

Induction of Pathogenesis-Related Proteins in Cucumber in Response to Infection with Soft Rotting *Erwinia*

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ABSTRACT

Three isolates of *Erwinia carotovora* subsp. *carotovora* were inoculated into three weeks old cucumber seedlings. Two of them, *Ecc* Cab21B and *Ecc* Cab45B, produced soft rot disease symptoms 24 hours after inoculation and one, *Ecc* 119A, failed to induce any disease symptoms. To determine if the difference in pathogenicity between these isolates is due to differences in the induction of proteins involved in plants defense against pathogens, RNA was extracted from inoculated seedlings as well as seedlings inoculated with sterile distilled water and non-inoculated, and subjected to reverse transcription (RT) – polymerase chain reaction (PCR). Peroxidase genes were detected in seedlings inoculated with the three (*Ecc*) isolates but not in seedlings inoculated with sterile distilled water or non-inoculated. On the other hand, β -1,3-glucanase genes were detected in cucumber seedlings inoculated with *Ecc* Cab21B and *Ecc* Cab45B but not in seedlings inoculated with *Ecc* 119A, sterile distilled water and non-inoculated.

Key words *Erwinia carotovora*, cucumber plant, pathogenesis-related proteins, peroxidase, β -1,3-glucanase.

Introduction

Bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* (syn. *Pectobacterium carotovorum* subsp. *carotovorum*) occurs worldwide in many crops. *Erwinia carotovora* subsp. *carotovora* (syn. *Pectobacterium carotovorum* subsp. *carotovorum*) is a soil borne, facultative anaerobic pathogen that causes maceration and rotting of parenchymatous tissue of all plant organs, eventually resulting in plant death (Perombelon & Kelman, 1980). It seems difficult to control this pathogen and to restrain it due to several reasons such as: absence of effective bactericides (Blom & Brown, 1999), its genetic variability (Gardan *et al.*, 2003), its wide host range, its broad array of virulence factors and because infection can be latent (Perombelon, 2002). Bacteria are spread mechanically during cultivation and symptoms can become visible during all stages of plant growth and development. Disease development could be reduced by cultural measures such as mulching, soil ventilation, drainage and time of crop harvesting tuber lifting can reduce disease development (Funnell, 1993; Wright & Burge, 2000 and Wright *et al.*, 2002).

The most effective disease control method could be achieved by the use of resistant cultivars. Many fungal and bacterial pathogens secrete a large number of cell wall degrading enzymes that digest the plant cell wall, allowing the pathogen to have access to nutrients (Salmond, 1994 and Walton, 1994). Such enzymes can release plant cell wall-derived elicitors that activate plant defense responses (Davis *et al.*, 1984). Plants possess different strategies to recognize and counteract pathogen attack (Jones & Dangl, 2006 and Boller & He, 2009). Pathogenesis related protein (PR proteins) are proteins which accumulate as a result of infection by pathogens or after treatment with elicitors and appear to have protective properties against pathogens (Broglie *et al.*, 1991; Alexander *et al.*, 1993; and Sela-Burlage *et al.*, 1993). PR proteins in the plant species are acid-soluble, low molecular weight, and protease-resistant proteins (Leubner-Metzger & Meins, 1999 and Neuhaus, 1999). Depending on their isoelectric points, they could be acidic or basic proteins but they have similar functions. PR proteins have been classically divided initially into 5 families based on molecular mass, isoelectric point, and localization and biological activity (Van Loon, 1985). Currently they are categorized into 17 families according to their properties and functions. PR proteins of cucumber have been detected before and identified as chitinases (Metraux *et al.*, 1989 and Van Loon & Van Strien, 1999) and peroxidases (Rasmussen *et al.*, 1995). The current challenge is how to apply the new knowledge to produce disease resistant crop cultivars (Li *et al.*, 2013). This study was undertaken to know whether cucumber resistance to isolate 119A is associated with peroxidase and β -1,3 glucanase accumulation or not.

Material and methods

Bacterial isolates

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Three bacterial isolates were used in this study; *Erwinia carotovora* subsp. *carotovora* 119A was isolated from rotted melon plant collected from Ismailia Governorate, Egypt and is not capable of infecting cucumber seedlings (El-Hendawy, 1983). *Ecc* Cab21B and *Ecc* Cab45B were isolated from rotted cabbage leaves collected from Giza and Cairo governorates, respectively and are capable of inducing soft rot disease symptoms on cucumber seedlings (El-Hendawy *et al.*, 2007).

Cucumber seeds and growth of seedlings

Seeds of cucumber (*Cucumis sativus*) of Eshraka variety were purchased from the Ministry of Agriculture, Egypt. Seeds were sown in sterile clay soil contained in plastic pots each of 20 cm diameter. Five seeds were sown in each pot at equal distances. All pots were placed on a bench at room temperature and watered as required.

Inoculations

Three weeks old cucumber seedlings were inoculated with a bacterial suspension prepared from an overnight culture and containing 0.7×10^6 cfu. Injection was carried out by using a 1 ml sterile disposable syringe fitted with a 25G needle.

Isolation of total RNA

The method used for RNA isolation is a modification of Chomczynsky and Sacchi (1987), which is based on guanidinium thiocyanate as an extraction buffer (4 M guanidinium thiocyanate, 100 mM HCl (pH 8.0), 75 mM EDTA and 2 (v/v) % β -mercaptoethanol) without phenol extraction step. The frozen cucumber tissues were transferred to a pre-cooled mortar and homogenized into a fine powder. 50 ml of the homogenizing buffer were added / 5 g homogenized tissues and mixed well, then 5 ml of 2M sodium acetate (pH 4.0) were added and mixed by inversion. An equal volume of chloroform- isoamyl alcohol (24-1) was added, and shaken vigorously for 3 minutes and chilled on ice for 20 min. The solutions were transferred to pre-cooled sterile 50 ml centrifuge tube, centrifuged at 10,000 rpm for 25 minutes at 4 °C. The RNA was present in the upper aqueous phase. DNA and protein were present in the lower organic phase and interphase. The aqueous phase was transferred to a fresh tube and the chloroform isoamyl alcohol extraction step was repeated, aqueous phases were transferred to a fresh tube and mixed with an equal volume of isopropanol, then placed at -70 °C for 30 min or at -20 °C for 2 h to precipitate RNA, centrifugation at 10,000 rpm for 25 min at 4 °C was performed. Supernatants were discarded. RNA present in the pellet. The pellets were dissolved in 5 ml of homogenizing buffer, gentle pipetting of the pellet is required. An equal volume of isopropanol was added and mixed. The solution was cooled at -70 °C for 15 min and was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatants were removed from RNA pellet, wash with 75% ethanol. Then dried under vacuum for 5 min and resuspended in 1 ml DEPC- treated water.

RNA was purified using tri-reagent RNA kit (Sigma, Lot No. T9424), then the purified RNA pellet was dissolved in 100 μ l DEPC- treated water. The extracted total RNA was electrophoresed on Formamide formaldehyde agarose gel (1.2 %) (50 ml 1X MOPS buffer, 2.7 ml formaldehyde 37% and 0.5 g agarose). Then 10 X MOPS was added (3- morpholinopropane sulfonic acid) running buffer (800 ml DEPC treated water , 41.8 g MOPS , 16.6 ml sodium acetate and 20 ml 0.5 M EDTA, pH 8.0 and formaldehyde (12.2 M) to give a final concentration 1X and 0.66 M respectively. Ethidium_bromide was added to give final concentration 0.5 μ g /ml before preparing the gel. The buffer reservoirs were filled with 1X MOPS running buffer, pH 7.0. After the samples were loaded (250 μ l Formamide, 38 μ l formaldehyde 37%, 50 μ l 10X MOPS buffer and 0.01 % Bromophenol blue) freshly prepared, the gel was run at 100 volts until the bromophenol blue has moved approximately half- way through the gel.

RT- PCR amplification

cDNA synthesis

cDNA synthesis was carried out in a reaction mixture of 40 μ l (final volume) containing 10 μ l of freshly prepared RNA as a template, 10 p mol of oligo (dT) antisense primer (Roche): 20 U of MM uLV reverse transcription (Promega, USA), 100 mM of each dNTP; 1 mM DTT; 50 mM Tris HCL, pH 8.3; 75 mM KCL and 6 mM MgCL2. The reaction mixture was incubated at 37°C for 1 h and stored at -20°C until used. Peroxidase gene primer was: The forward primer sequence (Prx1) was, 5'-TTG ACT GTG AGG CTC GGG AG-3' and the reverse primer sequence (Prx4) was, 5'- ACC ATG TCT GTT TCA CTA AGT CC-3'. β , 1-3 glucanase primers were designed according to the published sequence of *Acyrtosiphon pisum* clone IDOAAK9YP20 The

forward primer sequence (GluF) was, 5'-GCC GCAT AAC TTC GTA TAG CA-3' and the reverse primer sequence (GluR) was, 5'-GTC AAC ACA AGT CAT AGT TT-3'.

PCR amplification

Induced peroxidase and β ,1-3 glucanase genes, mRNA was amplified by PCR, using *T-GRADIENT* thermal cycler from Biometra in 25 μ l total volume containing 2.5 μ l of cDNA; 25 pmol of forward and reverse primers for each gene; 10 mM of each dNTPs; 1 U of Taq DNA polymerase; 10mM Tris-HCl, pH 9.0; 50 mM KCl; 1.5 mM Mg Cl₂. The cDNAs were amplified as: Denaturation at 94°C for 3 min. followed by 35 cycles of 1 min each at 94°C; 2 min at 45°C and 1 min at 72°C with a final extension step at 72°C for 7 min.

Agarose gel electrophoresis.

Agarose gel electrophoresis was performed in DNA electrophoresis submarine minicell. Agarose concentration was selected according to DNA size of expected PCR products and the electrophoresis was performed in 1 X TAE buffer (0.04 M Tris acetate, 0.001 EDTA, pH 8.0). DNA samples were mixed with 6 X gel loading dye (10 mM Tris- HCl, pH 7.0, 0.03% promophenol blue, 0.03 % xylene cynol FF, 60% glycerol, 60 mM EDTA). DNA was stained with ethidium bromide that was mixed with the gel and added both to the gel and to the buffer at a concentration of 0.5 g /ml. DNA was visualized on gel documentationsystem (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6C, Fullerton CA92631).

Nucleotide sequencing of β , 1-3 glucanase, peroxidase

Partial nucleotide sequences of β ,1-3 glucanase and peroxidase genes amplified from mRNA isolated from cucumber seedlings inoculated with *Erwinia carotovora* subsp. *carotovora* were sequenced by ABI-PRISM™ 3100 and ABI-PRISM™ 310 Genetic Analyzer by using dye-primer and dye terminator method at Gene Link DNA Sequencing service, New York, USA and Gene Analysis Unit. The resulting sequences for β ,1-3 glucanase and peroxidase genes were then compared to published sequences in the Gene Bank using the CLC Genomic Workbench version 3.6.5.

Results

Detection of Peroxidase gene by RT-PCR

The total RNA from cucumber seedlings healthy and infected with *Erwinia carotovora* subsp. *carotovora* were reverse transcribed using reverse transcription (RT) enzyme. PCR was performed on cDNAs synthesized on these mRNA samples using the forward primer (Prx1) and the (Prx4) as a reverse primer, the RT-PCR amplified fragment of 280 bp from peroxidase gene in the infected seedlings. No signal was detected in non-inoculated seedlings, lane (c) and seedlings inoculated with sterile distilled water lane (4), the same fragment was detected in lanes 1, 2, and 3 which represent cucumber seedlings inoculated with three *Ecc* isolates Cab21B, Cab45B and 119A. The size of the PCR products was estimated by comparing its electrophoretic mobility with those of standard 100 bp DNA marker (Fig. 1).

Nucleotide sequencing of peroxidase gene

The partial nucleotide sequence of peroxidase gene isolated from infected cucumber seedlings was aligned to the published peroxidase sequence at Gene Bank using CLC Genomic Workbench version 3.6.5. program. Comparison of partial nucleotide sequence of peroxidase gene showed 98.9% identity with other published peroxidase gene under accession No. X53675.1 for *Triticum aestivum* (bread wheat). As shown in Fig. 2.

Detection of β 1-3 glucanase gene by RT-PCR

The RT-PCR was used to amplify a fragment of 550 bp from β -1,3 glucanase gene in inoculated seedlings. No signals were detected in non-inoculated seedlings, lane C, or in seedlings inoculated with *Ecc* isolate 119A, lane 3. The fragment was detected in lanes 1 and 2 which represent cucumber seedlings inoculated with *Ecc* Isolates Cab21B and Cab45B, respectively. The size of PCR product was estimated by comparing its electrophoretic mobility with those of standard DNA marker (Fig. 3).

Nucleotide sequencing analysis of β 1-3 glucanase gene

The partial nucleotide sequence of β , 1-3 glucanase gene isolated from inoculated cucumber seedlings was aligned to the published β , 1-3 glucanase sequence in Gen Bank using CLC Genomic Workbench

version 3.6.5. Comparison of partial nucleotide sequence of β 1-3 glucanase gene with other published β , 1-3 glucanase gene under accession No.(U49454.1), (U49454.2), and (U49454.3) of *Prunus persica* (peach) showed 70% identity (Fig. 4).

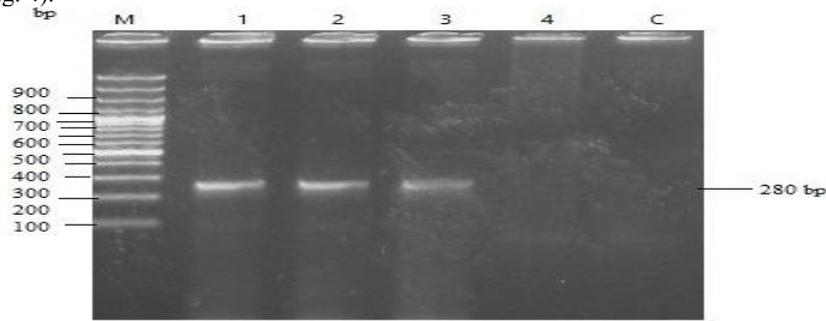


Fig. 1: Agarose gel showing the amplified RT-PCR product, using Peroxidase primer. Amplified total RNA extracted from cucumber leaves infected with *Ecc* isolates Cab21B, Cab45B, 119A and sterile distilled water; lanes 1, 2, 3 and 4, respectively, and non-inoculated seedlings (C). M: 100 bp DNA ladder marker.

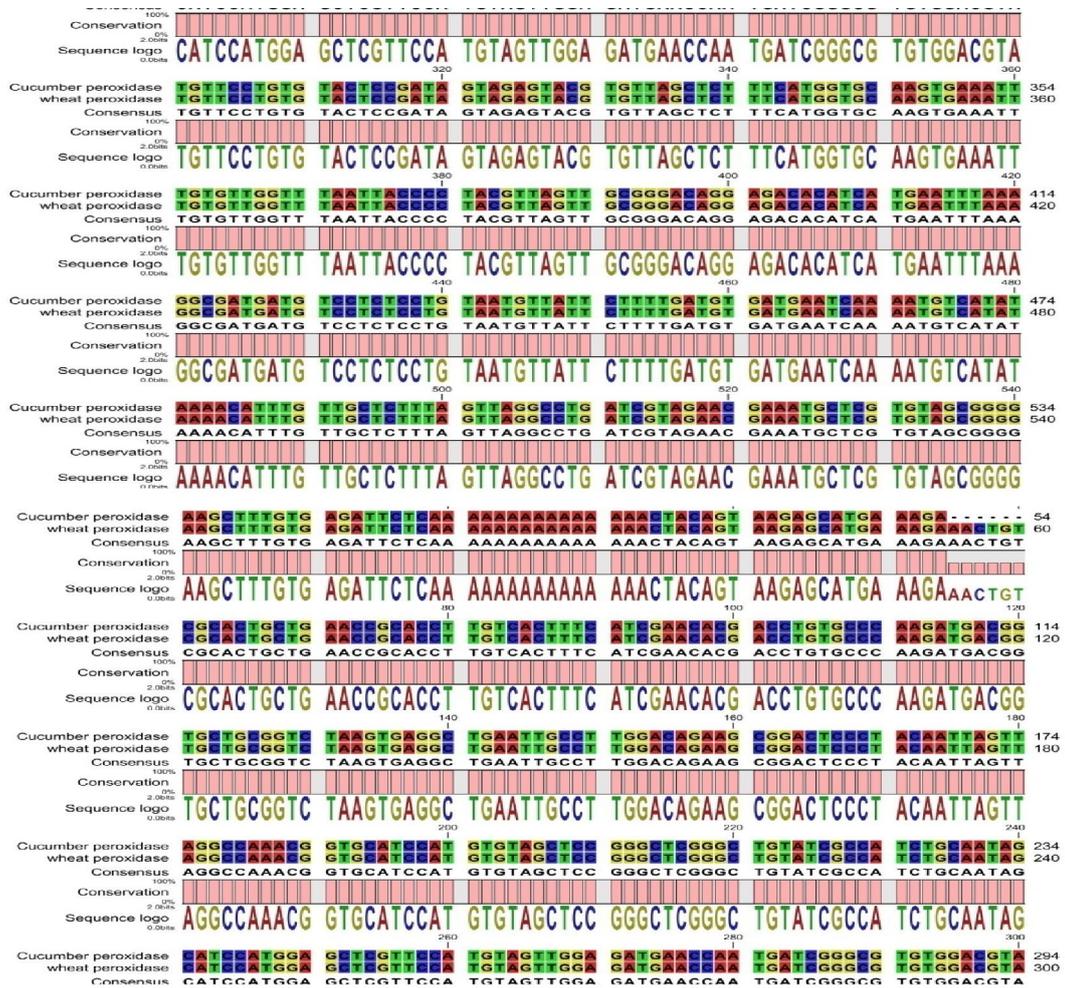


Fig. 2: Multiple sequence alignment of the partial nucleotide sequence of peroxidase gene isolated from inoculated cucumber seedlings compared with corresponding sequence of *Triticum aestivum* (bread wheat) in Gene Bank.

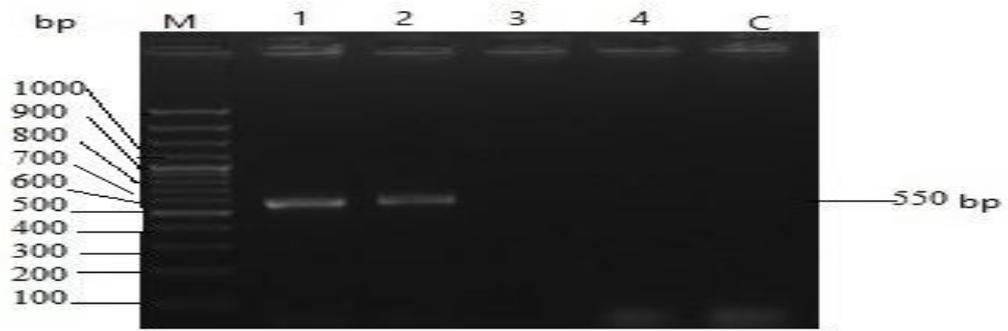


Fig. 3: Agarose gel showing the amplified RT-PCR product, using β 1-3 glucanase primer amplified from total RNA extracted from cucumber leaves inoculated with *Ecc* isolates Cab21B, Cab45B, 119A and sterile distilled water; lanes 1, 2, 3 and 4, respectively, and non-inoculated seedlings, lane (C) M: 100 bp DNA ladder marker.

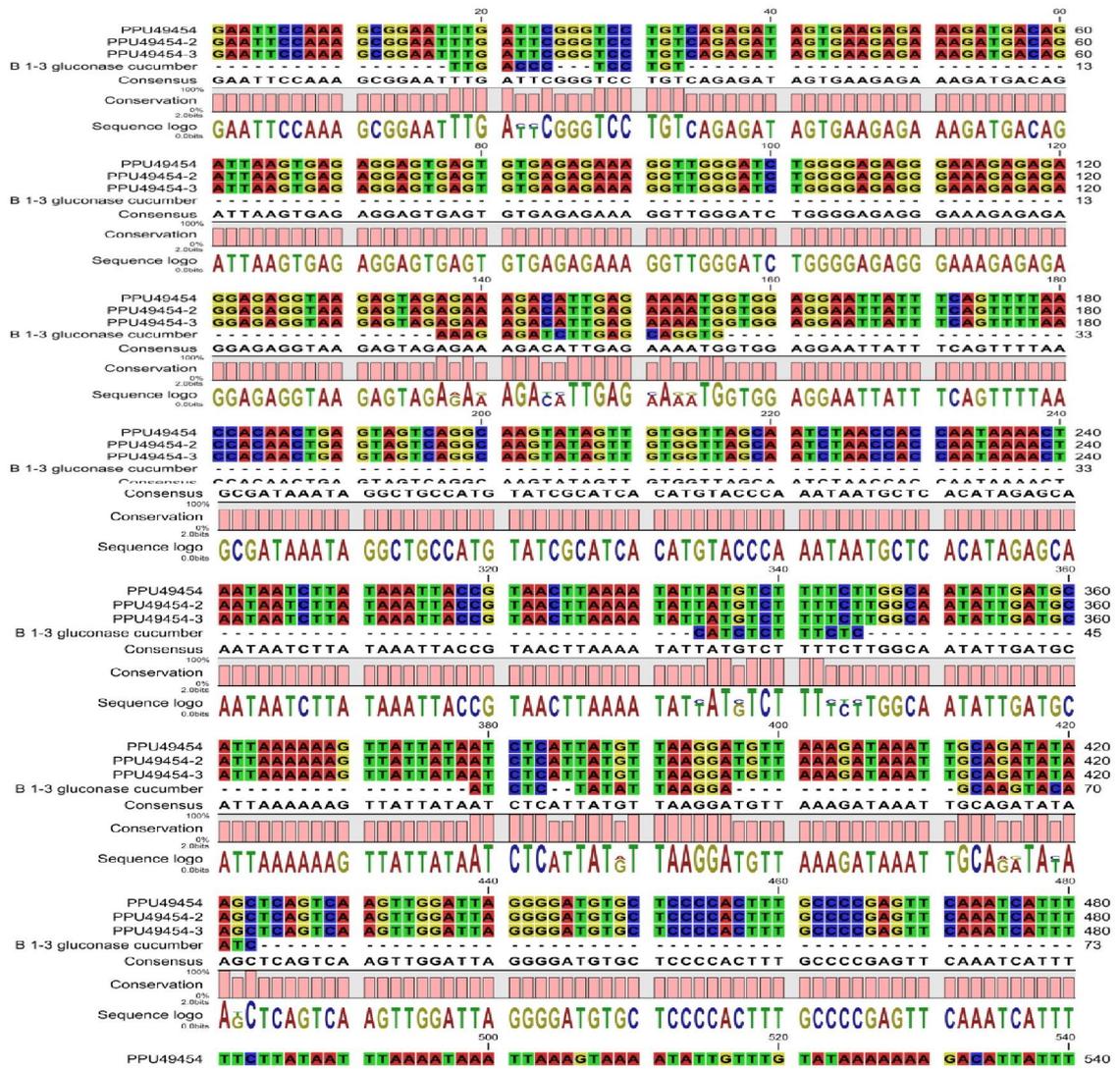


Fig. 4: Part of multiple sequence alignment of the partial nucleotide sequence of β , 1-3 glucanase gene isolated from inoculated cucumber seedlings with corresponding sequence of *Prunus persica* (peach) in Gene Bank.

Discussion

Plant pathogens usually express several virulence factors that enable them to colonize and damage host plant tissues, one of these factors is pectic enzymes that degrade the pectic fractions of the host plant cell wall (Collmer & Keen, 1986 and Sharma *et al.*, 2012), resulting in tissue degradation and nutrient release that facilitate growth of the pathogen. On the other hand, plants have different mechanisms of defense against pathogens, some of them are structural characteristics that could be present naturally in plants or induced after infection and biochemical reactions that take place in cells and tissues of the plant. Also, plants produce substances which either are toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant (Agrios, 2005). One of the important active defense systems in plants is hypersensitive response (HR). The expression of many plant genes is activated during HR, including genes encoding enzymes of phenolic pathway, peroxidase, glucanase and chitinases (Hahlbrock & Scheel, 1989).

Inoculation of three weeks old cucumber seedlings with *Erwinia carotovora* subsp. *carotovora* induced soft-rot disease symptoms only when cotyledons were inoculated with *Ecc* isolates Cab21B or Cab45B. In contrast, inoculation with 119A isolate did not produce any disease symptoms. However, it has been reported that soft rot erwinias attack a wide host range of plant hosts including economically important crops (Perombelon & Kelman, 1980; Perombelon, 2002 and Toth *et al.*, 2003) but the failure of some strains to infect some plant species was reported elsewhere (Arsenijevic & Obradovic, 1996 and El-Hendawy *et al.*, 2002). The inability of *Ecc* 119A to induce disease symptoms on cucumber seedlings from both stem or cotyledons inoculation could be attributed to induction of certain defense mechanisms which were developed against this pathogen. El-Hendawy (2005) found that cucumber seedlings inoculated with isolate 119A contained the highest level of DNA and RNA relative to all other treatments as well as control. Also the highest level of proteins was obtained in cucumber tissue inoculated with this isolate and it was suggested that these proteins play a role in the resistance of cucumber seedlings to this isolate.

To see whether or not some pathogenesis-related protein (PR) were induced in cucumber seedlings inoculated with different *Ecc* isolates, the expression of defense-related genes such as peroxidase and β -1,3-glucanase in inoculated seedlings was investigated using reverse transcription-polymerase chain reaction (RT-PCR). The obtained results indicated that either peroxidase and β -1,3-glucanase genes were expressed in cucumber seedlings inoculated with isolates Cab21B and Cab45B, whereas only peroxidase genes were expressed in seedlings inoculated with isolate 119A. Genes of both enzymes were not detected in seedlings inoculated with sterile distilled water and non-inoculated.

Vidal *et al.* (1997) reported that infection of tobacco plants with *Erwinia carotovora* subsp. *carotovora* or treatment with *Erwinia*-derived elicitor preparations resulted in induction of a number of genes thought to play a role in plant defense response to pathogens. In other studies, pectic enzymes produced by *Erwinia* induced plant defense reactions. For example, endopectate lyase from *Erwinia carotovora* subsp. *carotovora* have been used to elicit phytoalexin production by releasing cell wall fragments (Davis *et al.*, 1984).

Pectic enzymes from (*Ecc*) were found to be an efficient elicitors of plant response (Palva *et al.*, 1993). Vidal *et al.* (1998) had characterized in more details the role of individual enzymes and combination of isolated enzymes of *Ecc* in the induction of defense gene expression in tobacco plants. β -1, 3-glucanase was induced in *Nicotiana plumbaginifolia* tissue in response to *Erwinia* infection (Castresana *et al.*, 1990) and in *Arabidopsis* cell culture in response to culture filtrates from *Erwinia* (Davis & Ausubel, 1989). Also, the increased peroxidase activity was recorded in sweet potato plants due to infection with *Erwinia chrysanthemi* (Jang *et al.*, 2004).

Furthermore, Taheri & Tarighi (2012) detected elevated level of expression for peroxidase genes in the partially resistant tomato cultivar compared to the susceptible cultivar which support the involvement of plant peroxidases in defense. Although it has been reported that several defense-related proteins are induced as a response to wounding (Lagrimini, 1991 and Van Loon *et al.*, 2006), no change was detected in seedlings inoculated with sterile distilled water.

In this study, the presence of β -1,3- glucanase gene in cucumber seedlings inoculated with isolates Cab21B and Cab45B, and its absence in seedlings inoculated with isolate 119A did not affect the susceptibility or resistance of seedlings to these pathogens and this indicates that these proteins might serve essential functions in plant life, whether in defense or not (Van Loon *et al.*, 2006). Also gene of peroxidase are detected in seedlings inoculated with isolates Cab21B or Cab45B and produced soft rot disease symptoms and in seedlings inoculated with isolate 119A which did not produce any disease symptoms. However, it has been suggested that peroxidase activities in plants are involved in cell wall biosynthesis by the polymerization of cinnamyl alcohol into lignin (Lewis *et al.*, 1999), and catalyze polymerization of naturally occurring phenolics to produce a variety of bioactive product, suggesting that peroxidase reaction products might contribute to chemical as well as physical defenses (Kobayashi *et al.*, 1994).

Further work is required to characterize the defense mechanisms which are involved in resistance of cucumber seedlings to *Ecc* isolate 119A.

References

- Agrios, G.N., 2005. Plant pathology. Fifth Edition. Academic press, New York. PR.
- Alexander, R. M. Goodman, M. Gut-Rella, C. Glascock, K. Weymann, L. Friedrich, D. Maddox, D. Ahl-Goy, P., T. Luntz, E. Ward and J. Ryals, 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. Proc. Natl. Acad. Sci. USA. 90, 7327-7331.
- Blom, T.J. and W. Brown, 1999. Preplant copper-based compound reduce *Erwinia* soft rot on calla lilies. Hort Technol. 9, 56-59.
- Boller, T. and S.Y. He, 2009. Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. Science. 323, 742-744.
- Broglie, K., I. Chet, M. Holliday, R. Cressman, P. Biddle, S. Knowlton, C. J. Mauvais and R. Broglie, 1991. Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. Science. 254, 1194-1197.
- Castresana, C., F. De Carvalho, G. Gheysen, M. Habets, D. Inze, and M. Van Montagu, 1990. Tissue-specific and pathogen-induced regulation of a *Nicotiana plumbaginifolia* β -1,3-glucanase gene. Plant Cell. 2, 1131-1144.
- Chomczynsky, P. and N. Sacchi, 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Annals. Of Biotechnology. 162, 156-159.
- Collmer, A. and N.T. Keen, 1986. The role of pectic enzymes in plant pathogenesis. Ann. Rev. Phytopathol. 24, 383-409.
- Davis, K. R. and F. M. Ausubel, 1989. Characterization of elicitor-induced defense responses in suspension-cultured cells of *Arabidopsis*. Mol. Plant-Microbe Interact. 2, 363-368.
- Davis, K. R., G. D. Lyon, A. G. Darvill and P. Albersheim, 1984. Host-pathogen interactions. XXV. Endopolygalacturonic acid lyase from *Erwinia carotovora* elicits phytoalexin accumulation by releasing plant cell wall fragments. Plant Physiol. 74, 52-60.
- El-Hendawy, H.H., 1983. Biological studies on bacteria pathogenic to melons. M. Sc. Thesis. Cairo University, Egypt.
- El-Hendawy, H.H., 2005. Accumulation of proteins in cucumber seedlings resistant to *Pectobacterium carotovorum* subsp. *carotovorum* Phytopathol. Pol. 37, 11-21.
- El-Hendawy, H.H., M.E. Osman and H.A. Ramadan, 2002. Pectic enzymes produced *in vitro* and *in vivo* by *Erwinia* spp. isolated from carrot and pepper in Egypt. J. Phytopathol. 150, 431-438.
- El-Hendawy, H.H., M.O. Osman, and S.M. Korany, 2007. Pectic enzymes produced *in vitro* and *in vivo* by *Pectobacterium carotovorum* subsp. *carotovorum* isolated from cabbage in Egypt. Proceeding of the 11th International Conference on Plant Pathogenic Bacteria, 10-14 July 2006, Edinburgh, UK. p. 145 (Abstract).
- Funnell, K.A., 1993. *Zantedeschia*. In: A. De Hertogh & M. LeNard (Eds.), The physiology of flower bulbs Elsevier, Amsterdam. 683-703.
- Gardan, L., C. Gouy, R. Christen, and R. Samson, 2003. Elevation of three subspecies of *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. Int. J. Syst. Evol. Microbiol. 53, 381-391.
- Hahlbrock, K. and D. Scheel, 1989. Physiology and molecular biology of phenyl propanoid metabolism. Annual Review of Plant Physiology and Plant Molecular Biology, 40: 347-369.
- Jang, I.C., S.Y. Park, K.Y. Kim, S.Y. Kwon, J.G. Kim, S.S. Kwak, 2004. Differential expression of 10 sweet potato peroxidase genes in response to bacterial pathogen, *Pectobacterium chrysanthemi*. Plant Physiol. and Biochem. 42, 451-455.
- Jones, J.D.G. and J.L. Dangl, 2006. The plant immune system. Nature. 444, 323-329.
- Kobayashi, A., Y. Koguchi, H. Kanzaki, S.I. Kajiyama and K. Kawazu, 1994. A new type of antimicrobial phenolics produced by plant peroxidase and its possible role in the chemical defense systems against plant pathogens. Z. Naturforsch. 49, 411-414.
- Lagrimini, L.M., 1991. Wound-induced deposition of polyphenols in transgenic plants overexpressing peroxidase, Plant Physiol. 96, 577-583.
- Leubner-Metzger, G. and F.J. Meins, 1999. Functions and regulation of plant P-1,3-glucanases (PR-2). In: Datta, S. K., Muthukrishnan, S. eds. Pathogenesis-Related Proteins in Plants. CRC Press, Boca Raton, Florida. 77-105.
- Lewis, N.G., L.B. Davin, S. Sarkanen, 1999. The nature and function of lignins, in: D.H.R. Barton, K. Nakanishi, O. Meth-Cohn (Eds.), Comprehensive Natural Products Chemistry. 3. Elsevier. London. 3, 617-745.
- Li, Y., F. Haung, Y. Lu, Y. Shi, M. Zhang, J. Fan and W. Wang, 2013. Mechanism of plant-microbe interaction and its utilization in disease-resistance breeding for modern agriculture. Physiol. and Mol. Plant Pathol. 1-8.

- Mettraux, J.P., W. Burkhart, M. Moyer, S. Dincher, W. Middlesteadt, S. Williams, G. Payne, M. Carnes, and J. Ryals, 1989. Isolation of a complementary DNA encoding a chitinase with structural homology to a bifunctional lysozyme/ chitinase. Proc. Natl. Acad. Sci. USA. 86, 896-900.
- Neuhaus, J.M., 1999. Plant chitinases (PR-3, PR-4, PR-8, PR-11). In "Pathogenesis related proteins in plants", eds. Datta, S.K. and Mathukrishnan, S. CRC Press, Boca Raton. 77-105.
- Palva, T. K., K.O. Holmstrom, P. Heino, and E. T. Palva, 1993. Induction of plant defense response by exoenzymes of *Erwinia carotovora* subsp. *carotovora*. Mol. Plant-Microbe Interact. 6, 190-196.
- Perombelon, M.C.M., 2002. Potato diseases caused by soft rot *Erwinias*: an overview of pathogenesis. Plant Pathol. 51, 1-12.
- Perombelon, M.C.M. and A. Kelman, 1980. Ecology of the soft rot *Erwinias*. Ann. Rev. Phytopathol. 18, 361-387.
- Rasmussen, J.B., J.A. Smith, S. Williams, W. Burkhart, E. Ward, S.C. Somerville, J. Ryals and R. Hamerschmidt, 1995. cDNA cloning and systemic expression of acidic peroxidases associated with systemic acquired resistance to disease in cucumber. Physiol. Mol. Plant Pathol. 46, 389-400.
- Salmond, G. P. C., 1994. Secretion of extracellular virulence factors by plant pathogenic bacteria. Annu. Rev. Phytopathol. 32, 181-200.
- Sela-Buurlage, M. B., A. S. Ponstein, S. A. Bres-Vloemans, L. S. Melchers, P. J. M. Van den Elzen and B. J. C. Cornelissen, 1993. Only specific tobacco (*Nicotiana tabacum*) chitinases and p-1, 3-glucanases exhibit antifungal activity. Plant Physiol. 101, 857-863.
- Sharma, N., M. Rathore, and M. Sharma, 2012. Microbial pectinase: Source, characterization and application. Rev. Environm. Sci. Biotechnol. 12, 45-60.
- Taheri, P. and S. Tarighi, 2012. The role of pathogenesis-related proteins in the tomato *Rhizoctonia solani* Interaction. Journal of Botany. 2012, 1-6.
- Toth, I.K., S.B. Kenneth, M.C. Holeva and P.R.J. Birch, 2003. Soft rot *Erwiniae*: from genes to genomes. Molec. Plant Pathol. 4, 17-30.
- Van Loon, L. C., 1985. Pathogenesis-related proteins. Plant Mol. Biol. 4, 111-116.
- Van Loon, L.C. and E.A. Van Strien, 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol. Mol. Plant Pathol. 55, 85-97.
- Van Loon, L.C., M. Rep, and C.M.J. Pieterse, 2006. Significance of inducible defense-related proteins in infested plants. Ann. Rev. Phytopathol. 44, 135-162.
- Vidal, S., A.R.B. Eriksson, M. Montesano, J. Denecke and T. Palva, 1998. Cell Wall-Degrading Enzymes from *Erwinia carotovora* Cooperate in the Salicylic Acid-Independent Induction of a Plant Defense Response. Mol. Plant-Microbe Interact. 1, 23-32.
- Vidal, S., I.P. De Leon, J. Denecke and E.T. Palva, 1997. Salicylic acid and the plant pathogen *Erwinia carotovora* induce defense genes via antagonistic pathways. The plant J. 11, 115-123.
- Walton, J. D., 1994. Deconstructing the cell wall. Plant Physiol. 104, 1113-1118.
- Wright, P.J. and G.K. Burge, 2000. Irrigation, sawdust mulch, and Enhance (R) biocide affects soft rot incidence, and flower and tuber production of calla. N Z J Crop Hort. 28, 225-231.
- Wright, P.J., G.K. Burge and C.M. Triggs, 2002. Effects of cessation of irrigation and time of lifting of tubers on bacterial soft rot of calla (*Zantedeschia* spp.) tubers. N Z J Crop Hort. 30, 265-272.