台灣農業研究 (J. Taiwan Agric. Res.) 75(2):281–291 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0008

Research Article

Development of a Liquichip-Based Assay for Detection of *Paracidovorax citrulli* in Cucurbits

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Abstract

Tsai, C. H., Y. T. Liu, S. Y. Lin, C. S. Tseng, and C. P. Kuan. 2026. Development of a Liquichip-based assay for detection of *Paracidovorax citrulli* in cucurbits. J. Taiwan Agric. Res. 75(2):281–291.

Bacterial fruit blotch (BFB), caused by *Paracidovorax citrulli*, threatens cucurbit production worldwide and often results in severe economic losses. In this study, we developed a detection assay for sensitive detection of *P. citrulli*. The assays employ polymerase chain reaction (PCR) with specific co-amplification of a plant gene as an internal control from total nucleic acids, enabling parallel detection and minimizing the risk of false-negative PCR results during routine testing and eliminating the need to remove contaminating plant DNA from extracts. The assay exhibited high specificity, as no cross-reactions were observed with non-target bacterial species or with nucleic acid extracted from healthy cucurbit tissues. Sensitivity testing demonstrated reliable detection of low concentrations of pathogen DNA, and the method was validated using artificially inoculated seedlings and field samples. Compared with conventional PCR, the multiplex detection assay provides higher throughput and quantitative readouts based on mean fluorescence intensity. This platform offers a reliable tool for seed health testing, quarantine surveillance, and integrated management of BFB in cucurbit crops.

Key words: *Paracidovorax citrulli*, Bacterial fruit blotch, Cucurbits, Diagnosis.

INTRODUCTION

Bacterial fruit blotch (BFB) is a devastating seedborne disease of cucurbits, primarily affecting *Citrullus lanatus* (watermelon) and *Cucumis melo* (melon). The causal agent was historically identified as *Acidovorax citrulli* (Schaad *et al*. 1978; Burdman & Walcott 2012), but recent phylogenomic analyses led to its reclassification as *Paracidovorax citrulli* (Du *et al*. 2023). This taxonomic update reflects an improved understanding of the *Comamonadaceae* lineage and underscores the need to update diagnostic protocols and reference databases accordingly.

BFB has caused serious epidemics worldwide, resulting in significant economic losses in watermelon and melon seed production. The bacterium is primarily transmitted through contaminated seeds, which serve as the main inoculum source for field outbreaks and facilitate international spread via trade (Burdman & Walcott 2012). Infected seedlings exhibit cotyledon necrosis, seedling wilt, and fruit lesions under

Received: November 27, 2025; Accepted: March 6, 2026.
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warm and humid conditions, while latent seed infections remain symptomless but highly infectious. Therefore, sensitive detection of *P. citrulli* is critical for effective disease management and phytosanitary certification.

In Taiwan, BFB was first reported in muskmelon and watermelon in the late 1990s, where disease incidence reached economically damaging levels (Cheng *et al*. 2000). The pathogen remains an occasional but notable threat to local cucurbit production. However, despite national surveillance and quarantine measures, the lack of a rapid, high-throughput detection platform remains a key limitation for seed-health testing programs. Diagnostic methods for *P. citrulli* have traditionally relied on grow-out assays and serological methods (Wang & Cheng 2001; Azman Husni *et al*. 2021). The enzyme-linked immunosorbent assay (ELISA) has been widely used but often lacks sensitivity for detecting low bacterial titers (Hopkins *et al*. 1996). Polymerase chain reaction (PCR) and real-time PCR offer greater sensitivity and specificity; however, their throughput remains limited in large-scale seed testing programs (Song *et al*. 1995; Schaad *et al*. 2003). The novel detection method is based on Liquichip technology, using a chemiluminescent detection and analysis system, in combination with multiplex polymerase chain reaction. This assay can simultaneously detect multiple pathogens within a single reaction tube (Lim *et al*. 2016). Although PCR-based assays are widely used for detecting *P. citrulli*, they are generally limited to single-target analysis and can be affected by inhibitory substances present in seed extracts. Liquichip technology enables high-throughput, multiplex detection of multiple targets in a single reaction, making it more suitable for large-scale seed health and quarantine screening. In addition, the flexible design of Liquichip assays allows new targets to be incorporated without extensive re-optimization. Therefore, the development of a Liquichip-based assay provides a complementary and efficient diagnostic approach for bacterial fruit blotch detection.

The aim of this study was to develop a Liquichip-based assay for detecting *P. citrulli*. The assay enables simultaneous detection of the target pathogen and plant internal control genes, and its specificity and sensitivity were systematically evaluated. The performance of the Liquichip assay was further compared with that of conventional PCR methods.

MATERIALS AND METHODS

Plant and bacterial strains

Seedlings of watermelon (*Ci. lanatus* (Thunb.) Matsum. & Nakai) and melon (*Cu. melo* L.) at the two- to three-leaf stage were used for all experiments. Seeds were grown in a controlled-environment growth chamber maintained at 25 ± 2℃ with a 16 h light/8 h dark photoperiod and approximately 70% relative humidity. Plants were watered daily and fertilized weekly with a balanced nutrient solution until used for inoculation or sampling. The bacterial pathogen, *P. citrulli* (syn. *A. avenae* subsp. *citrulli*) AAC33 (strain AAC33) was isolated from infected watermelon tissues and maintained at the Taiwan Agricultural Research Institute (TARI), Taichung, Taiwan. For experimental use, the bacterial culture was grown on nutrient agar (NA) plates at 28℃ for 48 h and stored at 4℃ for short-term preservation. Liquid cultures were prepared in nutrient broth (NB) and incubated at 28℃ with shaking at 200 rpm overnight. Reference bacterial strains used for specific testing included *Xanthomonas axonopodis* pv. *vesicatoria* (XV43), *Pseudomonas* spp. (P3), *Erwinia carotovora* (BCRC13147), *Bacillus subtilis* (BCRC6633), and *Escherichia coli* (DH5α). These strains were obtained from the TARI culture collection. All bacterial isolates were routinely cultured on NA or NB at 28℃ and stored at -80℃ in 20% glycerol for long-term preservation.

Total DNA extraction

Genomic DNA of *P. citrulli* was extracted

from pure bacterial cultures using a commercial bacterial DNA extraction kit according to the manufacturer's instructions. Briefly, a single bacterial colony was cultured in nutrient broth at 28℃ for 24 h with shaking, then centrifugated to collect the cells. Genomic DNA was purified, eluted in nuclease-free water, and stored at -20℃ until use. DNA concentration and purity were measured using a spectrophotometer, and the extracted DNA was used as the template for subsequent PCR-based assays.

Primer design

Specific primers targeting *P. citrulli* (AAC) were designed for the ribosomal gene using PrimerPlex 2.0 (PREMIER Biosoft, Palo Alto, CA, USA), based on available nucleotide sequences of the pathogen and host plant genomes. Candidate primers were verified for specificity using Basic Local Alignment Search Tool (BLAST) searches against the National Center for Biotechnology Information (NCBI) database to avoid cross-reactivity with other cucurbit pathogens. PrimerPlex 2 (PRIMER Biosoft, Palo Alto, CA, USA) was used to design target-specific primer extension/allele-specific primer extension (TSPE/ASPE) primers for multiplex PCR reactions. Final primer sequences used in this study are listed in Table 1.

Specificity of PCR for *P. citrulli*

Each 20 μL reaction contained 1 U Taq DNA polymerase (Platinum II, Invitrogen, Carlsbad, CA, USA), 2 μL of 10x PCR buffer, 400 μM dNTPs, 0.4 μM of each primer, 5–10 ng of template cDNA, and ddH_2O to 20 μL and PCR amplification was performed with thermal cycling consisted of 94℃ for 5 min, followed by 35 cycles of 94℃ for 30 s, 55℃ for 30 s, and 68℃ for 2 min, with a final extension at 68℃ for 10 min. PCR products were resolved on 2% agarose gels, stained with ethidium bromide, and imaged under UV light.

TSPE/ASPE

The probes and primers used in this study are listed in Table 1. Each probe has two functional regions: the 3′ end (18–30 nts) acts as a primer in the TSPE reaction, complementary to the target gene, while the 5′ end contains a Target Amplification gene (TAG) sequence for xTAG bead hybridization. ASPE and hybridization to xTAG microspheres were performed in a 20 μL reaction containing 5 μL of PCR product, 1× TSPE buffer, 1.0 mM $MgCl_2$, 0.05 mM of each TAG-ASPE primer, 0.75 U Tsp DNA polymerase, 5 mM of each dNTP except dCTP, and 5 mM biotin-14-dCTP (Invitrogen, Carlsbad, CA,

Table 1. Primers used in this study.

Pathogen/gene target	Target region[z]	Sequence (5′-3′)[y]	Position[x]	Amplicon size	MagPlex-TAG bead address[w]
Paracidovorax citrulli	F1	CGACATATGCAACGACTTA	2066341-2066579	239 bp	53
	R1	TGACGGTGGAATCAAATC			
	T	TTAACAACTTATACAAACACAAACTGCCG AGATAGCCATTACCAGAC -3			
Cyoxid	F	GAGGCAACAACCAGAATGAAAT	75101-75403	303 bp	26
	R	TCTCCACTAACCACAAGGATATA			
	T	TACATTCAACACTCTTAAATCAAAGCCCAT CACTCCAGCAATG			

[z] F, R, and T represent forward primer, reverse primer, and Target Amplification gene-allele-specific primer extension (TAG-ASPE) primer, respectively.
[y] Underlined indicates the MagPlex-TAG sequences provided by Luminex Corporation.
[x] Position of primers for *P. citrulli* and Cyoxid are based on the accession number CP000512.1 and MF034193, respectively.
[w] The MagPlex-bead product number assigned by Luminex Corporation.

USA). TSPE thermocycling conditions were: 94℃ for 2 min, then 30 cycles of 94℃ for 30 s, 55℃ for 30 s, and 74℃ for 1 min.

PCR products were verified by 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light.

Liquichip analysis

Detection of TSPE products with biotin was carried out using flow cytometry and magnetic bead hybridization. All xTAG magnetic bead sets were obtained from Luminex (Austin, TX, USA). Each bead set carried complementary anti-TAG sequences paired with specific TSPE probes, enabling allelic-specific hybridization.

Hybridization reactions were performed in 96-well plates, each with a total volume of 50 μL. This included 25 μL of the xTAG bead mixture (100 beads μL^{-1} in 2× tetramethylammonium chloride (TMAC) buffer: 0.4 M NaCl, 0.2 M Tris, 16% Triton X-100, pH 8.0), 5 ul TSPE extension product, and each MagPlex-TAG bead set (Table 1). The final volume was adjusted with distilled water.

The hybridization procedure: 25 μL of bead mix, 5 μL of ASPE reaction product, and 20 μL of water were incubated at 37℃ for 30 min. No-template controls were included as background references.

After hybridization, the beads were collected with a magnetic separator (Luminex, Austin, TX, USA) and washed twice with 75 μL of 1× TMAC buffer. Beads were then resuspended in 75 μL of 1× TMAC buffer containing 4 μg mL^{-1} streptavidin-R-phycoerythrin (Invitrogen, Carlsbad, CA, USA) and incubated at 37℃ for 15 min.

The final products were analyzed on the Liquichip 200 workstation at 37℃. Results were expressed as mean fluorescence intensity (MFI). For each bead set, a signal three times higher than the highest background MFI was considered positive.

Hybridization and fluorescence detection

For hybridization, 5 μL of PCR product was combined with 2,500 coupled beads in 1.5× TMAC buffer (final volume 50 μL). Samples were denatured at 95℃ for 5 min, then hybridized at 50℃ for 20 min. Beads were incubated with streptavidin-R-phycoerythrin (Molecular Probes, Eugene, OR, USA) at 50℃ for 15 min. Fluorescence was measured on the Liquichip 200 system (Qiagen, Hilden, Germany), and MFI values were recorded.

Specificity and sensitivity assay

The specificity of the assay was evaluated using total DNA extracted from *P. citrulli* isolates, unrelated bacterial pathogens (*X. axonopodis* pv. *vesicatoria*, *Pseudomonas* spp., *E. carotovora*, *B. subtilis*, and *E. coli*, as well as healthy plant tissues. Each reaction was performed in triplicate and independently repeated.

To assess detection sensitivity, genomic DNA from *P. citrulli*-infected watermelon plants was used as a template. Ten-fold serial dilutions ranging from 10ng to 10^{-6} ng were prepared in diethyl pyrocarbonate (DEPC)-treated water. Each dilution was tested in parallel by conventional PCR and the Liquichip assay, with all reactions performed in triplicate. For Liquichip analysis, results were recorded as MFI values, and a reaction was considered positive when the MFI exceeded at least three times the background signal.

Inoculation and disease assessment

P. citrulli was cultured in NB medium, incubated overnight at 28℃, and adjusted to approximately 10^9 CFU mL^{-1}. Seedlings were inoculated by the needle-wound inoculation method, in which bacterial suspensions were applied to wounded leaf surfaces created with a sterile needle. Sterile distilled water was used as the negative control. Disease symptoms were recorded daily for 10 d post-inoculation, and symptomatic leaf tissues were collected for subsequent analyses. Leaf samples (~0.1 g) were collected from watermelon plants grown in fields in Taiwan. Total DNA extraction and PCR were performed as described above. Detection was performed using the Liquichip assay, and results were validated by conventional PCR.

RESULTS

Evaluation of analytical cross-reactivity in the Liquichip assay for AAC detection

To assess assay specificity, DNA extracts from melon and watermelon tissues were subjected to PCR with *P. citrulli*-specific primers, yielding a 239-bp amplicon. A plant cyoxid gene fragment (303 bp) was co-amplified as an internal control to verify DNA quality. The amplified products were hybridized with microsphere-conjugated probes and analyzed using the Liquichip system. Strong fluorescence signals were detected only in samples infected with *P. citrulli*, whereas no cross-reactivity was observed in healthy cucurbit tissues or negative controls (sterile water). The cyoxid internal control was consistently detected in all reactions, confirming successful DNA extraction and amplification (Fig. 1).

Specificity testing further demonstrated that the Liquichip assay reacted exclusively with *P. citrulli* and showed no signal with other bacterial species, including *E. coli*, *B. subtilis*, *Pseudomonas* spp., *X. axonopodis* pv. *vesicatoria*., or *E. carotovora* (Fig. 2).

Detection limit analysis of Liquichip and RT-PCR

To determine the detection limit, a 10-fold serial dilution of total DNA extracted from *P. citrulli*-infected watermelon was prepared and used as the template for both PCR and Liquichip assays. PCR amplification followed by agarose gel electrophoresis revealed that *P. citrulli* could be specifically detected up to a dilution of 10^{-5}, corresponding to approximately 10^{-4} ng of DNA. Similarly, the Liquichip system also produced detectable signals at the 10^{-5} dilution, corresponding to about 10^{-4} ng of target DNA. These results indicate that the sensitivity of the Liquichip assay is comparable to that of PCR assay (Figs. 3 and 4).

Field evaluation of AAC detection using the Liquichip assay

Disease symptoms developed progressively in both melon and watermelon seedlings after inoculation with *P. citrulli* (Table 2, Fig. 5). In melon, visible symptoms appeared by dpi (day of post infection) 2 in some plants and reached 100% incidence by dpi 7. Watermelon showed

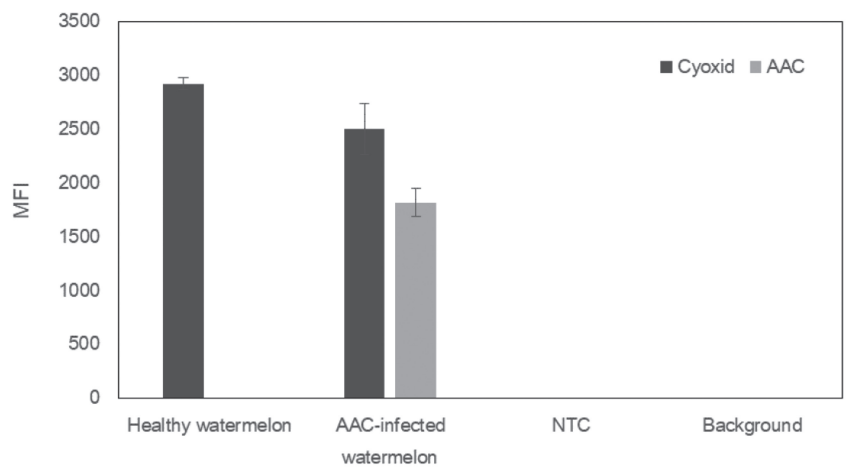

Fig. 1. Specificity assay of Liquichip for the detection of *Paracidovorax citrulli*-infected plant. Tested samples included *P. citrulli*-infected watermelon and healthy watermelon. All reactions were performed with dual probes simultaneously, and background signal values were subtracted. Each bar represents the average MFI of the Liquichip assay. NTC indicates no-template reaction, respectively. Error bars represent the standard deviations from three replicate reactions. MFI: mean fluorescence intensity. *Acidovorax avenae* subsp. *citrulli* (AAC)-infected watermelon: *P. citrulli*-infected watermelon.

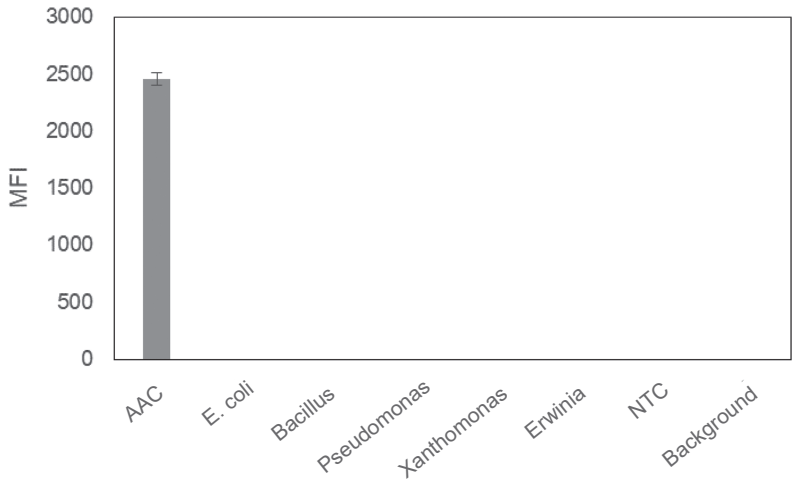

Fig. 2. Specificity assay of Liquichip for detection of *Paracidovorax citrulli*. Tested samples included *P. citrulli* (*Acidovorax avenae* subsp. *citrulli*; AAC), *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas* spp., *Xanthomonas axonopodis* pv. *vesicatoria*, *Erwinia carotovora*, and healthy watermelon. All reactions were performed with dual probes simultaneously, and background signal values were subtracted. Each bar represents the average MFI of the Liquichip assay. NTC indicates no-template reaction, respectively. Error bars represent the standard deviations from three replicate reactions. MFI: mean fluorescence intensity.

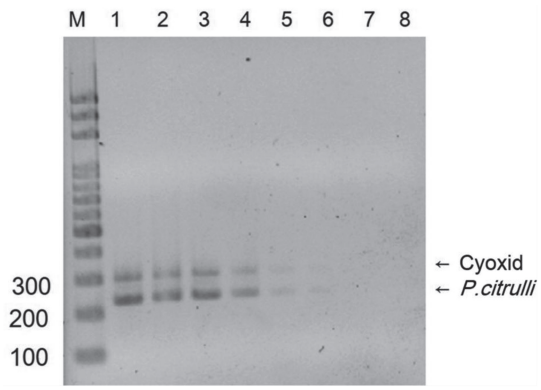

Fig. 3. Sensitivity evaluation of polymerase chain reaction (PCR) for the detection of *Paracidovorax citrulli*. PCR products amplified from a 10-fold serially diluted *P. citrulli*-infected watermelon. Lane M represents the 100-bp ladder. Lane 1–8: templates of total DNA were from 10 ng to 10^{-6} ng serial dilutions, respectively.

a similar disease progression but with slightly faster detection using the Liquichip assay. At dpi 2, Liquichip detected *P. citrulli* in all three watermelon plants (100%), while only two of three melon plants (67%) tested positive. By dpi 4, both hosts reached full detection with Liquichip, whereas PCR required until dpi 7 for complete detection in both species.

DISCUSSION

Bacterial fruit blotch remains a major limiting factor in cucurbit production (Burdman & Walcott 2012). The primary strategy for disease management is early and accurate detection, as no effective resistant cultivars are available. Traditional assays, such as isolation and ELISA, provide useful diagnostic tools but are often constrained by sensitivity (Hopkins *et al*. 1996; Schaad *et al*. 2003). PCR assays have improved sensitivity and specificity (Song *et al*. 1995; Walcott & Gitaitis 2000), but their use for routine large-scale testing remains limited. The International Seed Testing Association (ISTA) currently recommends ELISA and PCR-based methods for the detection of *P. citrulli* in seed health testing (ISTA 2022). While ELISA is widely adopted because of its simplicity and ability to screen many samples simultaneously, its sensitivity is insufficient when pathogen titers are low (Hopkins *et al*. 1996; Burdman & Walcott 2012). PCR improves detection sensitivity but

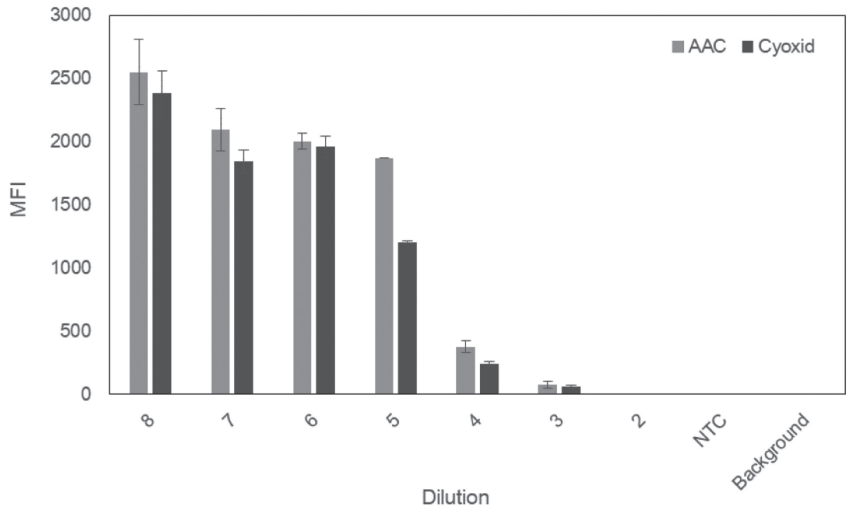

Fig. 4. Sensitivity evaluation of Liquichip for the detection of *Paracidovorax citrulli*. DNA extracted from *P. citrulli*-infected watermelon was serially diluted from 10 ng to 10^{-6} ng. Each bar represents the average MFI of the LiquiChip assay. NTC indicates no-template reaction, respectively. Error bars represent the standard deviations from three replicate reactions. MFI: mean fluorescence intensity. *Acidovorax avenae* subsp. *citrulli* (AAC)-infected watermelon: *P. citrulli* infected watermelon.

Table 2. Disease development and detection of *Paracidovorax citrulli* in melon and watermelon seedlings.

Host	dpi[z]	Symptom observation	PCR[y] positives (n/3, %)	Liquichip positives (n/3, %)
Melon	0	No visible symptom	0/3 (0%)	0/3 (0%)
Melon	2	Small water-soaked lesions	1/3 (33%)	2/3 (67%)
Melon	4	Necrotic spots with chlorotic	2/3 (67%)	3/3 (100%)
Melon	7	Expanded blotches	3/3 (100%)	3/3 (100%)
Melon	10	Expanded blotches	3/3 (100%)	3/3 (100%)
Watermelon	0	No visible symptom	0/3 (0%)	0/3 (0%)
Watermelon	2	Small water-soaked lesions	2/3 (33%)	3/3 (100%)
Watermelon	4	Necrotic spots with chlorotic	2/3 (67%)	3/3 (100%)
Watermelon	7	Expanded blotches	3/3 (100%)	3/3 (100%)
Watermelon	10	Expanded blotches	3/3 (100%)	3/3 (100%)

[z] dpi: day of post infection; n/3, %: percentage of positives = (n/3) × 100%.
[y] PCR: polymerase chain reaction.

requires labor-intensive nucleic acid extraction and electrophoretic analysis of products, which limits its applicability in high-throughput seed testing laboratories (Minsavage *et al*. 1990). The Liquichip assay developed here demonstrated high specificity and sensitivity comparable to PCR. In our evaluation, the Liquichip assay achieved detection sensitivity down to 10^{-4} ng of *P. citrulli* DNA, likewise, PCR reliably detected 10^{-4} ng as well. This level of sensitivity surpasses the detection capacity of ELISA and is comparable to, or better than, PCR-based assays (Bergervoet *et al*. 2008). The specificity assessment confirmed that the assay was highly selective for *P. citrulli*, with no cross-reaction observed with unrelated cucurbit pathogens or healthy plant extracts. Importantly, the inclusion of an internal control (plant gene) ensured DNA extraction quality,

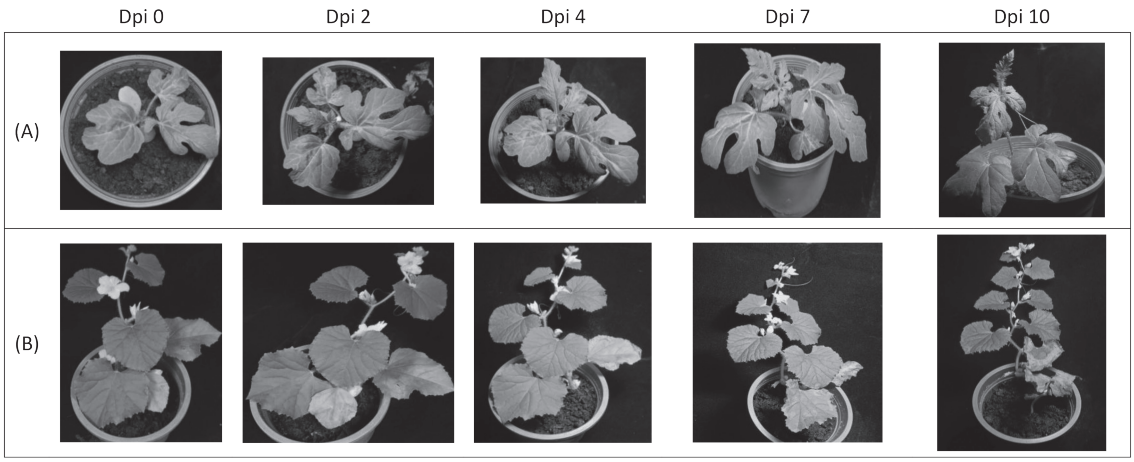

Fig. 5. Disease progression and sampling timeline of watermelon and melon plants following inoculation with *Paracidovorax citrulli* (*Acidovorax avenae* subsp. *citrulli*; AAC). Plants were monitored daily for symptom development from 1 to 10 d post-inoculation (dpi). Leaf samples were collected at 0, 2, 4, 7, and 10 dpi for subsequent pathogen detection and analysis. (A) watermelon; (B) melon.

thereby reducing the risk of false negatives, a key consideration in seed certification programs. Beyond sensitivity, the multiplexing capacity of the Liquichip system allows simultaneous detection of multiple pathogens in a single reaction. This greatly enhances testing efficiency, reducing reagent use and labor compared with conventional PCR or ELISA (Bergervoet *et al*. 2008). Although seed testing is not currently used in seed health laboratories, where a large number of samples must be screened, the semi-automated nature of the Liquichip platform further reduces dependence on specialized personnel and enables reliable, high-throughput testing.

Our results indicate that the differing symptom severity between watermelon and melon may be influenced by variations in pathogen concentration, which could reflect host-specific responses to BFB infection. Watermelon and melon are generally recognized as highly susceptible hosts, typically supporting rapid bacterial multiplication and producing typical BFB symptoms, whereas cucumber and squash often exhibit low-titer or latent infections with mild or absent symptoms (Hopkins *et al*. 1996; Burdman & Walcott 2012). These host-associated differences directly influence diagnostic assay performance because pathogen loads in different cucurbits can vary by several orders of magnitude. Quantitative analyses have shown that pathogen titers in symptomatic watermelon seedlings frequently exceed 10^6 CFU g^{-1}, while cucumber tissues often harbor only 10^2–10^3 CFU g^{-1}, levels that pose substantial detection challenges for ELISA and, in some cases, even for standard PCR assays (Walcott & Gitaitis 2000; Schaad *et al*. 2003). These limitations have resulted in false negatives in seed health certification programs, particularly when dealing with asymptomatic low-level infections (Song *et al*. 1995; Hopkins *et al*. 1996). Although earlier PCR-based assays provided higher sensitivity than serological methods, their analytical performance remained partially dependent on pathogen concentration and host species.

In Taiwan, BFB has been recognized as a persistent concern for both melon and watermelon production. Outbreaks were first documented in the 1990s, and periodic re-emergence continues to constrain seedling production and commercial fruit yields (Cheng *et al*. 2000). The pathogen has been detected in domestic and imported seed lots, underscoring the importance of rapid and highly sensitive diagnostic systems for

quarantine screening. Recent surveys in Taiwan have demonstrated considerable variability in bacterial loads among cucurbit hosts, consistent with global findings (Cheng *et al*. 2000). These host-dependent variations further underscore the necessity for diagnostic platforms that can accurately detect *P. citrulli* across a wide range of cucurbits, regardless of symptom expression or bacterial concentration.

The Liquichip assay developed in the present study offers clear advantages over conventional molecular and serological methods. By integrating multiplex PCR with bead-based hybridization and chemiluminescent detection, the diagnostic platform enhances signal-to-noise ratios and improves analytical sensitivity (Lim *et al*. 2016), particularly for low-titer infections or testing of asymptomatic cucurbits or seedling materials. This capacity for high-throughput detection is especially valuable for large-scale seed testing programs, where early detection of asymptomatic contamination is critical. Overall, combining molecular specificity with bead-based multiplex detection provides a powerful tool to strengthen BFB surveillance, support seed certification, and reduce the risk of pathogen dissemination.

In conclusion, the Liquichip assay demonstrated performance comparable to PCR for the detection of *P. citrulli*, providing sensitivity and greater diagnostic efficiency. Its advantages, including sensitivity and reduced labor requirements, make it a valuable alternative to conventional seed health assays. By enabling rapid and reliable detection of BFB, this technology has the potential to enhance disease diagnosis and contribute to the sustainable production of cucurbit crops.

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建立以 Liquichip 為基礎之葫蘆科作物中 *Paracidovorax citrulli* 檢測分析方法

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摘要

蔡佳欣、劉雅婷、林思妤、曾清山、關政平。2026。建立以 Liquichip 為基礎之葫蘆科作物中 *Paracidovorax citrulli* 檢測分析方法。台灣農業研究 75(2):281–291。

由 *Paracidovorax citrulli* 引起的細菌性果斑病 (bacterial fruit blotch; BFB) 對全球瓜類作物生產造成嚴重威脅，並經常導致顯著的經濟損失。本研究建立一套可高靈敏偵測 *P. citrulli* 的檢測方法。該檢測系統採用聚合酶連鎖反應 (polymerase chain reaction; PCR) 並同時共擴增植物基因作為內部控制，以全核酸進行分析偵測，有效降低例行檢測中產生偽陰性結果的風險，並降低樣品中植物 DNA 污染。結果顯示，本方法具有高度專一性，未與非目標細菌菌種或健康瓜類組織所萃取之 DNA 產生交叉反應。靈敏度測試證實本系統可穩定偵測低濃度之病原菌 DNA，且已成功應用於人工接種幼苗感染田間樣品之驗證。與傳統 PCR 方法相比，本檢測系統具備較高的檢測通量，並可依螢光強度提供定量化的分析結果。本平臺可作為瓜類果斑病於種子健康檢測、檢疫監測及整合性病害管理之可靠診斷工具。

關鍵詞：細菌性果斑病、瓜類、分子診斷、西瓜。

投稿日期：2025 年 11 月 27 日；接受日期：2026 年 3 月 6 日。

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