Available online at www.notulaebotanicae.ro
Print ISSN 0255-965X; Electronic ISSN 1842-4309
Not. Bot. Hort. Agrobot. Cluj 36 (2) 2008, 71-75
AcademicPres
Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Role of Certain Elicitors on the Chemical Induction of Resistance in Tomato against the Leaf Caterpillar *Spodoptera litura* Fab.

MELVIN JOE M.[1)], N. MUTHUKUMARAN[2)]

[1)] *Department of Microbiology, Faculty of Agriculture, Annamalai University, Tamilnadu, India*
[2)] *Department of Entomology Faculty of Agriculture, Annamalai University, Tamilnadu, India, e-mail: mel_vin@sify.com*

Abstract

Elicitors of chemical induction were used to manipulate the activities of several putative defense related proteins in the leaves of tomato, *Lycopersicon esculentum* Mill. The four presumptive defenses manipulated were protinase inhibitors, polyphenol oxidase, peroxidase and lipoxygenase. The elicitors used were jasmonic acid (JA), salicylic acid (SA) and PGPR *Pseudomonas aeruginosa*. These elicitors were tested against growth and development of *Spodoptera litura* Fab. In order to asses the relative roles of proteins in induced resistance against *S. litura*. When activities of proteinase inhibitors and/or polyphenol oxidase in leaf tissue were high, growth rates of *S. litura* were low and vise versa. In contrast, high activities of peroxidase and lipoxygenase have no effect on growth and development of *S. litura*. The association of high levels of proteinase inhibitors and polyphenol oxidase strongly implies these proteins as causal agent inducing resistance against *S. litura*.

Keywords: *Pseudomonas aeruginosa*, *Spodoptera litura*, jasmonic acid, salicylic acid, tomato, induced systemic resistance

Introduction

Tomato (*Lycopersicon esculentum* Mill.), an important fruit vegetable, commercially cultivated throughout the world, is an important source of vitamins and minerals. Among many fruits and vegetables, tomato occupies an area of about 20.445 hectares in Tamil Nadu and it's usually cultivated both an irrigated and rain fed crop (Man-Mohan attavar, 2000).

Among the various pests damaging tomato, Leaf caterpillar *Spodoptera litura* Fab. is one of the predominant polyphagous pest and one of the most important horticultural pests. It infests many crops including tomato. It develops throughout the year, rising about seven to eight generations annually (Avidor and Harpaz, 1969). The larvae cause significant damage to the foliage and cause fruit damage ranging from 11.8 to 23.01 present in rainy season and 9.4 to 27.4 percent in winter (Patnaik, 1998).

Chemical management to reduce the insect infestation is much limited in vegetable crops because of the resistance of larvae to insecticides and residual toxicity on the fruits. Induced Systemic Resistance (ISR) (or) Systemic Accured Resistance (SAR) is a good and viable alternative tool for the management of pests in crop plants.

ISR (or) SAR mechanism produces response to local attack by producing compounds thereby reducing or inhibiting further attack by herbivore or pathogens (Hunt et al., 1996; Sticher et al., 1997; Hammer Schimidt, 1999). Two types of responses such as jasmonic acid (JA) and salicylic acid (SA) mediated responses are necessary for plant resistance against insect pests.

Rhizobacteria are present in large numbers on plant root surfaces and they stimulate plant growth and are therefore called as Plant Growth Promoting Rhizobacteria (PGPR). PGPR strains were isolated from naturally disease suppressive soils mainly, among which Pseudomonas sp promoted plant growth by suppressing soil borne pathogens (Leeman et al., 1993). Some of these biological control strains are able to induce a plant mediated mechanism that is phenotypcially similar to pathogen indeed SAR as the induced resistance is often referred to as Rhizobacteria – Mediated Induced Systemic Resistance (RMISR) (Van Loon et al., 1998).

RMISR has been reported in many plant species, *viz.*, bean, carnelian, cucumber, radish, tobacco and tomato against a broad spectrum of plant pathogens including fungi, bacteria and viruses (Van Loon, 1990). But the reports regarding the induction of systemic resistance by PGPR against the insect pests particularly *Spodoptera litura* were scare, hence the present investigation was undertaken to evaluate the efficiency of PGPR in the induction of resistance against *Spodoptera litura* on tomato.

Materials and methods

The study was conducted during, Sep 2006 - Dec 2006 Department of Microbiology, Faculty of Agriculture, Annamalai University.

(I) Bacterial strains and culture conditions

The non pathogenic PGPR strain *Pseudomonas aeruginosa* was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. They were maintained in nutrient agar slants at 25 - 30°C with monthly transfer.

(II) Tomato plant raising

Tomato plant was grown in 500 ml pots. Natural light was supplemented with high pressure mercury lamps. Natural light supply 16 h for day time and 8 h for night time. Temperature 25 ± 3° C was maintained. When plants had three fully expanded leaves and the fourth was almost fully expanded, we moved them to a controlled environment chamber (27 ± 1°C, 14:10 light: dark) for evaluating the efficiency of different treatments against *S. litura*.

(III) Culture of leaf caterpillar

Culture of *S. litura* was initiated with field collected larvae. They were fed with fresh leaves of castor daily and were maintained in individual plastic buckets covered with muslin cloth. The full grown larvae were allowed to pupate in the sterilized soil spread at the bottom of plastic buckets. The pupae were then collected, surface sterilized with 0.1% sodium hypochloride, kept on a layer of saw dust spread in a Petri plate. The emerging moths were collected using test tubes and allowed inside plastic buckets for mating and oviposition. The moths were fed with 10% sucrose solution impregnated in cotton wool, placed in small Petri dish covered with wire gauge. Castor leaves with their stalks inserted into water in a small conical flask were placed inside the bucket. The egg masses found on the castor leaves were collected. After 2 or 3 days neonates emerge out from egg masses to continue their lifecycle.

(IV) Jasmonic and salicylic acid application

Induced resistance was attempted by the foliar application of jasmonic acid (JA) and salicylic acid (SA). JA and SA were dissolved in acetone at a rate of 1 g ml-1 and dispersed in water to achieve 1.5 ml JA and SA solution (Thaler et al., 1999). The carrier solution, which consists of acetone without JA or SA dispersed in water, was used as a control. Approximately, 6 weeks after germination the tomato plants were sprayed with JA, SA solution or carrier solution, applied at a rate of 1 ml per leaf using an atomizer. The eight leaves from the cotyledon of each plant were protected from treatment using a plastic bag and it was not removed until the solution on all the other leaves had dried. Infection studies could be was performed on this unsprayed leaf so that the systemic affect of JA, SA application could be measured independently of any effects from the residue.

(V) Challenge inoculation

Challenge inoculation consists of isolates of *P. aeruginosa* obtained from MTCC, Chandigarh. The cultures were grown on nutrient agar for 24 h at 28°C and single colonies were transferred to nutrient broth and incubated at 25°C for 24 h with shaking at 150 rpm. Bacterial cultures were centrifuged and pellets were re-suspended in distilled water, resulting in concentration of 10^7 CFU ml^{-1}. Fully expanded new leaves were challenge inoculated two weeks before transplanting by infecting 3.0 ml of inoculum suspension at each of the 30 sites per leaf.

(VI) Chemical assays

Extracts for assays of foliar polyphenol oxidase, peroxidase, and lipoxygenase, were prepared by homogenizing individual weighted leaflets in 1.25 ml of pH 7 K phos. (0.1 M) buffer containg 7% (w/v) polyvinyl polypyrrolidine. An aliquot (0.4 ml) of a 10% solution of Tritonx-100 was added with mixing and the homogenate was centrifuged at 6000 rpm for 15 min. The resulting supernatant was used directly as an enzyme source (Stout et al., 1996).

Assay for polyphenol oxidase and peroxidase were measured at the rate of formation of melanin like material from phenolic substrates (Stout et al., 1996) for polyphenol oxidase assays, 30 μL of enzyme extract were added to 1 ml of 2.92 mM caffieic acid in pH 8 K phos buffer (0.1 M) and the change in absorbance of the mixture at 470 nm measured for 30 sec. The procedure for assaying peroxidase activities was identical, but the substrate was 5 mμ giracol with 0.02 mM H2O2 added as a cofactor. Polyphenol oxidase and peroxidase activities are reported as ΔOD/mm/gm fresh weight. The lipoxygenase assay measured the formation of conjugated dienes from linoteic acid (Hildebrand and Hyrrowitz, 1981). The reaction mixture consisted of 15 –20 ml enzyme extract and 2.9 ml of 0.4 mM linoteic acid dispersed in pH 7K phos buffer containing 0.1% (v/v) tween-20. Rate of change in absorbance of this mixture was measured at 234 nm at 10 min.

The assay for serine proteinase inhibitors measured by the degradation of a peptide substrate of the plant extracts (Benzol tryosme ethyl ester, BTEE) by the proteinase chymotrypsin (Stout et al., 1998). Tomato plants contain two inhibitors of serine proteinases, one an inhibitor of chymotrypsin and another inhibitor of both trypsin and chymotripsin (Schaller and Ryen, 1995). The inhibition of chymotrypsin by leaf extracts provides a good measure of total proteinase inhibitor activity in tomato leaves. Individual leaflets were extracted by grinding them, using mortar and pestle, and dissolved in tris HCl (pH 7.8) extraction buffer (pH 7.8) (3ml/mg fresh wt.) Polyvinyl-polypyrrolidine, (7%) phenyl urea (1.67 μM), KCl (0.3 ml) and ascorbic acid (0.4 mM). Extracts were then freezed for further use. For protinase inhibitor as-

says, the frozen leaf extract was collected and 25 µL of the extract added to an identical volume of a 0.001 N solution of HCl containing 0.0015 mg of chymotrypsin. The mixture was allowed to incubate for 10 min in a quartz cuvette. Following the incubation, 2.9 ml of 0.5 ml of BTEE in a methanol K phos buffer (pH 8.0) mixture (12:13 ratio) was added to the cuvette and the increase in absorbance at 260 nm was monitored for 10 min.

For every sample run, a control was run with BTEE and chymotrypsin only. Proteinase inhibitor activities are identified by rates of change in absorbance in this assay. There is a linear relationship between protinase inhibitor concentration and chymotrypsin activity in this assay (Stout *et al.,* 1996).

(VII) Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) as per the procedures described by Gomez and Gomez, (1984).The values are mean $\pm$ SD for three samples in each group . P values $\leq$ 0.05 were considered as significant.

Results and discussion

In the present investigation, we used elicitors of plant chemistry i.e., jasmonic acid (JA) to selectively induce putative casual agents of induced resistance, and weak inhibitors of wound induction i.e., salicylic acid (SA), to mediate some of the plant responses of accrued resistance and a PGPR to obtain an additive effect on the level of resistance to trigger different signaling pathway in plants to provide attractive possibility for improvement of pest suppression.

The JA treatment strongly affected the activity of proteinase inhibitors, moderately the PPO activity and to a lesser amount the lipoxygenase activity but the peroxidase activity was even less as compared to control on the other hand SA treatment could induce the peroxidase activity to a higher extent. In order to obtain an additive effect of all the enzymes studied, including the protinase inhibitor, we tried the combination of SA + JA, which could include the activity of all enzymes but not of protinase inhibitor activity, which has been recorded low as compared to the control (Table 1). It has been found that SA application has inhibited some pathways which lead to the synthesis of protinase inhibitor. The results of our present study were in conformity with the earlier findings of Stout et al., (1998).

Interestingly, the pretreatment of seedlings with *P. aeruginosa* instead of salicylic acid has not inhibited the effect of JA exposure. It has been found that the protinase inhibitor activity and enzyme activity has been found to be increased when plants were pre-treated with *P. aeruginosa* before expose to JA. (v 1).

Recent advances in research on plant defense signaling pathway have shown that plants are capable of activating distinct defense pathways, depending on the type of pathogen encountered (Reymond and Farmer, 1998; Pieterse and Van Loon, 1999). It has been found that, different rhizobacteria utilize difference mechanisms for triggering systemic resistance. Some rhizobacteria trigger a SA – dependent SAR pathway and the JA depended ISR pathway, which has resulted in an additive effect on the level of induced resistance attained (Van Wees et al., 2000).

Delanay et al. (1994) reported the role of SA produced by P. aeruginosa 7NSK2 in induced resistance to Botryties cinerea in bean. SA is considered to mediate plant responses to pathogen and it's associated with pathogen induced SAR. By using Bean based model with *B. cinerea* as challenging pathogen it was shown that P. aeruginosa 7NSK2 can also induce systemic resistance (De Meyer and Hofte, 1997). ISR refers to an increased broad spectrum and plant mediated Induced Resistance induced throughout the whole plant after an abiotic or biotic stimulus applied to only a part of the plant (Kloepper et al., 1992). A specific form of ISR is rhizobacteria induced systemic resistance, triggered by non-pathogenic rhizobacteria that do not cause harmful affect to the plant (Van Loon et al., 1998).Hofte et al. (1993) reported that SAR pathway is involved in ISR by P. aeruginosa 7NSK2 because SA is implied in SAR and this SAR might mediate ISR pathway by *P. aeruginosa* .The accumulation of mg amounts of SA by *P. aeruginosa* is insufficient to affect the jasmonic acid dependent SAR pathway, which induces the proteinase inhibitors activity.

Many plant responses for activities of primary and secondary metabolites were induced by some chemical elicitors. In this study induced resistance was activated in

Table 1 Enzyme activity as influenced by SA, JA and *P. aeruginosa* treatment

Treatments	Protein activity			
	PPO	POD	LOX	PI^s
Control	17.4 ± 3.1c	13.7 ± 2.1d	5.6 ± 0.7c	32.1 ± 10.4c
SA	23.7 ± 2.9b	21.6 ± 3.1a	7.5 ± 0.4b	80.4 ± 1.7b
JA	25.8 ± 2.8b	9.1 ± 2.4e	7.5 ± 0.4b	80.4 ± 1.7b
SA + JA	17.4 ± 2.7c	16.2 ± 3.4c	5.8 ± 0.6c	4.4 ± 2.4d
P. aeruginosa + JA	29.6 ± 3.2^a	19.2 ± 3.1^b	8.9 ± 0.3^a	87.4 ± 1.9^a

Values are given as mean ±SD of three replications in each group. Values which are not sharing a common superscript (a,b,c,d) differed significantly at P≤0.05(DMRT) PPO -Polyphenol oxidase, POD-Peroxidase, LOX- lipoxygenase, PIs- proteinase inhibitors

tomato using foliar application of chemical elicitors on growth and development of *S. litura*.

Under the pot culture condition, it was observed that the tomato leaves treated with combined foliar application of JA and *P. aeruginosa* caused maximum larval mortality followed by JA and SA as compared to the untreated check. Pupation rate was reduced to the minimum incase of leaves treated with JA and *P. aeruginosa*. Adult emergence and adult longevity was also reduced on the same treatment. Surprisingly, pre-treatment with SA followed by treatment with JA has a very less effect on mortality of *S. litura* larvae (Table -2).

Hence, among the various treatments combining activity of JA and *P. aeruginosa* has a negative impact on growth and development of *S. litura* followed by JA. Our finding have shown that pre-treatment with PGPR followed by application of JA has not affected the effect of JA on *S. litura* during different stages of growth (Table 1). As compared to SA pretreatment, that showed inhibitory effect on JA application.

While the peroxidise level was found to be less as compared to the control. However polyphenol oxidase and inhibitors activity were often lower in SA treated plants than in control. Manipulation of chemical induction have resulted in changes in the suitability of plant tissue for growth of *S. litura* leaves in which the chemicals induced by JA were poor sources of food for *S. litura,* while the leaflets in which induction by JA was inhibitated by SA have less effect on mortality of *S. litura.* Moreover when this treatment of SA has been replaced by pretreatment with PGPR (*P. aeruginosa*) has shown better inhibition as compare to the SA treatment. Similar conclusion was made on basis of correlation between systemic increases in polyphenol oxidase, protinase inhibitors activity and systemic increase in resistance to *S. exigua* (Stout and Duffey, 1996).

Stout et al. (1998) reported that *S. exigua* growth rate were low when polyphenoloxidase and protinase inhibitors levels were high and suggested that polyphenol oxidase and proteinase inhibitors are important contributors to induce resistance to *S. exigua* in tomato foliage but that inducible peroxidase and lipoxyginase are negligible contributors to induce resistance to *Spodoptera exigua*. In our present study similar conclusion were made on the basis of correlation between systemic increases in polyphenloxidase and proteinase inhibitors activity and systemic increase of resistance in tomato plant to *S. litura*.

Conclusions

The results of our study have showed that the PGPR pretreatment, has no inhibitory effect on JA application. This study has also showed that it is possible to exhibit an additive effect of both the pathways. This suggest that newer and more effective elicitors of SAR and ISR will surely be developed, perhaps in part as the result of our growing understanding of the underlying mechanisms of these pathways within the plant. In the future, it may be possible to apply elicitor cocktails that induce a balance of defenses regulated by salicylic acid and jasmonic acid, against specific pests or complexes of threats. Therefore a detailed knowledge of the molecular mechanisms underlying induced disease resistance will be instrumental in developing a biologically-based environmentally friendly and durable crop protection. However, this future will require a shift in conventional agriculture away from the total reliance on pesticides to solve pest problems, and a concerted effort to manage pests as opposed to eliminating them.

References

Avidov, Z., I. Harpaz, 1969, Plant pests of Israel. Israel Universities Press Jerusalem, 235.

Berlinger, M. J., 1986, Pests, 391-442, Atherton, J. G., J. Rudich (Eds.), The tomato crop, A scientific basis for improvement,. Chapman and Hall, London.

Table 2 Plant defense mechanism of tomato on *S. litura* growth and development

Sl. No.	Treatments	Growth and development of *S. litura*				
		Larval mortality (%)	Larval* duration (Days)	Pupation (%)	Adult emergence (%)	Adult* longevity (Days)
1.	Control	8.00 (12.68) †	17.00± 0.80ᵃ	92.00 (77.31)†	82.00 (67.72) †	4.80± 0.74ᵃ
2.	SA	25.00 (24.94) †	16.00± 0.89ᵇ	85.00 (67.98)†	72.00 (62.06) †	4.40± 0.38ᵇ
3.	JA	26.00 (28.93) †	13.80± 0.89ᵈ	78.00 (14.06) †	68.00 (62.08) †	3.40± 0.38ᵈ
4.	SA + JA	27.00 (16.38) †	14.80± 1.16ᶜ	88.00 (74.06) †	78.00 (61.07) †	3.80± 0.40ᶜ
5.	*P. aeruginosa* + JA	32.00 (29.22) †	13.20± 1.16ᶜ	74.00 (59.45) †	64.00 (52.23) †	2.90± 0.48ᶜ

*Values are given as mean ±SD of three replications in each group; Values which are not sharing a common superscript (a,b,c,d) differed significantly at P≤0.05(DMRT); † Figures in parenthesis are arc sine transformed values

De Meyer, M. Hofte, 1997, Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by by *Botrytis cinerea* in bean. Phytopathology 87, 588-593.

Delanay, T. P., S. Uknes, B. Vernovij, L. Frieelrich, K. Weymann, D. Negrottos, T. Daffney, M. Gut-Rella, H. Kessmann, E. Word, J. Ryalis, 1994, A control role of salicylic acid in plant disease resistance. Science 266, 1247-1250.

Gomez, K. A., A. A. Gomez, 1984, Statistical procedures for agricultural research. John Wiley and Jons, Inc. New York, 20-29.

Hammer Schimidt, R., 1999, Induced disease resistance: how do induced plants stop pathogens? Physiological and Molecular Plant Pathology 55, 77-84.

Hildebrand, D.F. and T. Hyrrowitz, 1981, Two soybean genotypes lacking lipoxygenase. Journal of the American Oil Chemistry Society 58, 583-586.

Hofte, M., S. Buysens, N. Koedam, P. Eornelis, 1993, Zinc affects siderophore – mediated high affinity ion uptake system in the rhizosphere *Pseudomonas aeruginosa* 7NSK2. Biometals 6, 85-91.

Hunt, M. D., U. H. Neuen Schwander, T. P. Dalany, K. B. Weymann, L. B. Freidrich, K. A. Lawton, H. Y. Stemer, J. A. Ryalis, 1996, Recent advances in systemic acquired resistance research – a review. Gene 179, 89-95.

Juvik, J. A., M. J. Berlinger, T. Ben Davi, J. Rudich, T. David, 1982, Resistance among accessions of the genera *Lycopessicon* and solanam to four of the main insect pests of tomato in Israel. Phytoparasitica 10, 145-156.

Kloepper, J. W., S. Tuzun, J. A. Kuc, 1992, Proposed definitions related to induced disease resistance. Biocontl. Sci. Technol. 2, 349-351.

Leeman, M., J. A. Van dell, M. J. Handrick, R. J. Schefter, P. A. Bakker, B. Schippers, 1993, Biocontrol of *Fusarium* wilt of radish in commercial greenhouse trials by seed treatment with *Pseudomonas fluorescens* WCS 374. Phytopathology 10, 123-127.

Manmohan attavar, 2000, Hybrid vegetables tools for productivity gains. The Hindu Survey of Indian agriculture (ed) N. Ravi National Press, Chennai, 145-149.

Patnaik, H. P., 1998, Pheromone trap catches of *Spodoptera litura,* F. and extent of damage on hybrid tomato in Orissa. Reddy. P. P., N. K. K. Kumar and A. Varghese (Eds) Proc. I. Natl. Symp. On Advances in IPM. For Horticultural crops, Environmental implications and Thrusts, Bangalore Oct 15-17, 68-72.

Pieterse, C. M. J., L.C. Van Loon, 1999, Salicylic acid – independent plant defense pathways. Trends in Plant Science 4, 52-58.

Reymond, P., E. E. Farmer, 1998, Jasmonate and Salicylate as global signals for defense gene expression Curr. Opinion Plant Biol. 1, 404-411.

Rick, C. M., 1978, The tomato. Sci. Amer. 239, 76-87.

Schaller, A., C. A. Ryen, 1995, Systemic polypeptide defence in plants. Bioresource 18, 27-33.

Sticher, L., B. Mauch-Mani, J. P. Metraax, 1997, Systemic acquired resistance. Annual Review of Phytopathology 35, 235-270.

Stout, M. J., S. S. Duffey, 1996, Characterization of induced resistance in tomato plants. Entomologia Experimentalist Applicata 79, 273-283.

Stout, M. J., K. V. Workman, S. S. Duffey, 1996, Identification, special distribution and variability of induced chemical responses in tomato plants. Entomological Experimentalist Applicata 79, 255-271.

Stout, M. J., K.V. Workman, R. Cherd, M. Bostock, S. S. Duffey, 1998, Stimulation and attenuation of induced resistance by elicitors and inhibitors of chemical induction in tomato (*Lycopersicon esculentum*) foliage. Entomologia Experimentalist Applicata 86, 267-279.

Thaler, J. S., A. L. Fidantsef, S. S. Duffey, R. M. C. Bostock, 1999, Tradeoffs in plant defense against pathogens and herbivores: a field demonstration using chemical elicitors of induced resistance. J. Chem. Ecol. 23, 1597-1609.

Van Loon, L. C., 1990, Induced resistance in plants and role of pathogenesis related proteins. Eur. J. Plant Pathl. 103, 753-765.

Van Loon, L. C., P. A. H. M. Bakker, C. M. J. Pieterse, 1998, Systemic resistance induced by rhizosphere bacteria. Annual Review of Phytopathology 36, 453-483.

Van Wees, S. C. M., E. A. M. De Swort, J. A. Pelt, L. C. Van Loon, C. M. J. Pieterse, 2000, Enhancement of induced disease resistance by simultaneous activation of Salicylate and Jasmonate dependent defense pathways in *Arabidopsis thaliana.* Proc. Natl. Acad. Sci., USA 97, 8711-8716.

Vermes, M., 1976, *Spodoptera littoralis*. Encycl. Agric. 3, 517-579.