pp. 307–312.
- [28] Chen, Z., Zheng, Z., Huang, J., Lai, Z., & Fan, B. (2009). Biosynthesis of salicylic acid in plants. *Plant Signaling & Behavior*, 4(6), 493-496.
- [29] Y. Takeuchi, M. Yoshikawa, G. Takeba, K. Tanaka, D. Shibata, and O. Horino, "Molecular cloning and ethylene Induction of mRNA encoding a phytoalexin elicitor-releasing factor, β -1,3- endoglucanase, in soybean", *Plant Physiology* 93(2), 1990, pp. 673–682.
- [30] L.C. van Loon. "Induced resistance in plants and the role of pathogenesis-related proteins". *Eur J Plant Pathol* 103, 1997, pp. 753–765.

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