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Full Length Research Paper

Induction of phenolics, lignin and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors

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Elicitors are capable of mimicking the perception of a pathogen by a plant, thereby triggering induction of a sophisticated defense response in plants. In this study, we investigated an induced resistance in eggplant in respect to cell wall strengthening and defense enzyme activation affected by four elicitors such as, chitosan (CHT), salicylic acid (SA), methyl salicylate (MeSA) and methyl jasmonate (MeJA). The increase in total phenolic content of eggplant roots by the elicitors was significantly higher. Lignin deposition in the cell wall of eggplant roots was increased 5, 4, 3 and 3 times by SA, CHT, MeJA and MeSA at 96 h of elicitation, respectively. Phenylalanine ammonia-lyase (PAL) activity showed an increase of 4.7, 3.7, 3.5 and 3.2 times by SA, CHT, MeJA and MeSA at 36 h of elicitation, respectively. Highest activity of peroxidase (POD) was observed at 24 h after elicitation under the precise influence of CHT and SA. The activities of polyphenol oxidase (PPO), cinnamyl alcohol dehydrogenase (CAD) and catalase (CAT) were also increased several folds by the elicitors. Accumulation of phenolics and lignin in high amounts, together with higher level activity of major defense enzymes in response to the elicitors, may bolster eggplants in mounting practical and effective resistance against *Ralstonia solanacearum*, the devastating wilt pathogen.

Key words: Catalase, chitosan, cinnamyl alcohol dehydrogenase, lignin, methyl jasmonate, methyl salicylate, phenylalanine ammonia lyase, peroxidase, salicylic acid, *Solanum melongena*.

INTRODUCTION

Eggplant or brinjal (*Solanum melongena* L.) is an important and widely consumed vegetable crop of India grown round the year. It is stated that plant diseases pose a threat to crop production, because, at least, 10% of global food production is lost to plant diseases (Strange and Scott, 2005). Eggplant suffers heavy yield losses due to many diseases like bacterial wilt, little leaf, *Phomopsis* blight, *Verticillium* wilt, sclerotinia blight, *Fusarium* wilt and leaf spots. Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi is one of the most serious diseases of eggplant and many other crops in tropical, subtropical and warm temperate regions of the world (Hayward, 1991). The pathogen is soil-borne with a wide host-range and very difficult to control. Management strategies like crop rotation, adjusting the date of planting, cultural methods and soil treatment are not effective against the pathogen (Chellemi et al., 1997). Though biological control of bacterial wilt disease has been reported using antagonistic bacteria, no effective control measure is available for this pathogen in any of its hosts.

Elicitors are molecules that are capable of mimicking the perception of a pathogen by a plant, thereby triggering induction of a sophisticated defense response in plants. Chitosan (CHT), a deacetylated chitin derivative, behaves like a general elicitor, inducing a non-host resistance and priming a systemic acquired immunity (Iriti

Abbreviations: CHT, Chitosan; **SA,** salicylic acid; **MeSA,** methyl salicylate; **MeJA,** methyl jasmonate; **PAL,** phenylalanine ammonia-lyase; **PPO,** polyphenol oxidase; **CAD,** cinnamyl alcohol dehydrogenase; **CAT,** catalase; **POD,** peroxidise; **JA,** jasmonic acid; **AIR,** alcohol insoluble residue; **TGA,** thioglycolic acid; **PVP,** polyvinyl pyrrolidone; **ASM,** acibenzolar-*S*-methyl; **SABP2,** salicylic acid-binding protein 2.

and Faoro, 2009). CHT treatment induced a significant increase in the activities of polyphenol oxidase (PPO) and peroxidase (POD), and enhanced the content of phenolic compounds in tomato fruits, thus providing protection against both gray and blue mould diseases (Liu et al. 2007). Mandal et al. (2009) demonstrated that exogenous application of salicylic acid (SA) could induce resistance against *Fusarium oxysporum* f. sp. *lycopersici* in tomato. The volatile derivative of SA and methyl salicylate (MeSA), is also involved in plant defense and recent grafting experiments, which suggested that MeSA is a critical, long-distance, phloem-mobile SAR signal in tobacco (Park et al., 2007). It has been shown that jasmonic acid (JA) plays a crucial role in protecting *Arabidopsis* from pathogens such as *z mastophorum* (Vijayan et al., 1998). However, JA is unlikely to be a solitary signal *in vivo*; whereas its volatile counterpart, methyl jasmonate (MeJA), is a powerful cellular regulator in plant tissues (Farmer et al., 1998).

The constitutive defenses of plants include structural barriers, such as the plant cell wall, as well as inhibitory compounds including phenolics (Nürnberger et al., 2004). Phenolic compounds can be formed in response to the ingress of pathogens and their appearance is considered as part of an active defense (Nicholson and Hammerschmidt, 1992). Evidence strongly suggests that esterification of phenols to cell-wall materials is a common theme in the expression of plant resistance and it has been suggested that crosslinking of such phenylpropanoid esters leads to the formation of lignin-like polymers (Fry, 1987). Phenylalanine ammonia lyase (PAL) is a key enzyme of phenylpropanoid metabolism in plants. PAL activity in plant tissue may rapidly change under the influence of various factors, such as pathogen attack and treatment with elicitors (Dixon and Lamb, 1990). However, POD is a phenol oxidizing enzyme. The activities of PAL and POD may rapidly be enhanced under the influence of elicitors or pathogen attack. Enhancement of PAL and POD activities was reported in response to *Rhizoctonia solani* inoculation in cowpea pretreated with SA (Chandra et al., 2007). PPO is a copper-containing enzyme with molecular oxygen as co-substrate. The role of PPO has been demonstrated in phenol metabolism and in defense mechanisms against pathogens (Lax and Cary, 1995). In the last step of monolignol biosynthesis, coniferaldehyde and sinapaldehyde are converted into their corresponding alcohols by cinnamyl alcohol dehydrogenase (CAD) (Santos et al., 2006). This enzyme is an indicator of lignin biosynthesis because of its specific role at the end of the monolignol biosynthetic pathway (Moerschbacher et al., 1990). Catalase (CAT) was shown to be involved in the regulation of H_2O_2 levels in plant tissues, thus avoiding cellular disintegration (Bolwell and Wojtaszek, 1997).

Induction of resistance in plants by elicitors is increasingly becoming a promising approach for management of plant diseases. However, there have been no reports on induction of resistance in eggplant against *R. solanacearum* by elicitors. In this study, we investigated an induced resistance in eggplant in respect to cell wall strengthening and defense enzyme activation affected by CHT, SA, MeSA and MeJA.

MATERIALS AND METHODS

Chemicals

All chemicals used in sample preparation were of analytical grade, while all authentic standards were procured from Sigma-Aldrich Chemical Co. Ltd (Bangalore, India).

Plant material

Seeds of eggplant (cv. Utkal Anushree) were obtained from Orissa University of Agriculture and Technology, India. Plants were grown in a fertile and irrigated plot in an open ambient climate. Roots were collected from uniformly grown plants at flowering stage for use in all elicitation experiments (Mandal and Mitra, 2007).

Preparation of elicitors

Four elicitors used in the elicitation experiment were CHT, SA, MeSA and MeJA. CHT was prepared essentially as described by Villegus and Brodelius (1990). SA (Mandal et al., 2009), MeSA and MeJA were dissolved in sterile distilled water (with 10% methanol) at 0.01% concentration.

Elicitation of eggplant roots

Elicitation of eggplant roots was performed according to the cotton leaf disk elicitation experiment of Dubery and Slater (1997). Small root fragments (approximately 50 mm) were cut from eggplants at flowering stage. The root fragments were washed several times with sterile distilled water to remove dirt and wound metabolites and then immersed in 20 ml of elicitor solution under sterile conditions in test tubes. The elicitor solution consisted of 2 ml of elicitor in 18 ml of sterile distilled water. Control root fragments were immersed in 20 ml sterile distilled water. Petri dishes with root fragments were incubated for 0, 12, 24, 36, 48, 72, 96 and 120 h at 25°C in the dark. All experiments were performed in three replications.

Estimation of total phenolics in the roots of eggplant

The total phenolic content was determined as described by Waterman and Mole (1994) using Folin-Ciocalteau reagent. The reaction mixture contained 100 µl methanolic extract of eggplant root tissues and 200 µl sterile distilled water with 500 µl of Folin-Ciocalteau reagent. After 5 min, 800 µl of 20% sodium carbonate (Na_2CO_3) was added, and after 1 h of incubation, the absorbance was measured at 254 nm in a BioMate^{TM} 3 spectrophotometer (Thermo Spectronic, USA). As a result, the standard curve was prepared with *p*-hydroxybenzoic acid in 50% (v/v) methanol. The total phenolic content was expressed as micrograms of *p*-hydroxybenzoic acid equivalent/g FW of tomato root tissues.

Determination of lignin in the roots of eggplant

Lignin was extracted according to the method of Bruce and West (1982) with modifications. Root samples were homogenized in 80% methanol. The homogenate was filtered through Whatman no. 4

filter paper and rinsed with methanol; then the residue was dried at 60°C for 24 h. The dried alcohol insoluble residue (AIR) was used for lignin determination. Subsequently, 5 ml HCl (2 N) and 0.5 ml thioglycolic acid (TGA), respectively, were added to 50 mg of AIR in glass screw-cap vials and the mixture was placed in boiling water for 4 h. The mixture was then centrifuged at 10 000 × g for 30 min and the pellet was washed with 5 ml deionised water. The resulting pellet was suspended in 5 ml of 0.5 N NaOH, shaken at 25°C for 2 h and then centrifuged at 10 000 × g for 30 min. Concentrated HC1 (1 ml) was added to the supernatant, and the lignin-thioglycolic acid was allowed to precipitate at 4°C for 4 h. After centrifugation at 10 000 × g for 10 min, the orange-brown pellet was dissolved in 10 ml of 0.5 N NaOH. It was again centrifuged and the absorbance of TGA-derivatives in the supernatant was measured at 280 nm. Results were expressed as the increase in $A_{280\ nm}$/g of AIR FW.

PAL activity assay in the roots of eggplant

Enzyme extraction steps were carried out at 4°C. However, 1 g fresh weight of root tissue was crushed in liquid nitrogen in the presence of 20% (w/w) polyvinyl pyrrolidone (PVP) and then extracted with 5 ml of 100 mM homogenization buffer (Tris-HCl, pH 8.0). The suspension was homogenized for 1 min and then centrifuged at 10 000 × g for 30 min. PAL was assayed directly in the supernatant after concentration through AmiconR™ Ultra-4 CFU membrane (Millipore, Bedford, USA). Subsequently, 200 mM Tris-HCl (pH 7.0) was used as assay buffer and 20 mM L-phenylalanine as substrate of the enzyme in the assay. PAL activity was assayed using a modified method of Sainders and McClure (1975). The reaction was carried out for 60 min at 37°C and the increase in A_{290} nm was recorded at every 15 min interval. The rate of formation of *t*-cinnamic acid was taken as a measure of enzyme activity using an increase in absorbance of 0.01 at A_{290} nm as 3.09 nmol of *t*-cinnamic acid formed. The PAL activity was expressed in nkat/mg protein. Protein concentration was measured according to the standard method of Bradford (1976).

Assay of POD activity in the roots of eggplant

POD activity was assayed as per Chance and Maehly (1955). The enzyme activity was determined by measuring the increase in absorbance at 470 nm due to oxidation of guaiacol to tetraguaiacol. The reaction mixture consisted of 20 mM guaiacol (0.5 ml), 0.1 mM acetate buffer (pH 5.0; 2.1 ml), 40 mM H_2O_2 (0.2 ml) and enzyme extract (0.2 ml) with a final volume of 3 ml. The linear portion of the activity curve was used to calculate enzyme activity. One unit of enzyme activity represented the amount of enzyme catalyzing the oxidation of 1 µmol of guaiacol in 1 min.

PPO activity assay in the roots of eggplant

Eggplant roots were homogenized (1:2 w/v) in 0.1 M potassium phosphate buffer (pH 6.8). The homogenate was centrifuged at 10 000 rpm for 30 min at 4°C. The supernatant was used for enzyme assay. The reaction mixture consisted of 1 mM catechol in 0.05 M sodium phosphate buffer (pH 6.5) and 500 µl enzyme extract, whereas the reference contained only catechol. PPO activity was determined by monitoring the increase in absorbance at 405 nm (Gauillard et al. 1993). The linear portion of the activity curve was used to express enzyme activity. One unit was defined as a change in absorbance of 0.001 under the assay conditions.

Assay of CAD activity in the roots of eggplant

The enzyme was extracted in 0.1 M Tris-HCl (pH 7.5) containing 15 mM β-mercaptoethanol, polyethylene glycol (10% v/v) and 5% polyclarT. Root fragments were homogenized (1:2 w/v) and centrifuged at 10 000 rpm for 30 min at 4°C. The supernatants were used for enzyme assay. The reaction mixture consisted of 2.5 ml 0.1 M Tris-HCl (pH 8.8), 200 µl 0.1 M Tris-HCl (pH 8.8) containing 3 mM coniferyl alcohol, 200 µl 0.1 M Tris-HCl (pH 8.8) containing 6 mM NADP and 100 µl enzyme extract. CAD activity was measured following the oxidation of coniferyl alcohol at 30°C and the formation of coniferaldehyde at 400 nm (Wyrambik and Grisebach, 1975).

CAT activity assay in the roots of eggplant

Eggplant roots were homogenized in 10 ml of chilled 0.1 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 4°C for 30 min at 10 000 rpm. After concentration through AmiconR™ Ultra-4 CFU membrane (Millipore, Bedford, USA), the supernatant portion was used as enzyme extract for the determination of CAT activity. The activity was assayed by measuring the rate of disappearance of H_2O_2 at 240 nm using a BioMate™ 3 spectrophotometer (Thermo Spectronic, USA). The reaction mixture (2 ml) consisted of 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.2 ml enzyme extract. One unit was defined as a change in absorbance of 0.1 under the assay conditions (Cakmak and Marschner, 1992).

RESULTS

Effect of elicitors on total phenolic content in the roots of eggplant

Total soluble phenolic content was measured in the eggplant roots from elicited and control samples (non-elicited roots). All four elicitors such as, CHT, SA, MeSA and MeJA could increase the total phenolic content in the roots from 12 h of elicitation when compared to the corresponding control. However, SA emerged the most effective elicitor as total phenolic content was highest in SA-treated eggplant roots through the time course study (Figure 1). At 48 h of elicitation, the increase in total phenolic content of roots was 10 times by SA, 8 times by CHT, 7.5 times by MeJA and 6.5 times by MeSA in comparison to control. There was a gradual decrease in the levels of phenolic content with passage of time after 48 h, but the level returned to the level of control in the case of none elicitors.

Effect of elicitors on lignin deposition in the roots of eggplant

Lignin deposition was measured in the eggplant roots from elicited and control samples after elicitation on a time course. It was observed that all elicitors could increase lignin deposition in eggplant roots in varying degrees, starting from 12 h of elicitation, when compared to the corresponding control. Similar to the total phenolic content, it was found that the maximum increase in lignin synthesis was orchestrated by SA (Figure 2). Lignin deposition in the cell wall of eggplant roots was increased

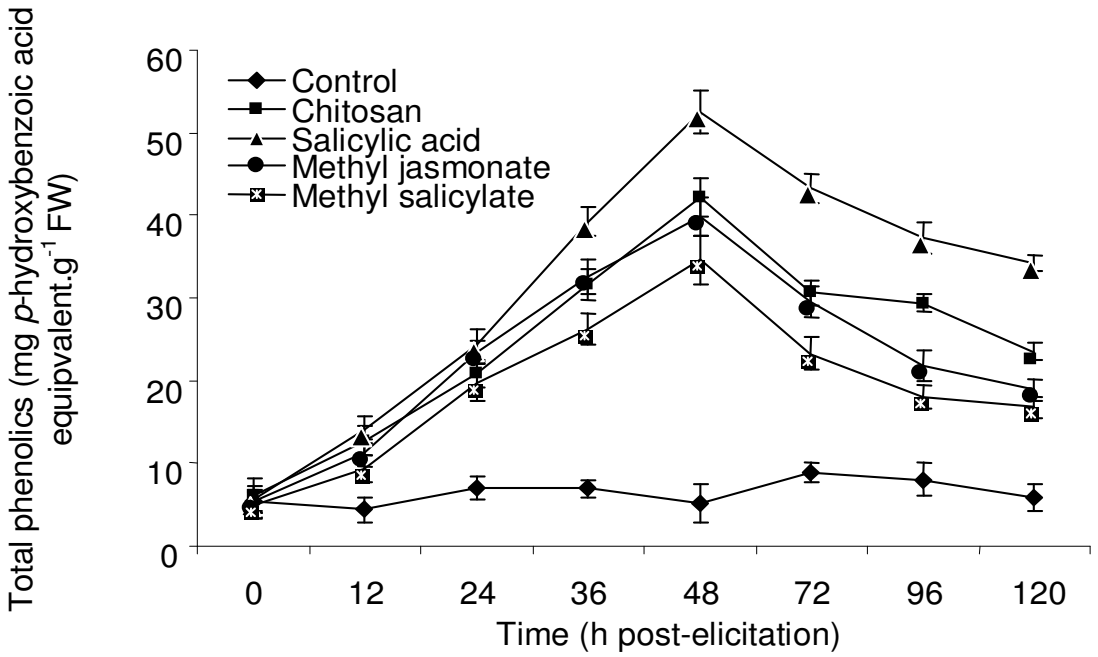


Figure 1. Total phenolics content (expressed as mg *p*-hydroxybenzoic acid equivalent/g FW) in roots of eggplants on a time course after elicitation of the plants with different elicitors as compared with the control. Data presented in graphs are the means ± SD of three replicates.

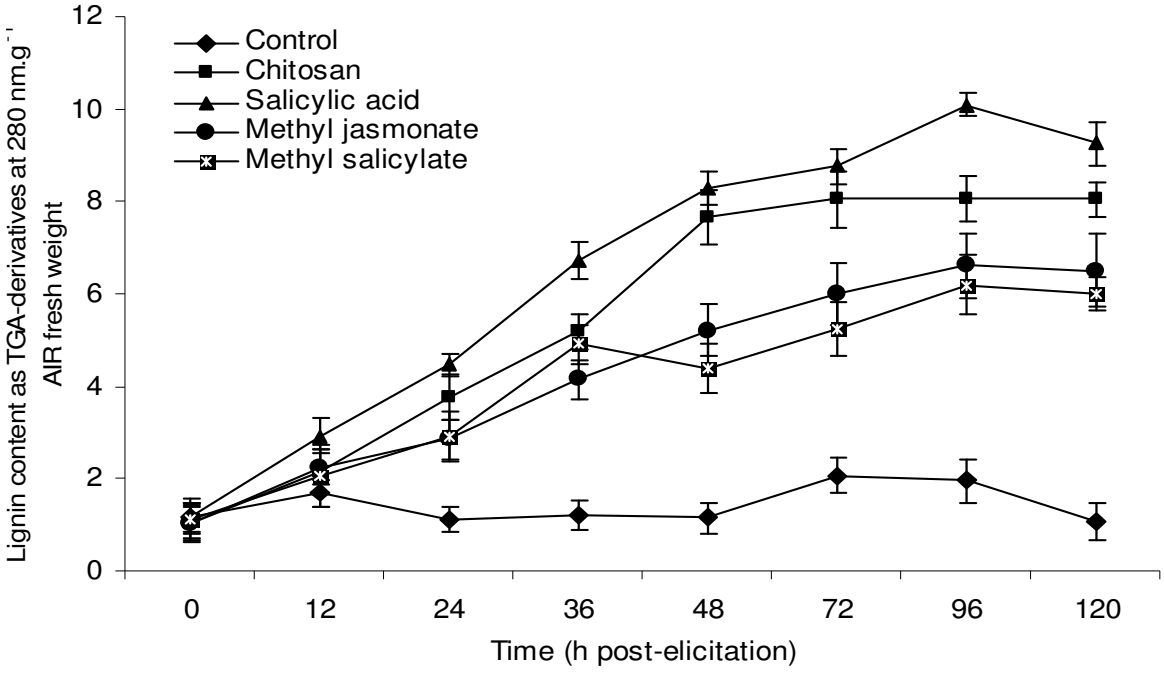


Figure 2. Deposition of lignin (expressed as thioglycolic acid derivatives at 280 nm/g alcohol insoluble residue (AIR) FW) in the cell wall of eggplants' roots on a time course after elicitation of the plants with different elicitors as compared with the control. Data presented in graphs are the means ± SD of three replicates.

5, 4, 3 and 3 times by SA, CHT, MeJA and MeSA at 96 h of elicitation, respectively. After this point, there was marginal fall in lignin deposition in the root cell walls till the end of the experiment at 120 h of elicitation.

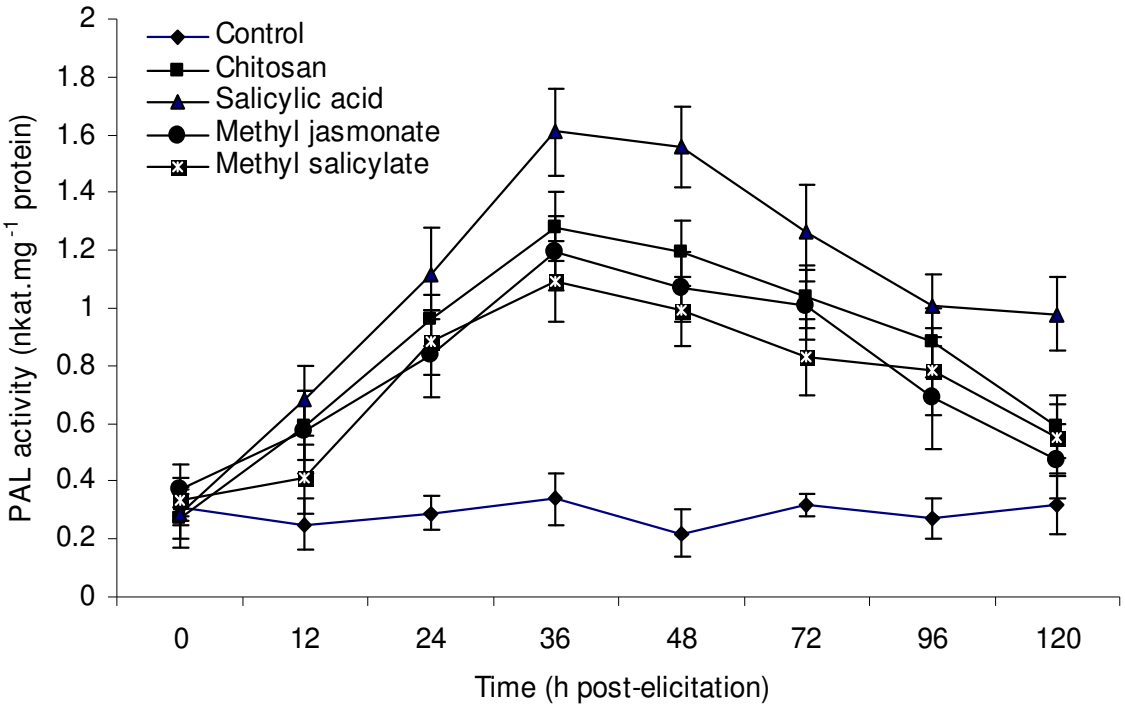


Figure 3. Phenylalanine ammonia lyase (PAL) activity (expressed as nkat/mg protein) in eggplant roots on a time course after elicitation of the plants with different elicitors as compared with the control. Values are mean ± SD of triplicate analysis.

Elicitation of PAL activity in the roots of eggplant

PAL is considered a key defense enzyme of the plants and its activity was studied after elicitation of the eggplant roots on a time course. The activity of PAL was found to increase from 12 h of elicitation and was highest at 36 h post elicitation under the influence of the elicitors (Figure 3). PAL activity showed an increase of 4.7, 3.7, 3.5 and 3.2 times by SA, CHT, MeJA and MeSA at this point of elicitation, respectively, with reference to the control; then there was a gradual decrease in PAL activity in the root tissues. At 120 h post elicitation, PAL activity of elicited roots was found to be less than double than that of the control roots in the case of CHT, MeJA and MeSA. However, the activity of the enzyme remained higher than double under the influence of SA at this point of elicitation of eggplant roots.

Effect of the elicitors on POD activity of eggplant roots

POD is another key plant defense enzyme. It was observed that its activity rose sharply at 12 h post elicitation in the eggplant roots. The highest activity of POD was observed at 24 h after elicitation under the precise influence of CHT and SA compared to the corresponding control (Figure 4). At this point in time, POD activity was 3.8 and 3.6 times higher than the control in the case of CHT and SA, respectively. However, the peak of POD activity was recorded at 36 and 48 h post elicitation in the case of MeJA and MeSA, respectively. After attaining peak activity, there was a gradual decrease in the activity of the enzyme up to 120 h of elicitation, but still higher than the level of control.

Elicitation of PPO activity in the roots of eggplant

PPO is another enzyme playing important role in plant defense. In this experiment, it was found that all the four elicitors increased PPO activity in eggplant roots beginning with 12 h of elicitation as compared to the control. The peak of PPO activity was attained at 36 h of elicitation in respect to three of the four elicitors, such as, SA, MeJA and CHT (Figure 5). The activity of the enzyme was 4.5, 3.5 and 3.4 times higher than the control in respect to the above elicitors at this point of elicitation. After attaining peak activity, a sharp decrease in the activity of the enzyme was observed in the case of SA up to 120 h of elicitation; whereas the decrease in activity was rather gradual in the case of the other two elicitors. PPO activity attained its peak at 48 h (2.9 times higher than control) in the case of MeSA-treated roots; then there was a gradual decrease in the activity of the enzyme in this case as well. However, in all cases, the activity remained above the level of control up to 120 h of

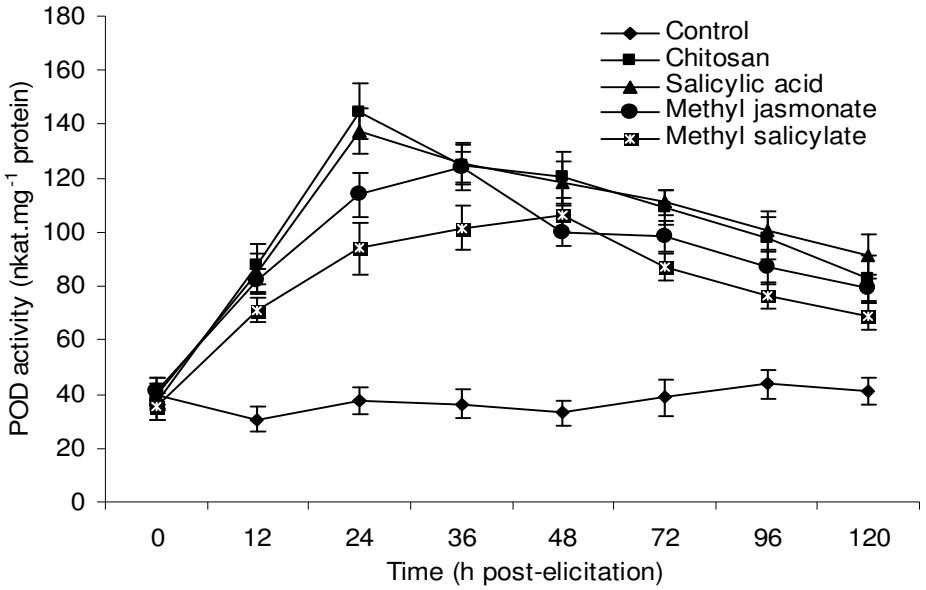


Figure 4. Peroxidase (POD) activity (expressed as nkat/mg protein) in eggplant roots on a time course after elicitation of the plants with different elicitors as compared with the control. Values are mean ± SD of triplicate analysis.

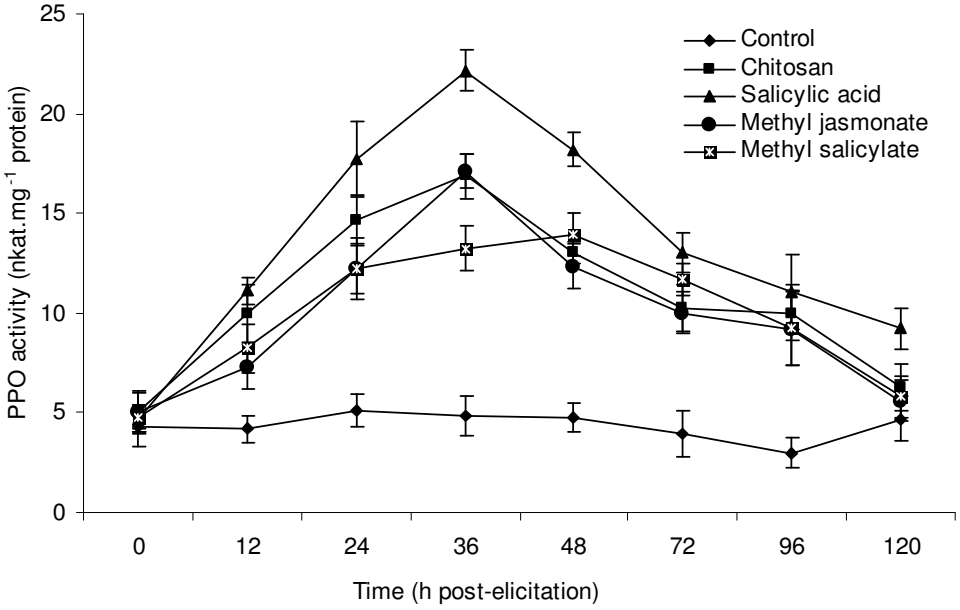


Figure 5. Polyphenol oxidase (PPO) activity (expressed as nkat/mg protein) on a time course in elicited and non-elicited roots of eggplants. Values are mean ± SD of triplicate analysis.

elicitation.

Effect of the elicitors on CAD activity of eggplant roots

CAD is the key enzyme in lignin synthesis. In this experiment, it was observed that CAD activity started increasing gradually from the twelfth hour of elicitation. The activity of the enzyme reached the highest level at 72h post elicitation in respect to all elicitors except CHT where it attained the highest level at 96 h (Figure 6). CAD activity was 4.0, 3.6 and 3.4 times higher than the control at 72 h post elicitation in respect to SA, MeSA

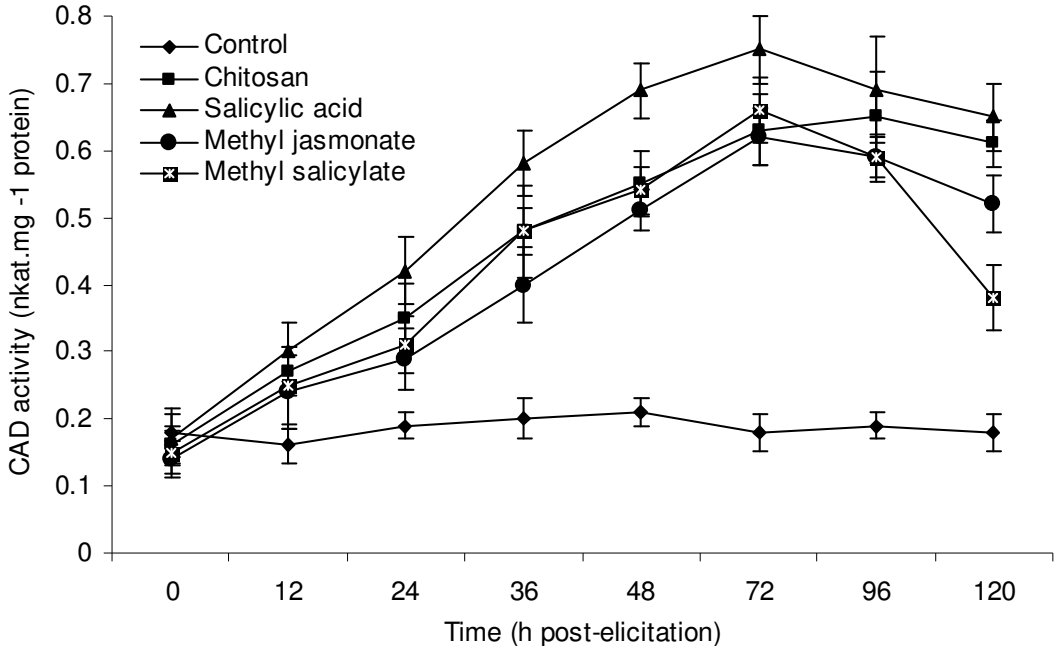


Figure 6. Cinnamyl alcohol dehydrogenase (CAD) activity (expressed as nkat/mg protein) on a time course in elicited and non-elicited roots of eggplants. Values are mean ± SD of triplicate analysis.

Similarly, the increase of CAD activity was 3.4 times higher at 96 h post elicitation in respect to CHT. There was a marginal fall in the activity of CAD, after 72 and 96 h of elicitation in respect to SA and MeJA, and CHT, respectively. However, the decrease in CAD activity was sharp in the case of MeSA after 72 h post elicitation.

Elicitation of CAT activity in roots of eggplant

Increase in CAT activity was observed to be rather slow from 12 h as a result of elicitation of eggplant roots by four elicitors. At 24 h however, there was a sharp increase of CAT activity with respect to CHT and SA. Overall, CAT activity was found to be at its peak at 48 h post elicitation in respect to all four elicitors except MeJA where it peaked at 36 h (Figure 7). CAT activity was 5, 4 and 3.4 times higher than the control at 48 h post elicitation in respect to SA, CHT and MeSA. The increase of CAT activity was 4.5 times higher at 36 h post elicitation in respect to MeJA. Thereafter, a sharp fall in the activity of CAT was observed in respect to all the elicitors in the eggplant roots, and the activity of the enzyme returned almost to the control level at 120 h of elicitation.

DISCUSSION

Elicitation of plants with elicitor molecules results in the activation of a series of defense responses, including cell wall reinforcement by deposition of lignin and induction of an array of defense enzymes (Desender et al., 2007). Cell wall strengthening, through the deposition of lignin, preceded by the induction of the synthesizing enzymes played an important role in the defense response of *Lycopersicon esculentum* in reaction to a variety of elicitors (Mandal and Mitra, 2007). In the present study, it was demonstrated that four elicitors such as, CHT, SA, MeJA and MeSA could induce cell wall strengthening of eggplant roots through lignin deposition and induction of several defense enzymes such as PAL, POD, PPO, CAD and CAT. However, SA was found to be the most efficient among the four elicitors.

Plant phenolics are formed through phenylpropanoid metabolism, and free phenolics can be cytotoxic in the cytoplasm. Playing safe, plants deposit them in the cell wall. In the cell wall, they may be ester-/ether-linked to the polysaccharides or hemicelluloses, or be polymerized into lignin (Lewis and Yamamoto, 1990). The cross-linked cell wall polysaccharides provide a backbone to the matrix within which various proteins, enzymes, metabolites and inorganic ions are associated. Localized responses to bacterial or fungal attacks often result in structural alterations, such as lignification, involving multiple matrix components that include callose, phenolics and hydroxyproline-rich proteins (McLusky et al., 1999). In this study, manifold increase of phenolics and lignin deposition in response to the elicitors is significant in respect to crosslinking of phenolic acids to cell wall materials. The increase in total phenolics resulted in increased deposition of lignin in elicitor-treated eggplant roots.

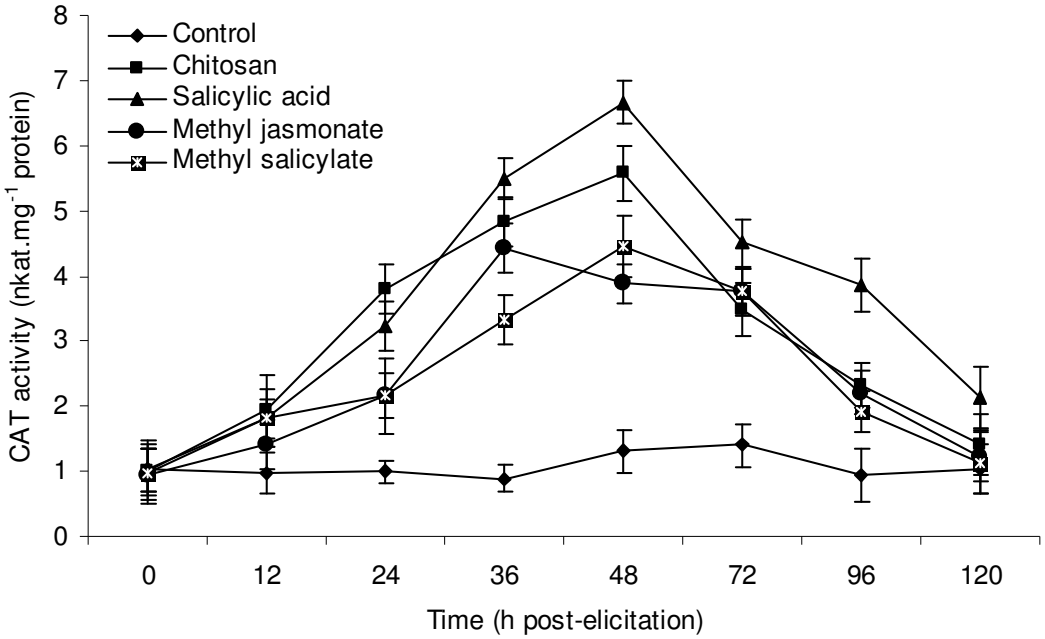


Figure 7. Catalase activity (expressed as nkat/mg protein) in roots of eggplants on a time course after elicitation of the roots of the plants with different elicitors. Data presented in graphs are the means ± SD of three replicates.

PAL activity in plant tissue may rapidly change under the influence of various factors such as treatment with elicitors (Dixon and Lamb, 1990). PAL mRNA is accumulated in both the incompatible interaction between tobacco cells and *Pseudomonas syringae* pv. *tomato* and the compatible interaction with *P. syringae* pv. *syringae*, in response to flagellin (Tagushi et al., 2003). In this investigation, the rapid increase in PAL activity in elicited eggplant roots supports published reports and correlates with the accumulation of phenolics and lignin. It has been proposed that the last step in the synthesis of lignin is catalyzed by PODs, possibly with the involvement of other proteins (Quiroga et al., 2000). This is a known fact that lignin is highly resistant to pathogen attack by virtue of being an effective barrier to pathogen entry and spread. Significant increase in the activities of POD helped achieve enhanced resistance against *Clavibacter michiganensis* ssp. *michiganensis* in tomato plants treated with acibenzolar-*S*-methyl (ASM) (Baysal et al., 2003). Results obtained in this study indicated that the eggplant roots responded actively to the elicitors by a rapid induction of POD activity. PPO plays a defensive role in plants in that it oxidizes phenolic compounds to quinones that are more toxic to pathogens (Campos-Vargas and Saltveit, 2002). The possible involvement of PPO in the defense of eggplant roots was suggested by highly increased levels of PPO activity after elicitation. CAD is a true indicator of lignin biosynthesis because of its specific role at the end of the monolignol biosynthetic pathway (Moerschbacher et al., 1990). It has been shown that elicitors made from fungal mycelium extracts induced a rapid stimulation of the monolignol pathway in flax cell suspension cultures, as confirmed by CAD gene expression (Hano et al., 2006). In this study, the huge increase in CAD activity in eggplant roots confirms the important role it plays in the lignification process. Exogenous application of chemical elicitors, like SA and CHT during different stages in tomato fruit development, notably increased the level of CAT and POD enzymes activity in fruit tissue (Ortega-Ortiz et al., 2007). A fungal glycoprotein elicitor increased CAT activity in the localized acquired resistance zone of tobacco leaves. Induction of CAT activity in the localized acquired resistance zone could play a protective role against possible oxidative damage (Dorey et al., 1998). Consequently, a higher level of CAT activity in eggplant roots in response to elicitors may be a pointer to the above observation.

SA treatment was found to induce resistance to viral, bacterial and fungal pathogens in many plant species. It also induced the same set of genes in tobacco and *Arabidopsis* as was activated during systemic acquired resistance (Vlot et al., 2009). Moreover, an addition of 20 µM salicylic acid to *Saussurea medusa* cell cultures resulted in 7.5-fold increase in PAL activity (Yu et al., 2006). SA spraying on *Ya Li* pear plants increased PAL and POD activities greatly and contributed in the protection of pear fruits against postharvest diseases (Cao et al., 2006). This has proved that besides its antifungal activity, CHT induces defense-related enzymes (Bautista-Baños et al., 2006). A report found that when CHT was injected in date palm roots, it could induce increase in the

activities of POD and PPO (El Hassni et al., 2004). Various CHT derivatives induced and raised lasting PAL and POD activities in tobacco leaves and roots as local or systemic responses, which could lead to the accumulation of secondary metabolites and formation of barriers that altogether enhance plant resistance against pathogens (Falcón-Rodríguez et al., 2009). It has been reported that elicitation of grapevine leaves by CHT contributed to enhanced induction of PAL activity (Trotel-Aziz et al., 2006). In a report, Seskar et al. (1998) concluded that tobacco tissue, MeSA, might play a role similar to that of volatile MeSA in the pathogen-induced defense response. Application of gaseous MeSA to healthy tobacco plants increased the expression of the PR-1 gene (Shulaev et al., 1997). The failure of some of the transgenic lines to mount *P. syringae*-induced SAR was taken as a supportive evidence for the notion that MeSA represents a universal mobile SAR signal in plants (Vlot et al., 2008). In compatible interactions, MeSA production depends on the *P. syringae* virulence factor (coronatine), suggesting that the phytopathogen uses coronatine-mediated volatilization of MeSA from leaves to attenuate the SA-based defense pathway (Attaran et al., 2009). Results from recent studies confirm the importance of salicylic acid-binding protein 2 (SABP2) and MeSA for SAR development in tobacco, and establish similar roles for MeSA and the orthologs of SABP2 in *Arabidopsis* (Park et al., 2009). Recent studies show that the conversion of SA to MeSA can affect both direct and indirect plant defense responses (Ament et al., 2010). Accumulation of secondary metabolites has been reported in response to treatment of germinating soybean seedlings with MeJA (Franceschi and Grimes, 1991). Exogenously applied MeJA induced *de novo* transcription of the key enzyme's gene (Lois et al., 1989) of the PAL pathway, resulting in elevated levels of active enzyme in soybean cell suspension cultures. Thirty six plant species tested in cell suspension culture could be elicited with respect to the accumulation of secondary metabolites by exogenously supplied MeJA. An addition of MeJA initiated *de novo* transcription of genes, such as PAL, that are known to be involved in the chemical defense mechanisms of plants (Gundlach et al., 1992). Another study in barley has found that the systemic protection provided by MeJA against *Blumeria graminis* accompanied significant increases in activities of PAL and POD (Walters et al., 2002). Response of MeJA-treated Norway spruce stems involves tissue-specific differentiation of traumatic resin ducts, terpenoid accumulation and induction of defense enzyme activities in the developing xylem tissues (Martin et al., 2002).

The results described above are in good stead for understanding the biochemical basis of resistance of eggplants evoked in response to the elicitors. Mechanical isolation of the pathogen during determinative phases of colonization coupled with response within and outside the cellular system is required to give resistance to wilt pathogens such as *R. solanacearum*. Accumulation of phenolics and lignin in high amounts together with higher level of activity of major defense enzymes in response to the elicitors may bolster eggplants in mounting practical and effective resistance against the devastating wilt pathogen. These results also demonstrate the integral role of the SA, MeJA and MeSA in the intracellular signal cascade that initiates the interaction of an elicitor molecule with the plant cell surface. As a consequence, this results in the accumulation of secondary metabolites.

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