Int. J. Phytopathol. 13 (02) 2024. 191-198 DOI: 10.33687/phytopath.013.02.4763

Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

RALSTONIA PSEUDOSOLANACEARUM INFECTING PUMPKIN (*CUCURBITA MAXIMA*) AND ITS PATHOGENICITY ON CUCURBITACEAE FAMILY

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ARTICLE INFO

Article History
Received: August 22, 2023
Revised: May 11, 2024
Accepted: June 12, 2024

Keywords
Bacterial wilt
Biovar
Pumpkin
Phylotype
R. pseudosolanacearum
Sequevar

ABSTRACT

Ralstonia solanacearum species complex (RSSC) is a devastating phytopathogen with wide host range in many economically important crops all over the world. Bacterial wilt on a pumpkin (*Cucurbita maxima*) caused by *Ralstonia pseudosolanacearum* was observed in Chiang Rai, Thailand. The symptoms that appeared were wilting, drooping of half leaves, and oozing from crosscut stems of these diseased plants. The *R. pseudosolanacearum*-specific 280 bp amplicon, and sequevar 17 confirmed by sequencing the *hrpB* gene. The *hrpB* gene have been shown to be key determinants for pathogenicity in the phytopathogenic bacterial wilt. The susceptibility of different Cucurbitaceae species to bacterial wilt were tested. Members of the *Cucurbita, Cucumis, Citrullus*, *Momordica*, and *Lagenaria* genera were susceptible to bacterial wilt, with disease incidence ranging from 30% to 100%. The susceptibility of different Cucurbitaceae species to bacterial wilt, which can provide valuable information for the plant breeding programs to the improvement of pathogen-targeted.

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INTRODUCTION

Bacterial wilt caused by the *Ralstonia solanacearum* species complex has a very wide plant host range of more than 200 species. Traditionally, *R. solanacearum* has been categorized into four biovars based on the metabolism of disaccharides (maltose, lactose, cellobiose and D-trehalose) and hexose alcohols (mannitol, sorbitol and dulcitol), however, biovar 2 was further divided in two different phenotypes: biovar 2T as phenotype A and biovar 2A as phenotype B (French *et al.*, 1993). Due to the significant genetic diversity the *R. solanacearum* species complex was identified by Fegan and Prior (2005) resulting in a new classification for *R. solanacearum*. The species complex is comprised of species, phylotype and sequevar. Phylotype can be further classified into sequevar based on the sequence analysis of *hrpB*, *egl* and *mutS* genes. The phylotypes were separated into four genetic clusters associated with their respective geographical origin by phylogenetic analysis of the ITS region. The *R. solanacearum* species complex is composed of at least 4 phylotypes corresponding to their respective geographical origin. Phylotype I contains strains primarily from Asia; phylotype II includes strains that originated in America; phylotype III originated from Africa and surrounding

islands and phylotype IV includes strains native to Indonesia (Safni *et al.*, 2014; Prior *et al.*, 2016). Phylotype classification is based on the sequevar analysis of the DNA sequence of the intergenic transcribed spacer (ITS) region of the ribosomal RNA gene and is further subdivided by the DNA sequence of the endoglucanase gene (*egl*) (Fegan and Prior, 2005). Polymorphisms in the ribosomal DNA region and functional genes can be further classified into sequevar based on the sequence analysis, including *egl* and *hrpB* genes (Xu *et al.*, 2009). The transcriptional activator *hrpB* gene, encoding type III secretion system (T3SS), has been shown to be key regulator for pathogenicity in the vascular phytopathogenic bacterium *R. solanacearum* species complex (Vasse *et al.*, 2000). The part of *hrpB* pathogenicity that also contains the T3SS genes induces a defense reaction similar to a vascular hypersensitive response (Genin and Boucher, 2004). However, there is a lack of identification by molecular characterization of the *R. solanacearum* species in Thailand. Therefore, the aim of this research was to detect bacterial wilt on pumpkin, and also conducted phylogenetic analyses by *hrpB* gene and examined pathogenicity on Cucurbitaceous plants.

MATERIALS AND METHODS

Pathogen Isolation

Pumpkin (*Cucurbita maxima*) showing symptoms of bacterial wilt was collected in Northern Thailand. Bacterial wilt exhibited symptoms such as wilting and drooping of half leaves. Wilted pumpkin stems were rinsed in water and dipped in sterilized distilled water to obtain bacterial oozes. The isolate Pu6 of bacterial wilt of pumpkin was used in this study from the Bacterial Plant Disease Laboratory, Faculty of Agriculture, Chiang Mai University (Akarapisan *et al.*, 2021). The stems were surface sterilized with 70% ethanol followed by rinsing with sterile distilled water. The bacterial ooze were streak on NA (Nutrient Agar). The plates were incubated at 28-30°C for 2-3 days and then observed the plates for the presence of bacterial colonies. Ralstonia solanacearum colonies typically appear as small, smooth, and creamy-white. Pure isolates were sub-cultured, and glycerol stocks were prepared for long-term storage at -20°C. For the hypersensitive reaction test, tobacco leaves (*Nicotiana tabacum*) were used. Bacterial wilt strain cultivated on NA at 28ºC for 48 hours were utilized in the preparation of the bacterial suspensions (10^8 cfu/ml). The bacterial suspensions were inoculated into an area of approximately 3-5 cm^2 in the leaf intercellular space using hypodermic syringes. Leaves were inoculated with distilled water for the negative control. The reactions on the leaves were recorded at 24 hours after incubation.

Pathogenicity Test on Cucurbitaceous Plants

Bacteria were inoculated on 14-day-old Cucurbitaceous plants for the pathogenicity test including pumpkin, squash 'Mini ball', cucumber, melon, young water-melon, bitter melon, bitter cucumber, bottle gourd and wax gourd. The commercial varieties of Cucurbitaceous plants were collected and used for test resistance to bacterial wilt strain. To prepare inoculum, the bacteria were cultured on NA at 28°C for 48 hours and then bacterial suspensions (10^8 cfu/ml) were prepared. The pumpkin was inoculated by injuring the root. Afterward, 5 ml of the bacterial suspension was poured into each pot. Each strain was inoculated on 4 plants and inoculated at room temperature (32-35°C). The experiment was repeated three times. At 7, 14 and 28 days after inoculation, the disease incidence was calculated as DI (%) = 100 × number of disease plant / total number of inoculated plants in each plot experiment (She *et al.*, 2017). The DI ranged from 0% (no disease) to 100% (dead). The resistance of 9 Cucurbitaceous plants to bacterial wilt from pumpkin was evaluated as follows: DI value of (i) 0%, highly resistant (-); (ii) 1-30%, resistant (+); (iii) 31-60%, moderated resistant (++); and (iv) 61-100%, susceptible (+++) (Yang *et al.*, 2012).

DNA Sequencing of Transcriptional Regulator (*hrpB*) Gene

For identification by molecular analysis, bacterial isolates were cultivated on a rotary shaker at 30ºC and 180 rpm in Luria-Bertani broth (LB) for 18 hrs. A modification of the protocol by Cheng and Jiang (2006) was used to extract the DNA from Gram-negative bacteria. The DNA sample was stored at -20°C until use. For partial sequencing of the *hrpB* gene, the partial nucleotide sequences were done in 50 µl of a final volume of the reaction mixture, containing Quick Taq HS DyeMix (TOYOBO CO., LTD., Japan) and 1.6 pM of the primers hrpBF 5´ TGC CAT GCT GGG AAA ATC T-3´ and hrpBR 5´-GGG GGC TTC GTT GAA CTG C-3´ for the transcriptional regulator (hrpB) genes (Poussier *et al.*, 2000). Thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles consisting at 94°C for 30 s, 67°C for 30 s and 72°C for 1 min, followed by a final extension at 72°C for 10 min. Amplification products were separated in a 1.5%

(w/v) agarose gel and viewed by gel electrophoresis (Opina *et al.*, 1997). The PCR products were purified and directly sequenced by fluorescent dye-terminator sequencing ABI PrismTM 3730xl DNA sequencer (Bio Basic Inc). DNA sequences were aligned by using MEGA10 software and assembled and then compared with available sequences in GenBank using BLASTn for nucleotide alignments. The DNA sequences were transformed into phylogenetic trees by using the neighbor-joining program.

RESULTS

Pathogen Isolation

The bacterial wilt bacterial on pumpkin isolate Pu6 was collected from the Bacterial Plant Disease Laboratory, Faculty of Agriculture, Chiang Mai University (Akarapisan *et al*., 2021). The isolate was identified as *R. pseudosolanacearum* (biovar 2T, phylotype I, sequevar 17 by *egl* gene). The symptoms showed that bacterial wilt-infected pumpkin demonstrated wilting and drooping of half leaves (Figure 1a). The bacterial ooze from stems was milky-white. The isolate showed fluidal colonies, either entirely white on NA or with a pale pink center on TZC (Figure 1b). The pathogenicity tests resulted in the wilting of the lower leaves of 14-day-old pumpkin plants at 3 days after inoculation. After that, wilting continued, and then the lower leaves turned yellow and died (Figure 1c). In the hypersensitive test in tobacco leaves, the isolate caused necrosis that appeared after 24 hours. Tobacco leaves infiltrated with sterile water showed no reaction (Figure 1d). The pathogenicity of this isolate was confirmed on pumpkin under greenhouse conditions to fulfil the Koch's postulates, and re-isolates from the inoculated pumpkin plants same as the colony characteristics of the inoculated isolate.

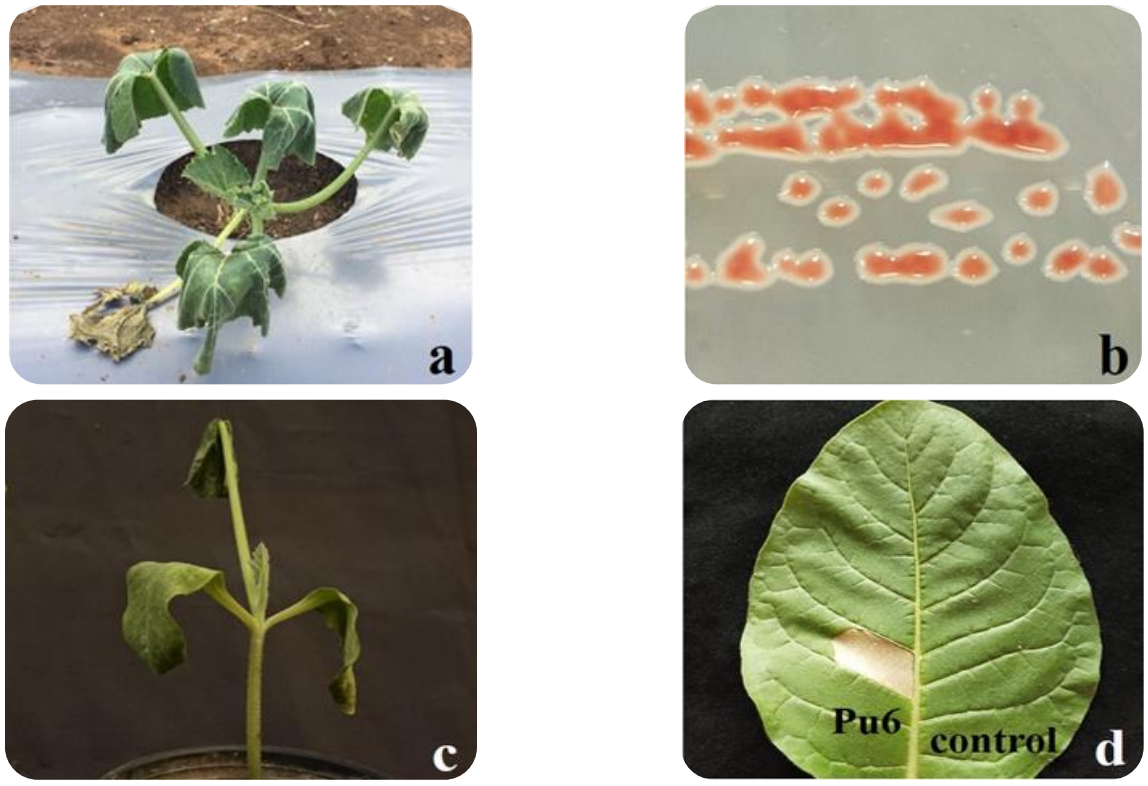

Figure 1. Bacterial wilt disease on pumpkin: (a) symptoms of bacterial wilt on pumpkin, (b) bacterial colony on TZC medium after incubation for 2 days, (c) inoculated pumpkin showing wilt symptoms, (d) hypersensitive reaction test on tobacco with bacterial isolate and sterile water as the negative control at 24 hours after infiltration.

Pathogenicity Test on Cucurbitaceous Plants

In a host range study, the isolate was pathogenic on 9 Cucurbitaceous plants. Seven of the inoculated plants began to wilt at 3 days after inoculation, but melon and young water-melon plants began to wilt at 14 days after inoculation. After inoculation for 28 days, the melon and young water-melon plants still showed fewer symptoms and no death. Therefore, seven of the Cucurbitaceous

plants including pumpkin, squash 'Mini ball', cucumber, bitter melon, bitter cucumber, bottle gourd and wax gourd were susceptible to bacterial isolate. Whereas, melon and young water-melon were resistant to the bacterial wilt isolate at 28 days after inoculation (Table 1). Members of the *Cucurbita, Cucumis, Citrullus*, *Momordica*, and *Lagenaria* genera were susceptible to bacterial wilt, with disease incidence ranging from 30% to 100%.

Phylogenic Analysis Based on *hrpB* Sequences

The isolate Pu6 was identified as *R. pseudosolanacearum* sequevar 17 by *egl* gene (Akarapisan et al. 2021). However, the partial sequence of the *hrpB* gene was analyzed by phylogenetic relationships to confirm. The *hrpB* gene represents a key regulator for the pathogenicity of bacterial wilt. In the study, the *hrpB* gene sequence with 26 reference strains, were retrieved from GenBank. The *R. pseudosolanacearum* strain of pumpkin was classified as sequevar 17. The sequence similarity to sequevar 17 with reference strains Pe11, RS348, E1118 and T01 (Table 2, Figure 2).

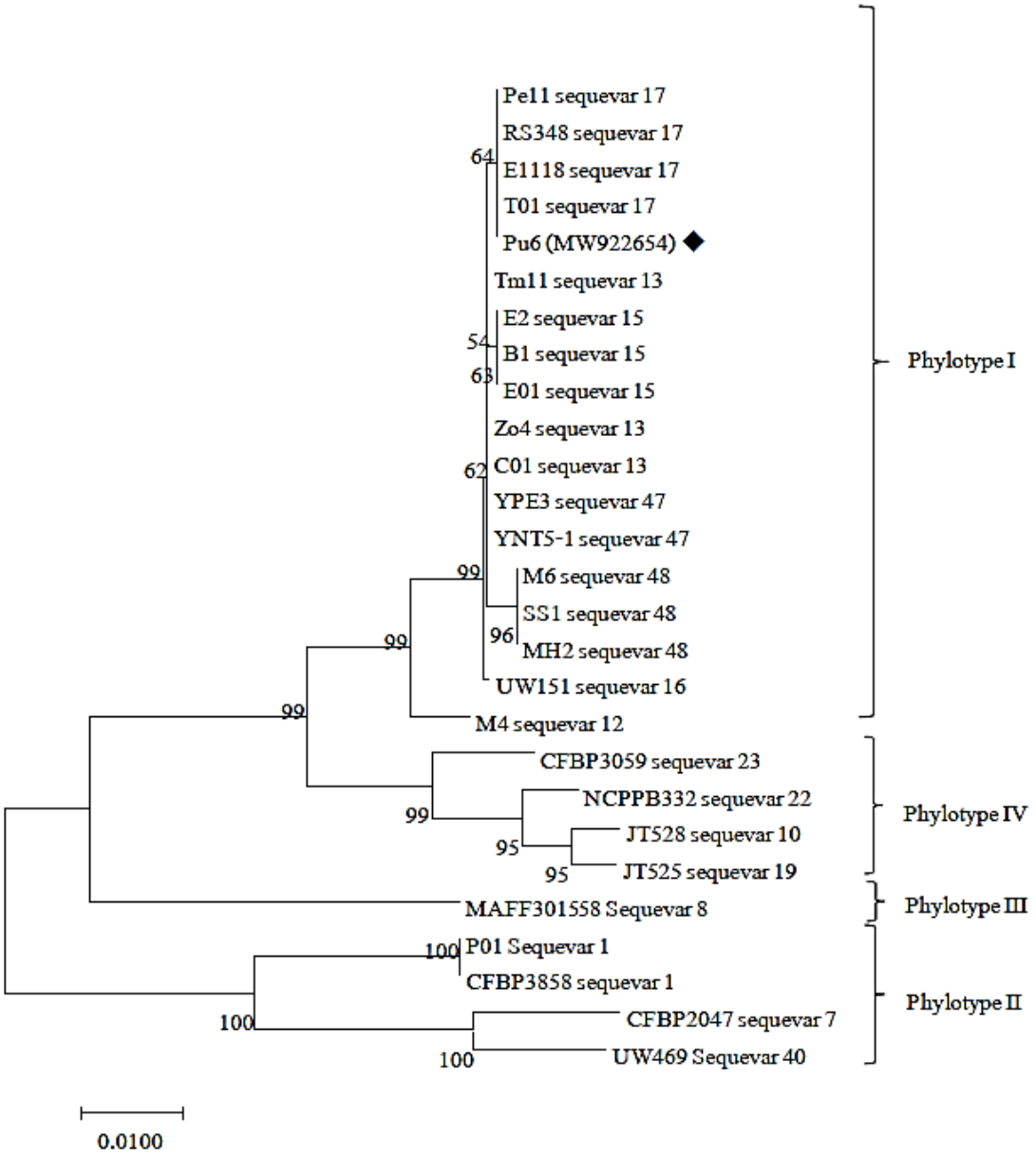

Figure 2. Phylogenetic tree of *Ralstonia pseudosolanacearum* isolated from pumpkin based on the *hrpB* gene sequence obtained by the neighbor-joining method. The numbers at the branching points are the percentages of occurrence in 1000 bootstrapped trees. The scale bar represents 0.1% sequence dissimilarity.

Table 1. Pathogenicity of *R. pseudosolanacearum* on Cucurbitaceous plants.

Varieties	Cultivars	Disease incidence value[a]		
		7 days after inoculation	14 days after inoculation	28 days after inoculation
Cucurbita maxima	Pumpkin	+++	+++	+++
Cucurbita moschata	Squash 'Mini ball'	+++	+++	+++
Cucumis sativas	Cucumber	++	+++	+++
Cucumis melo	Melon	-	+	+
Citrullus lanatus	Young water-melon	-	+	+
Momordica charantia	Bitter melon	+++	+++	+++
	Bitter cucumber	+++	+++	+++
Lagenaria siceraria	Bottle gourd	++	+++	+++
	Wax gourd	+++	+++	+++

[a] DI value of 9 Cucurbitaceous plants to bacterial wilt from pumpkin: 0%, highly resistant (-); 1-30%, resistant (+); 31-60%, moderated resistant (++); and 61-100%, susceptible (+++)
DI (%) = 100 × number of disease plant / total number of inoculate plant in each plot experiment

Table 2. *Ralstonia solanacearum* strains complex used in the phylogenic study based on sequence variation of *hrpB* gene.

Strain	Host	Origin	Phylotype	Sequevar	GenBank No.
Pu6	*Cucurbita maxima*	Thailand	I	17	MW922654
Reference strains					
Pe11	*Capsicum frutescens*	China	I	17	FJ561191
RS348	*Cucurbita moschata*	China	I	17	KY594816
E1118	*Solanum melongena*	China	I	17	FJ561173
T01	*Lycopersicon esculentum*	Thailand	I	17	MG136860
Tm11	*Lycopersicon esculentum*	China	I	13	FJ561226
Zo4	*Zingiber officinale*	Philippines	I	13	FJ561242
C01	*Capsicum frutescens*	Thailand	I	13	MG136859
E2	*Solanum melongena*	China	I	15	FJ561243
B1	*Ipomoea batatas*	China	I	15	FJ561169
E01	*Solanum melongena*	Thailand	I	15	MG136862
UW151	*Zingiber officinale*	Australia	I	16	AF295607
YPE3	*Capsicum annuum*	Myanmar	I	47	LC375922
YNT5-1	*Solanum lycopersicum*	Myanmar	I	47	LC375921
M6	*Morus alba*	China	I	48	FJ561183
SS1	*Curcuma alismatifolia* gagnep	Thailand	I	48	MG136858
MH2	*Curcuma alismatifolia* gagnep	Thailand	I	48	MG136857
M4	*Morus alba*	China	I	12	FJ561181
P01	*Solanum tuberosum*	Thailand	II	1	MG136863
CFBP3858	*Solanum tuberosum*	Netherlands	II	1	AF295612
CFBP2047	*Lycopersicon esculentum*	United-States	II	7	AF295615
UW469	*Solanum tuberosum*	Brazil	II	40	AF295622
MAFF301558	*Lycopersicon esculentum*	Japan	III	8	AY465037
CFBP3059	*Solanum melongena*	Burkina Faso	IV	23	AF295623
NCPPB332	*Solanum tuberosum*	Zimbabwe	IV	22	AF295629
JT528	*Solanum tuberosum*	Island	IV	10	AF295626
JT525	*Pelargonium asperum*	Island	IV	19	AF295625

DISCUSSION

Northern Thailand is located in a tropical region with a warm climate, which provides favorable for the prevalence of bacterial wilt. The bacterial wilt caused by *R. solanacearum* is a major limiting factor to many food productions. The pathogen is pathogenic on more than 200 plant species belonging to over 50 botanical families worldwide (Genin and Denny, 2012). Bacterial wilt disease can cause a complete loss of economic plant yields in Thailand, and it was observed on potato (Dhital *et al.*, 2001). Later, the disease was reported in potato, pepper, tomato, marigold, sesame, ginger and Patumma (Thammakijjawat *et al.*, 2001). Over time, even more hosts were found to be susceptible to *R. solanacearum* infection. In 2018, the occurrence of sequevar member strains by sequence analysis on Patumma, brinjal, chili, tomato and potato of bacterial wilt was reported in Thailand (Thano and Akarapisan, 2018). Recently, Akarapisan *et al.* (2021) reported of 19 bacterial wilt strains that infecting 10 plant species. In this study, isolate Pu6 were obtained from bacterial wilt symptom of pumpkin. The isolate reported in 2021 assessed its pathogenicity, biovar 2T, phylotype I and sequevar 17 of genetic diversity (*egl* gene). The species was identified based on *R. pseudosolanacearum*. This is consistent with previous reports that were pathogenic to pumpkin, cucumber, tomato eggplant, chili and marigold. She *et al.* (2017) reported in 2016 that bacterial wilt on *C. maxima* was first observed in China. They reported that it was caused by *R. pseudosolanacearum* (race 1, biovar 3, phylotype I, sequevar 17), *R. pseudosolanacearum* (race 1, biovar 4, phylotype I, sequevar 45) and *R. pseudosolanacearum* (race 1, biovar 4, phylotype I, sequevar 56). Phylogenic analysis of *R. pseudosolanacearum* clustered into three sequevars, including sequevar 17, 45 and 56. Only sequevar 17 has been report to infect pumpkin in Thailand.

Our results also indicated that the PCR and phylogenetic of *hrpB* gene positioning results confirmed the isolate from the pumpkin in Chiang Rai Province belongs to sequevar 17 proposed by Fegan and Prior (2005). The transcriptional activator *hrpB* gene represents a key regulator for the pathogenicity of bacterial wilt causing *R. solanacearum* species complex. The *hrpB* regulates genes for initiating a hypersensitive reaction and pathogenicity gene, which is produced symptoms in susceptible hosts and hypersensitive reaction on the resistant host or non-host (Poussier *et al.*, 1999). In a host range study, the isolate Pu6 was virulent on pumpkin, squash 'Mini ball', cucumber, bitter melon, bitter cucumber, bottle gourd and wax gourd, but showed fewer symptoms on melon and young water-melon plants. Delaspre *et al.* (2007) reported that the *hrpB* regulon extends beyond this T3SS. The regulator induces 3-hydroxy-oxindole synthesis. The *hrpB* gene, also known as hypersensitive response and pathogenicity B, is a key component of the type III secretion system (T3SS) in certain plant pathogenic bacteria, including *Ralstonia pseudosolanacearum*. The T3SS is a molecular syringe-like apparatus that injects bacterial effector proteins into host plant cells, facilitating the bacteria's ability to cause disease (Liu *et al.*, 2014). In *Ralstonia pseudosolanacearum*, the *hrpB* gene is involved in regulating the expression of virulence factors and pathogenicity-related genes. It controls the expression of various effector proteins that manipulate host cell processes to promote bacterial colonization and disease development, particularly in the context of bacterial wilt (Aldon *et al.*, 2000). These results revealed the genetic structure and phylogenic relationships and will be helpful in bacterial wilt-resistance breeding (She *et al.*, 2017). Breeding programmers for bacterial wilt resistance are considered the most effective strategic measures for controlling the disease.

Therefore, we report that the occurrence of *R. pseudosolanacearum* belonging to biovar 2T, phylotype I, sequevar 17 from the pumpkin in Thailand. Then, the isolate Pu6 was checked and confirmed part of the hrpB gene, which also belongs to sequevar 17. In the virulence assay of this study, compared with the 9 Cucurbitaceous plants, melon and young watermelon showed resistance to the bacterial wilt isolate. The results presented here may help breeding programmers for phylotype-specific resistant host plants in Thailand to be more successful. It will be favorable for the plant breeding program to the improvement of pathogen-targeted and geographically targeted management.

ACKNOWLEDGEMENTS

This research was partially supported by the Innovation Agriculture Research Center, Faculty of Agriculture, Chiang Mai University and the Center of Excellence on Agricultural Biotechnology: (AG-BIO/MHESI), Bangkok, Thailand.

AUTHOR CONTRIBUTIONS

All the authors have contributed equally to research and compiling the data as well as editing the manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

Akarapisan, A., A. Kumvinit, C. Nontaswatsri, T. Puangkrit and W. Kositratana. 2021. Phylotype, sequevar and pathogenicity of *Ralstonia solanaceaum* species complex from Northern Thailand. Journal of Phytopathology, 170: 176-84.

Aldon, D., B. Brito, C. Boucher and S. Genin. 2000. A bacteriai sensor of plant cell contact controls the transcriptional induction of *Ralstonia solanaceaum* pathogenicity gene. The EMBO Journal, 19: 2304-14.

Cheng, H.-R. and N. Jiang. 2006. Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnology Letters, 28: 55-59.

Delaspre, F., C. G. Nieto Peñalver, O. Saurel, P. Kiefer, E. Gras, A. Milon, C. Boucher, S. Genin and J. A. Vorholt. 2007. The *Ralstonia solanacearum* pathogenicity regulator *Hrp*B induces 3-hydroxy-oxindole synthesis. Proceedings of the national academy of sciences, 104: 15870-75.

Dhital, S. P., N. Thaveechai and S. K. Shrestha. 2001. Characteristics of *Ralstonia solanacearum* strains of potato wilt disease from Nepal and Thailand. Nepal Agriculture Research Journal, 4: 42-47.

Fegan, M. and P. Prior. 2005. How complex is the *Ralstonia solanacearum* species complex. In, Bacterial wilt disease and the *Ralstonia solanacearum* species complex.

French, E., P. Aley, E. Torres and U. Nydegger. 1993. Diversity of *Pseudomonas solanacearum* in Peru and Brazil. Australian Centre for International Agricultural Research. Place Published. pp.70-70.

Genin, S. and C. Boucher. 2004. Lessons learned from the genome analysis of *Ralstonia solanacearum*. Annual Review of Phytopathology, 42: 107-34.

Genin, S. and T. P. Denny. 2012. Pathogenomics of the *Ralstonia solanacearum* species complex. Annual Review of Phytopathology, 50: 67-89.

Liu, Z. Y., L. F. Zou, X. B. Xue, L. L. Cai, W. X. Ma, L. Xiong, Z. Y. Ji and G. Y. Chen. 2014. HrcT is a key component of the Type III secretion system in Xanthomonas spp. and also regulates the expression of key hrp transcriptional activator HrpX. . Applied Environment Microbiology, 80: 3908.

Opina, N., F. Tavner, G. Hollway, J. Wang, T. Li, R. Maghirang, M. Fegan, A. Hayward, V. Krishnapillai and W. Hong. 1997. A novel method for development of species and strain-specific DNA proves and PCR primers for identifying *Burkholderia solanacearum* (formerly *Pseudomonas solanacearum*). Asia Pacific Journal of Molecular Biology and Biotechnology, 5: 19-30.

Poussier, S., P. Prior, J. Luisetti, C. Hayward and M. Fegan. 2000. Partial sequencing of the *hrp*B and endoglucanase genes confirms and expands the known diversity within the *Ralstonia solanacearum* species complex. Systematic and Applied Microbiology, 23: 479–86.

Poussier, S., P. Vandewalle and J. Luisetti. 1999. Genetic diversity of African and worldwide strains of *Ralstonia solanacearum* as determined by PCR-restriction fragment length polymorphism analysis of the hrp gene region. Applied and Environmental Microbiology, 65: 2184-94.

Prior, P., F. Ailloud, B. L. Dalsing, B. Remenant, B. Sanchez and C. Allen. 2016. Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. BMC Genomics, 17: 1-11.

Safni, I., I. Cleenwerck, P. De Vos, M. Fegan, L. Sly and U. Kappler. 2014. Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: Proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii* subsp. nov., *R. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. nov. International Journal of Systematic and Evolutionary Microbiology, 64: 3087-103.

She, X., L. Yu, G. Lan, Y. Tang and Z. He. 2017. Identification and genetic characterization of *Ralstonia solanacearum* species complex isolates from *Cucurbita maxima* in China. Frontiers in Plant Science, 8: 1794.

Thammakijjawat, P., N. Thaveechai, W. Kositratana, J. Chunwongse, R. D. Frederick and N. W. Schaad. 2001. Genetic analysis of *Ralstonia solanacearum* strains from different hosts in Thailand using PCR-restriction fragment length polymorphism. Agriculture and Natural Resources, 35: 397-408.

Thano, P. and A. Akarapisan. 2018. Phylotype and sequevar of *Ralstonia solanacearum* which causes bacterial wilt in *Curcuma alismatifolia* Gagnep. Letters in Applied Microbiology, 66: 384-93.

Vasse, J., S. Genin, P. Frey, C. Boucher and B. Brito. 2000. The *hrpB* and *hrpG* regulatory genes of *Ralstonia solanacearum* are required for different stages of the tomato root infection process. Molecular plant-microbe interactions, 13: 259-67.

Xu, J., Z. Pan, P. Prior, J. Xu, Z. Zhang, H. Zhang, L. Zhang, L. He and J. Feng. 2009. Genetic diversity of *Ralstonia solanacearum* strains from China. European Journal of Plant Pathology, 125: 641-53.

Yang, W., Q. Xu, H.-X. Liu, Y.-P. Wang, Y.-M. Wang, H.-T. Yang and J.-H. Guo. 2012. Evaluation of biological control agents against *Ralstonia* wilt on ginger. Biological Control, 62: 144-51.