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Etiology, diagnostic approaches and management strategies of *Acidovorax citrulli*, a bacterial fruit blotch pathogen of cucurbits

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Abstract: Bacterial fruit blotch (BFB) caused by *Acidovorax citrulli,* represents one of the most destructing diseases of cucurbits, especially to watermelon- and melon producing-regions. This disease has been spread sporadically to many countries globally, due to the unintentionally dispersal of contaminated commercial seeds. The BFB causes massive yield losses up to 100% under conducive conditions. Once infected, all parts of the host plants are extremely susceptible to this bacterium, especially the seedlings and fruits parts. In recent years, various management approaches and detection tools have been employed to control *A. citrulli*. Genotypic characterization methods revealed two distinct groups of *A. citrulli* strains; (*i*) group I strains primarily isolated from non-watermelon cucurbits and consist of moderate to highly aggressive strains from wide range of cucurbit hosts, and (*ii*) group II strains isolated from watermelon which are highly aggressive on watermelon, but mildly aggressive on non-watermelon hosts. In this paper, an attempt has been made to review research findings where the impact of diverse methods and management approaches were applied in detection and controlling of *A. citrulli* infection. A better understanding of this devastating bacterium will serve as guidelines for agricultural practitioners in developing the most efficient and sustainable BFB control strategies.

Keywords: control strategies; detection; disease; phytopathogenic bacteria

Acidovorax citrulli, previously known as *Acidovorax avenae* subsp. *citrulli* is a Gram-negative, mesophilic, aerobic and seed-borne bacteria belongs to the β-proteobacteria class and *Acidovorax* genus. This pathogen causes bacterial fruit blotch (BFB) (Schaad et al. 1978, 2008) that severely affects the production of cucurbits crop, particularly watermelon (*Citrullus lanatus* L.) and melon (*Cucumis melo* L.) worldwide (Bahar & Burdman 2010; Burdman & Walcott 2012). In the mid-60s, the BFB disease was first discovered in several watermelon plantations in Georgia, United States (Webb & Goth 1965). However, during the late 1980s and early 1990s, the disease generated global attention after the prevalence of devastating BFB epidemics in fruit plantations in several states in the US and Mariana Island (Latin & Rane 1990; Somodi et al. 1991; Islam et al. 2019).

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Since then, the pathogen has spread sporadically, mainly via contaminated commercial seeds and posed a significant economic threat to global cucurbits industry. The BFB disease affects the economic losses up to 100% under conducive conditions (Burdman & Walcott 2012; Melo et al. 2014) particularly during rainy seasons and high fluctuated temperature regimes (Islam et al. 2019). To date, BFB disease has been reported in more than 22 countries and infected other cucurbits family members such as cucumber, citron melon, squash, pumpkin and several types of gourds (Sowell 1981; Isakeit et al. 1997; Langston et al. 1999; Martin et al. 1999; Martin & Horlock 2002; Bahar & Burdman 2010). Currently, there are no systematic and sustainable disease management available to control this devastating disease. In this article, we emphasize the current status of BFB disease, epidemiology of the pathogen and its associated disease symptoms, diagnostic tools for identification and detection of *A. citrulli* and management recommendation to combat BFB disease.

History of bacterial fruit blotch disease

In 1965, an unknown and seed-borne bacterium was isolated from several infected watermelon plantations in Georgia, United States (Webb & Goth 1965). The symptoms were large and irregular water-soaked lesions which slowly turned necrotic on the cotyledons and stems, leading to necrosis and seedling blight. The pathogen was discovered to be seed-borne and seed-transmissible with long-term survival in the seeds. Years later, Crall & Schenck (1969) reported the symptoms of watermelon fruit blotch (WFB), including the presence of multiple large and dark green water-soaked lesions on the rind of the fruits together with leaf spots in Leesburg, FL, USA, in 1967 and 1968. The isolated pathogen was later identified as *Pseudomonas pseudoalcaligenes* ssp. *citrulli* ATCC 29625 by Schaad et al. (1978). This bacterium produced non-fluorescent colonies on King's B medium, unable to produce a hypersensitive response (HR) in tobacco as well as incapable to induce watermelon fruit rot (Schaad et al. 1978). According to Sowell and Schaad (1979), BFB disease exhibited a low destructive potential on watermelon in the field, unless the seed was severely infested.

In the late 1980s, BFB disease became a primary concern after the occurrence of disastrous outbreak in watermelon fields in Mariana Islands and the USA in the early 1990s (Latin & Rane 1990; Somodi et al. 1991; Schaad et al. 2003). Infected fruit showed water-soaked lesions on the rind of the fruit that led to fruit rot. The outbreaks have caused mistrust amongst watermelon seed producers and fruit growers (Walcott 2008; Dutta 2011). Therefore, many watermelon growers filed over price lawsuits against seed companies when they encountered the disease. The BFB outbreak affected economic losses to the small seed companies and other growers. Besides, they were required to verify if the seed has been tested free from BFB infection (Latin & Hopkins 1995).

Willems et al. (1992) recommended to reclassify some of *Pseudomonas* species to the genus *Acidovorax* according to the DNA/DNA and DNA-rRNA homologies and phenotypic characterization. Hence, *Acidovorax avenae* subspecies *citrulli (Acidovorax avenae* subsp. *citrulli*) was the new nomenclature for *Pseudomonas pseudoalcaligenes* subspecies *citrulli*. Furthermore, in 2008, *A. avenae* subsp. *citrulli* was exalted to the species level, diverted as *Acidovorax citrulli* (*A. citrulli*) based on the internally transcribed spacer region sequences (16S rDNA and the 16S-23S rDNA), DNA/DNA reassociation assays, AFLP analysis and phenotypic data (Schaad et al. 2008). In 1995, several researchers developed integrated management approaches to verify pathogen exclusion through seed health testing that brought to the reduction in BFB outbreak (1995–1998). Hence after 1999, BFB disease was reported in other cucurbit hosts including melon, cucumber, pumpkin and squash (Isakeit et al. 1997, 1998; Langston et al. 1999; Martin et al. 1999; Islam et al. 2019).

Walcott et al. (2000) characterized the genotype difference between *A. citrulli* strains of melon, squash and watermelon. Molecular analyses using fatty acid methyl ester (FAME) and DNA fingerprinting pulse field gel electrophoresis (PFGE) technique revealed two genetically and pathogenically distinct groups among those strains (Walcott et al. 2000). Likewise, Walcott et al. (2004) obtained a similar result using the BOX-AIR fingerprinting method for DNA strands from infected watermelon. Group I strains were primarily isolated from non-watermelon cucurbits of cantaloupe melon (*Cucumis melo* var. *cantalupensis*), whereas group II strains were isolated from watermelon (*Citrullus lanatus*) (Walcott et al. 2000, 2004). Burdman et al. (2005) later confirmed that *A. citrulli* strains isolated from Israel were separated into group I and group II strains. Group I strains consist of moderate to highly aggressive strains from wide range of cucurbit hosts, and group II strains are highly aggressive on watermelon, but mildly aggressive

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on non-watermelon hosts (Walcott et al. 2000, 2004; Burdman et al. 2005). Similar observations were reported by Wen et al. (2008) and Feng et al. (2009) via amplified fragment length polymorphism (AFLP) and multilocus sequence typing (MLSA). However, recently, both of these groups (group I and group II) were able to distinguish based on the difference in the arsenal and sequencing of type III-secreted virulence effectors which contributed to the host preference association (Eckshtain-Levi et al. 2014).

Currently, the host range of BFB disease was restricted to only Cucurbitaceae family (Burdman & Walcott 2012). The BFB disease have widely spread through the seeds, transplants, and fruit production systems, and was reported to other cucurbit hosts including melon, citron melon, pumpkin, cucumber and squash (Table 1). In 2017, *A. citrulli* was added to the EPPO quarantine A1 List due to the devastating BFB outbreak occurred worldwide (EPPO 2017).

BFB disease etiology and symptomatology

Acidovorax citrulli is a Gram-negative bacterium with obligate aerobes, rod-shaped cells with average dimensions of 0.5 by 1.7 μm that is motile by a single polar flagellum of approximately 5.0 μm long (Schaad et al. 1978). It belongs to the class of β-proteobacteria and *Acidovorax* genus. This bacterium produced smooth, round, cream-colored, non-fluorescent colonies on

Table 1. Reports of bacterial fruit blotch disease caused by *Acidovorax citrulli* around the world

Year	Countries	Fruits	References
1965	Georgia (USA)	watermelon	Webb & Goth (1965)
1969	Florida (USA)	watermelon	Crall & Schenck (1969)
1988	Mariana Island	watermelon	Wall & Santos (1988)
1990	Indiana (USA)	watermelon	Latin & Rane (1990)
1991	Korea	watermelon	Song et al. (1991)
1991	Delaware (USA)	watermelon	Evans & Mulrooney (1991)
1992	Oklahoma (USA)	watermelon	Jacobs et al. (1992)
1994	Texas (USA)	watermelon	Black et al. (1994)
1995	Delaware, Iowa, Maryland (USA)	watermelon	Latin & Hopkins (1995)
1996	Turkey	watermelon	Demir (1996)
1997	Texas (USA)	honeydew	Isakeit et al. (1997)
1998	Texas (USA)	citron melon	Isakeit et al. (1998)
1998	China	watermelon	Zhang et al. (1998)
1999	Brazil	melon	Assis et al. (1999)
1999	Australia	rockmelon	O'Brien & Martin (1999)
1999	Georgia (USA)	pumpkin	Langston et al. (1999)
1999	Australia	cucumber	Martin et al. (1999)
2000	Japan	watermelon	Shirakawa et al. (2000)
2000	Taiwan	melon	Cheng et al. (2000)
2001	China	hami melon	Zhao et al. (2001)
2002	Illinois (USA)	watermelon	Babadoost & Pataky (2002)
2003	Thailand	watermelon	Schaad et al. (2003)
2005	Israel	watermelon, melon	Burdman et al. (2005)
2006	China	watermelon	Ren et al. (2006)
2007	Iran	Christ's thorn	Harighi (2007)
2008	Hungary	watermelon	Palkovics et al. (2008)
2009	Nigeria	watermelon	Amadi et al. (2009)
2010	Greece	watermelon	Holeva et al. (2010)
2013	China	watermelon	Tian et al. (2013a)
2014	California (USA)	melon	Kumagai et al. (2014)
2015	Serbia	watermelon	Popović & Ivanović (2015)
2019	France	melon	Cunty et al. (2019)

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King's B medium (Schaad et al. 1978), and consists of 67.2 to 68.53% GC contents (Johnson 2010). Various biochemical test of this seed-borne pathogen have been done and was described in Table 2.

All aerial parts of the host plants are highly potential to be infected by this bacterium. Likewise, young seedlings and fruits are susceptible due to the fact that the pathogen is a natural seed-borne and is basically transmitted by the seed (Dutta et al. 2012; Giovanardi et al. 2018). Hence, infected or contaminated seeds are pivotal sources of inoculum for BFB disease outbreaks. *Acidovorax citrulli* spreads rapidly among seedlings in transplant houses as well as in fruit production fields through infested seeds under conducive environmental conditions, preferably high temperature with high humidity and overhead irrigation (Schaad et al. 2003; Dutta et al. 2012; Sharma et al. 2016).

Symptoms on seedlings

Symptoms on seedlings were characterized by dark water-soaked lesions on the cotyledons and hypocotyl (Figure 1A), which gradually turned necrotic and resulted in the collapse of the emerging seedling or seedling blight (Dutta 2011). Cotyledon and leaf lesions on melon were tan brown in color and necrosis more extensive in melon compared to watermelon (Kurowski et al. 2015). According to Sharma et al. (2016), symptoms on the seedlings can be observed after five to eight days portraying greasy, water-soaked lesions on the cotyledon and subsequent coalescing on the cotyledon veins. In most severe cases, seedling may collapse and die during the damping-off stage (Sharma et al. 2016).

Symptoms on leaves

Symptoms on infected leaves displayed small, angular and tan to dark reddish-brown lesions surrounded by a yellow halo (Figure 1B and C) (Dutta 2011; Horuz et al. 2014). Melo et al. (2014) stated that the BFB symptoms are hard to distinguish on leaves since it did not lead to leaf fall. Interestingly, the stem, petiole and root parts are not commonly affected by this bacterium (Horuz et al. 2014; Melo et al. 2014; Rahimi-Midani et al. 2018).

Symptoms on fruits

Infected fruits produced small, greasy appearing, water-soaked lesions on the fruit's rinds that extended into the flesh and necrotic spot leading to internal fruit rot, which resulted in unmarketable fruits and causes severe economic losses (Figure 1D and E) (O'Brien

Table 2. Summary of selected tests done on the seed-borne bacterium, *Acidovorax citrulli*

Tests	Results
Gram staining	Gram-negative
Fluorescent pigment	–
Tobacco hypersensitivity	+
Arginine dihydrolase	–
Urease	+
Oxidase	+
Catalase	+
Lipase	+
Levan formation	–
Gelatin hydrolysis	–
Aesculin hydrolysis	–
Utilization of:	
Citrate	+
Glucose	+
L-arabinose	+
Ethanol	+
Fructose	+
Lactate	+
β-alanine	+
α-aminopentanoic	+
Ethanolamine	+
Glutarate	+
L-leucine	+
Levinulate	+
n-propanol	+
Succinate	+
D-xylose	+
D-serine	+
Mannose	+
Ribose	+
Salicin	+
Sorbitol	+
Adonitol	+
Benzoate	+
Cellobiose	+
Dextrin	+
Dulcitol	+
Erythritol	+
Inositol	+
Inulin	+
Lactose	+
Maltose	+
Melezitose	+
Raffinose	+
Rhamnose	+
Sucrose	+
D-tartrate	+
Trehalose	+

Source: Schaad et al. 1978; Cunty et al. 2019

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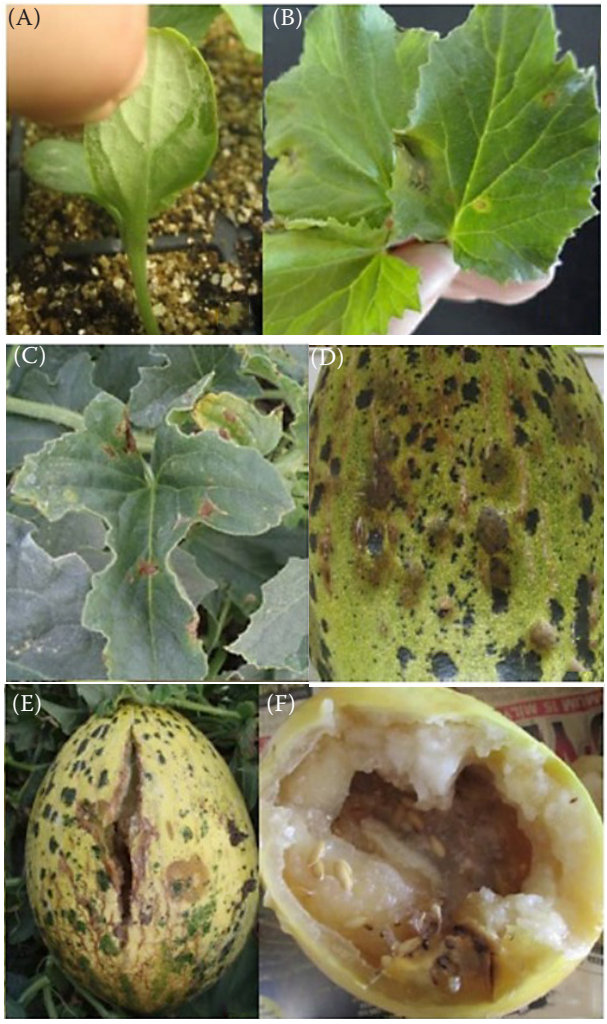

Figure 1. General symptoms of infected melon bacterial fruit blotch caused by *Acidovorax citrulli*; (A) water-soaked lesion on cotyledons, (B) angular spots following brown to dark necrotic lesions on limited by the veins, (C) irregular, brown to reddish-brown colored necrotic lesions on leaves, (D, E) water-soaked, the sunken lesions on the rind of the fruit, followed by browning in fruit flesh and crack, and (F) softening and rotting on melon fruit

Source: Horuz et al. 2014

& Martin 1999; Melo et al. 2014; Cunty et al. 2019). The previous report from infected melon showed water-soaked lesions on the surface of fruit, cracks, softening with effervescent oozing and rotting (Figure 1F) (Horuz et al. 2014). At the late stage of infection, fruit might be infected by secondary metabolites such as saprophytic bacteria and fungi (Horuz et al. 2014). According to Kurowski et al. (2015), bacterial fruit blotch symptoms differ with fruit type. The symptoms on infected melon fruit showed lesions can vary between pinpoint spots to small raised circular areas and result water-soaked around the sunken lesion. Internal lesions can lead to secondary fruit rot. Similarly, the infected watermelon fruit showed dark, gray-green, water-soaked lesions or blotches on rind surfaces not in contact with the soil. The blotches that in contact with soil are most prevalent associated with fungal infection. The infected parts on the fruit rinds may crack and rupture (Kurowski et al. 2015).

Epidemiology of BFB disease

It is known that *Acidovorax citrulli* is a natural seed-borne and seed-transmitted bacterium that infested or contaminated seeds which is a crucial inoculum source for BFB outbreak in seedling, transplant and fruit production system (Rane & Latin 1992; Kucharek 1993; Dutta et al. 2012; Feng et al. 2013; Tian et al. 2013a). Tian et al. (2013a) indicated that *A. citrulli* transmitted from grafted pumpkin rootstock to watermelon seedling and cause seedling blight. According to Lessl et al. (2007), *A. citrulli* colonized watermelon stigma within 96 hours post-inoculation, where this pathogen could reach up a population of 10^9 colony forming units (CFU/blossom). A low level of *A. citrulli* (10^3 CFU/blossom) may also resulted in seed infestation. Until today, factors on why *A. citrulli* fruit infection through flowers did not produce any fruit symptoms remain unclear. Seeds from pericarp-inoculated fruit by *A. citrulli* disclosed external contamination of the testae and perisperm-endosperm layers. In contrast, seeds from stigma-inoculated fruit have been proven to contain *A. citrulli* cells inside the seed embryos (Dutta et al. 2012).

BFB symptoms of emerging seedling may be developed by six to ten days after the inoculation pathogen into the transplant houses or greenhouses via contaminated seed (Burdman & Walcott 2012). The risks of BFB infection can be increased when the seedling is grown under conducive conditions such as high temperature, high humidity and overhead irrigation (Dutta et al. 2012; Giovanardi et al. 2018). The pathogen spreads sporadically and rapidly among the seedlings in greenhouses and fruit production fields causing severe outbreaks and unmarketable fruits (Dutta et al. 2012). With overhead irrigation and high temperature, *A. citrulli* is dispersed throughout a transplant house and rapidly infects healthy seedling, escalading the disease severity up to 100% (Burdman & Walcott 2012). This bacterium also sustains as an epiphyte on asymptomatic seedling and when transferred to the field under favorable condition, outbreaks may occur (Dutta et al. 2012).

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In the greenhouse, *A. citrulli* is spread by overhead irrigation and wind-driven rain, where the pathogen enters through open stomata into sub-stomatal intercellular spaces, multiples and causes foliar lesions and blight symptoms. Accumulation of foliar lesions and *A. citrulli* populations contributed to the BFB development of BFB disease on fruit. At early stages of fruit development, BFB symptoms were absent but developed shortly before harvest as large water-soaked lesions on the fruit, followed by fruit rot in the fields. Bacteria in the decaying tissue or infested seeds could serve as a source of contamination during the following season (Dutta 2011; Burdman & Walcott 2012). According to Block and Shepherd (2008), *A. citrulli* is highly resistant to drought and can survive on dried seeds over 35 years. The summary BFB disease cycle was described in Figure 2.

Diagnostic tools for identification and detection of *A. citrulli*

Owing to the high potential risk of BFB disease development under transplant house and greenhouse conditions, it is crucial to develop high sensitivity, reliable, quick and precise tools for detecting *A. citrulli* strain from symptomatic fields (Tian et al. 2016; Islam et al. 2019). Several seed assay protocols are currently available, such as isolation on differential or semi-selective agar media (Schaad & Sechler 1999; Zhao et al. 2009; Feng et al. 2013) seedling grow-out assay (SGO), sweat box or "dome assay" (Venette et al. 1987), carbon source utilization profiles, fatty acid methyl esters (FAME) and serological assays (Walcott et al. 2004; Feng et al. 2006; Matsuura et al. 2008; Himananto et al. 2011; Puttharungsa et al. 2011; Horuz et al. 2014; Melo et al. 2014; Kuo et al. 2018).

Years later, multiple detection tool have been employed to detect *A. citrulli* in the seeds such matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Fourier transform infrared (FTIR) spectra (Wang et al. 2012), padlock-probe (PLP) and dot-blot hybridization (Tian et al. 2013b), a self-paired colloidal gold immunochromatographic test strip (Sa-GICS) (Zeng et al. 2016), cross-priming amplification (CPA)-based isothermal DNA amplification (Zhang et al. 2012), Raman hyperspectral imaging (Lee et al. 2017), visual loop-mediated isothermal amplification (LAMP) (Yan et al. 2019), surface plasmon resonance (SPR) imaging (Puttharungsa et al. 2011) and lateral flow immunochromatographic strip (ICS) (Zeng et al. 2018). Each of these methods have their specificity and have including time-consuming, costly, laborious, require large greenhouse space and comprehensive knowledge on the morphological and biochemical properties of the strains.

On the other hand, DNA-based techniques have become the key tools for identification and detection of plant pathogenic bacteria. Polymerase chain reactions (PCR) is beneficial as it offers high specificity, fast and sensitive reaction compared to the conventional cultivation-based practices. Some PCR-based assays have been proven as useful techniques for detection of *A. citrulli* strains including conventional PCR, ethidium monoazide (EMA)-PCR (Jian-Jun et al. 2008), propidium monoazide (PMA)-PCR (Tian et al. 2016), SYBR green-based real-time PCR (Cho

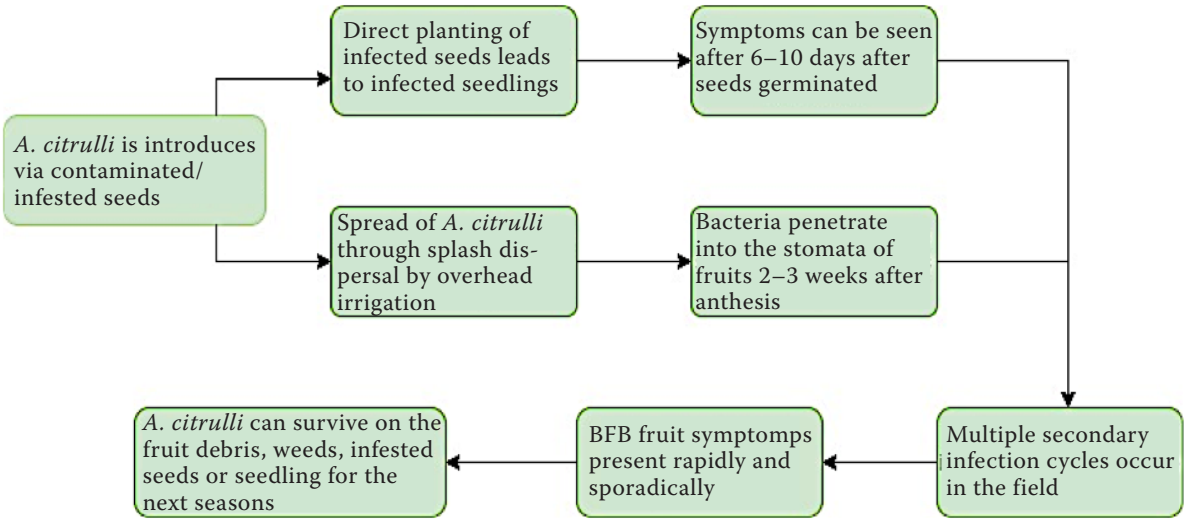

Figure 2. The cycle of seed-borne bacterium disease infected in the seedling, transplant houses and greenhouses

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et al. 2015), and enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) (Cho et al. 2015; Choi et al. 2016). In addition, PCR assays combined with immunological techniques (Xu et al. 2008; Rui et al. 2009) e.g. immunomagnetic separation (IMS) (Walcott & Gitaitis 2000) and magnetic capture hybridization (Amagliani et al. 2006) have been applied for detection of *A. citrulli* strains. These PCR assays were developed to target specific genes such as 16S rDNA, internal transcribed spacer (ITS) regions, *hrp* genes and YD-repeat proteins, which frequently lack of substantial polymorphism between closely related bacterial species, thus they not suitable for the detection of virulent *A. citrulli* strains (Eckshtain-Levi et al. 2016; Song et al. 2018; Slovareva et al. 2019).

Recently, reports on whole-genome sequence of *A. citrulli* strains from melon and watermelon (Copeland et al. 2006; Wang et al. 2015a; Wang et al. 2015b; Eckshtain-Levi et al. 2016; Park et al. 2017). Most of the assays were developed to detect *A. citrulli* strains at the species level (Song et al. 2004; Feng et al. 2013; Wang et al. 2015b; Choi et al. 2016; Giovanardi et al. 2019), or to distinguish between group II model strain AAC00-1 (virulent to watermelon hosts) from the model group I model strain M6 (virulent to all non-watermelon cucurbit hosts) (Eckshtain-Levi et al. 2016; Zivanovic et al. 2017). Islam et al. (2019)successfully designed three markers (AcM13, AcM380, and AcM797) which are reliable and sensitive to detect *A. citrulli* strains that are associated with melon hosts using variation inside the whole genome sequences of the pathogen.

Agar plating techniques

A. citrulli can be isolated from infected parts of the plants including water-soaked lesions of fruit and seedlings and cultured onto the surface of differential or semi selective agar media. For example, on King's B medium (KB), this bacterium produced round and smooth cream-colored colonies after 48 h at 41 °C (Schaad et al. 1978), while on yeast extract-dextrose-$CaCO_3$ (YDC) agar, it produced beige-to-tan colored, round, transparent, non-mucoid and spreading colonies (Jones et al. 2001; Schaad et al. 2008).

To date, various selective media are available for detection of *A. citrulli*, e.g. the watermelon fruit blotch No. 08 and No. 44 (WFB08, WFB44) (Gitaitis 1993; Jones et al. 2001), ethanol bromcresol purple/brilliant blue R (EBB) (Schaad & Sechler 1999) and EBB containing amplicin (EBBA) (Zhao et al. 2009). Antibiotics including cefaclor, boric acid, or ampicillin were added in those media to inhibit the growth of saprophytes bacteria e.g. *Bacillus myciodes*. Moreover, brilliant blue R and bromocresol purple solution were included in EBB agar to improve the differentiation of *A. citrulli* colonies (Schaad & Sechler 1999). A proper seed extraction buffer (SEB) was also extensively applied for isolation of *A. citrulli* strains from infected seeds. Zhao et al. (2009) added SEB and vancomycin (SEB-V) mix into EBBA or EBB agar to eradicate the saprophytic bacteria in seed. This method, however, revealed low sensitivity and accuracy as some strains of *A. citrulli* failed to grow or the medium unsuccessful to completely suppress the saprophyte bacteria completely (Zhao et al. 2009).

Seedling grow-out (SGO) assay

A simple greenhouse grow-out assay, which has been approved by the USDA National Seed Health System is widely used by industries for detection of *A. citrulli*. In this assay, a total of 10 000–50 000 seeds were planted under the greenhouse conditions for observation of BFB symptoms. Seedlings were exposed to light for 12 h and watered daily to promote BFB symptoms (Feng et al. 2013). Positive and negative controls were included to guarantee the BFB symptom development. Even though this SGO is a simple technique and is based on visual inspection, it is an expensive technique which is also time-consuming, requires large greenhouse space and trained technicians (Dutta 2011). Walcott et al. (2006) reported that SGO assay detected only 12.5% (1/8) and 37.5% (3/8) of seed lots (n = 10 000 seeds) with percentage of 0.01 and 0.1% infested seeds, respectively.

A sweat box or 'dome' assay (Venette et al. 1987) was utilized as a substitute technique for the SGO assay. In this technique, seedlings were grown in close containers to inspect for *A. citrulli* infection under growth chamber conditions that were optimal for symptom development. The seeds were then soaked into water and was vacuum-infiltrated into pre-germinated seeds from the same lot. Nonetheless, this method is not suitable for the detection of *A. citrulli* as a high potential for cross-reaction by other pathogens or saprophytic organisms (Zhao et al. 2009). Besides, a false-negative and false-positive result may occur even though two SGO tests were conducted together. Consequently, SGO methods were expensive and costly (Hopkins 1995).

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Serological assays

Serological assay is required to confirm the identity of *A. citrulli* isolated from infected seedlings. This technique was originally developed to detect viruses have been successfully used for bacterial detection (Hampton et al. 1990). These assays include enzyme-linked immunosorbant assays (ELISA), immunofluorescence (IF), direct agar double diffusion (DADD), immuno-isolation (IIS) and immuno-strip tests (Alvarez 2004). To date, various commercial ELISA kits as well as polyclonal and monoclonal antisera are available for detection of *A. citrulli* (Walcott & Gitaitis 2000; Wang & Hu 2005; Walcott et al. 2006; Hui et al. 2007; Matsuura et al. 2008; Himananto et al. 2011; Puttharugsa et al. 2011). An IIS assay was developed for the detection of *A. citrulli* with a sensitivity of 10^2–10^3 CFU/mL (Wang & Hu 2005). Although immuno-strip tests were carried out for a rapid preliminary detection of *A. citrulli* in the field since they were cheap and practical to apply, it was evidence that it displayed a low level of sensitivity towards the pathogen (~ 10^6 CFU/mL) (Feng et al. 2006). A modified membrane filter immunostaining (MFIS) method was the generated to detect *A. citrulli* in watermelon seeds and displayed a sensitivity of several CFU per 100 mL phosphate buffer with a sample of 1 000 commercial watermelon seeds (Matsuura et al. 2008). Serological assays should only be practiced as preliminary steps for the detection of *A. citrulli* due to the fact that the results obtained depend on the specificity of the method employed (Feng et al. 2013). Thus, these techniques were limited by lack of sensitivity and accuracy, time-consuming and require large greenhouse space (Feng et al. 2013).

MALDI-TOF MS and Fourier transform infrared (FTIR) spectra

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Fourier transform infrared (FTIR) spectra have been successfully implemented in the classification and identification of bacteria (Ayyadurai et al. 2010). Wang et al. (2012) reported that both of these methods were introduced as novel tools for rapid identification and differentiation between two closely related species of *Acidovorax; A. oryzae* and *A. citrulli*. The MALDI-TOF MS and FTIR spectroscopy are more practical and emergent physico-chemical techniques in bacterial research compared to other time-consuming detection techniques (Wang et al. 2012). However, these methods are limited to information that related to plant pathogenic bacteria (Wang et al. 2012).

Padlock-probe (PLP) and dot-blot hybridization

PLPs was developed to improve the detection sensitivity of *A. citrulli* and reduce the cost and time required for diagnosis *A. citrulli* in cucurbit seeds (Nilsson et al. 1994). Tian et al. (2013b) documented that PLP was designed based on the 16S–23S internal transcribed spacer ribosomal DNA sequence from *A. citrulli* and the target-specific products were detected by dot-blot hybridization. The detection threshold for the PLP assay was 100 fg of genomic DNA, and *A. citrulli* was detected in 100% of artificially infested seedlots with 0.1% infestation or greater. Furthermore, using the PLP assay, 4 of 8 melon seedlots collected from Xinjang province and 15 of 47 watermelon seedlots collected from Ningxia province were positive for *A. citrulli*. These data indicated that the PLP and dot-blot hybridization technique was more effective and reliable than conventional PCR for seed health testing (Tian et al. 2013b).

Self-paired colloidal gold immunochromatographic test strip (Sa-GICS)

The GICS was developed in 1971 (Faulk & Taylor 1971), using a gold-labelled antibody and nitrocellulose (NC) as a tracker and carrier. This method is rapid, high specificity, stable and convenient for results interpretation (Niu et al. 2014). Hence, this method is suitable for on-site applications in commercial facilities or in sites where sophisticated molecular tools are unavailable. Zeng et al. (2016) developed a single self-paired colloidal gold immunochromatographic test strips (Sa-GIS) by using a single monoclonal antibody, the McAb 6D which acts as a nanogold-labelled antibody and test antibody. The Sa-GIS method was successfully employed as a diagnostic tool for detection of *A. citrulli* since it was convenient, sensitive, rapid and easy to operate with sensitivity 10^5 CFU/mL. Moreover, this method is of high accuracy as it is capable to detect *A. citrulli* strains without cross-reaction with other pathogenic microorganisms, and produced results that can be observed within 10 min by naked eyes (Zeng et al. 2016).

Raman hyperspectral imaging

The Raman scattering phenomenon was discovered by Sir C.V. Raman (Schmid 1978), where it entailed analysis of Raman scattering excited by a strong light

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source (Wen 2007). The Raman hyperspectral imaging technique was known as a novel technology that combines imaging and spectroscopy and was first applied for the detection of bacteria with high detection sensitivity (Lu et al. 2011; Qin et al. 2013). Lee et al. (2017) reported that Raman hyperspectral imaging was an excellent candidate to substitute conventional methods for identification of bacteria in infected watermelon seeds as it provides high accuracy, rapid and non-destructive detection tools. Although this technique is recommended for detection of *A. citrulli*, it is however, requires extremely expensive equipment and skilled manpower (Lee et al. 2017).

Surface plasmon resonance (SPR) imaging

SPR imaging is one of the SPR tools that uses a broad incident monochromatic light beam to evaluate the change of intensity on reflected light, by using a charge-coupled device (CCD) camera at a fixed angle (Steiner 2004). This technique has potential to obtain a microscopic image of the sensor surface and to specify particular regions of interest (ROI) for evaluation of various biomolecular interactions and identification of non-specific binding, or cross-reaction in real-time PCR (Campbell & Kim 2007). Puttharugsa et al. (2011) developed SPR imaging using specific monoclonal antibody 11E5 (Mab 11E5) for the detection of *A. citrulli.* The level of sensitivity of SPR imaging detection was 10^6 CFU/mL, and 5×10^5 CFU/mL when using a polyclonal antibody (PAb) with SPR signal. Even though the level of sensitivity of this method is lower than ELISA, it is proved that this technique is more practical in terms of providing a rapid result, surface regeneration and multichannel analysis with an application on multiplex detection (Puttharugsa et al. 2011).

Lateral flow ommunochromatographic strip (ICS)

The ICS is suitable for personal detection and was recommended as an assay that avoids time-consuming and complex application steps. According to Zeng et al. (2018), ICS has been a powerful method for the identification and detection of *A. citrulli* strains using fluorescein isothiocyanate (FITC) labelled antigen and McAb antibody as dual fluorescent probes. The FITC is a fluorochrome dye that absorbs blue or ultraviolet light then emits visible yellow-green light (Zeng et al. 2018). The ICS enhanced the-sensitivity for the detection of *A. citrulli* by offering a simple, rapid, high stability, convenient and low-cost methods. The detection level of this technique could generated result up to 10^5 CFU/mL, which was 10-fold more sensitive compared to FITC strip labelled with antigen or antibody. This ICS strip could detect the majority of *A. citrulli* strains without cross-reaction with other pathogen (Zeng et al. 2018).

Cross-priming amplification (CPA)-based isothermal DNA amplification

The CPA is an isothermal DNA amplification system developed by Ustar Biotechnologies Co., Ltd., China. The CPA was carried out using a strand displacement DNA polymerase without any initial denaturation step or addition of a nicking enzyme (Xu et al. 2012). Result was later visualized on a lateral flow strip housed in a sealed plastic device to prevent leakage of amplicons (Kong et al. 2007). According to Zhang et al. (2012), the level sensitivity of CPA assays for pure bacterial culture was 3.7×10^3 CFU/mL. This method was easy to perform with self-contained amplicons detection technology. However, it is low of discriminative which unable to differentiate between closely-related *Acidovorax* strains (Zhang et al. 2012).

Loop-mediated Isothermal Amplification (LAMP)

The LAMP is a novel nucleic acid amplification method for the detection of hepatitis B virus DNA (Mori & Notomi 2009). The LAMP technique was allowed rapid amplification of target DNA using *Bst* DNA polymerase with strand displacement activity of specific inner and outer primer (Notomi et al. 2000). This tool was characterized as a cheap and easy to perform as it uses simple equipments (Notomi et al. 2000). Moreover, the LAMP technique has been widely used for detection of many plant pathogenic fungi (Tian et al. 2016), bacteria (Hodgett et al. 2016) and viruses (Tomlinson et al. 2013). During 2008, this technique was used to detect the *hrpG-hrpX* gene of *A. citrulli* (Oya et al. 2008). Yan et al. (2019) generated a LAMP protocol with a level of sensitivity at 100-fold and novel primers designed to recognized the non-ribosomal peptide synthetase (NRPS) gene from A. *citrulli*. The LAMP technique serve as a valuable tool for detection of *A. citrulli* in seed since it is highly specific without cross-reaction with other pathogens, despite requires much time for completion (Yan et al. 2019).

Polymerase chain reaction (PCR)

Numerous PCR-based assays have widely developed as a reliable alternative for specific detection and identification of seed-borne *A. citrulli* to defeat

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the diagnostic limitations associated with screening methods and pathogen diversity. The PCR amplification consists of countless advantages compared to the conventional methods e.g. fast, highly sensitive and convenience molecular tool.

The EMA is a binding dye that has been used to prevent the amplification of DNA from dead cells of food-borne bacteria cells in cytometry and PCR assays (Wang & Levin 2006). The EMA invade the damaged membranes of dead cells, but not the viable cells. This dye binds to DNA, restricts PCR amplification thus promoting differentiation between viable and dead cells. According to Nogva et al. (2003), DNA from dead cells treated with EMA could not be amplified by PCR. The EMA was used for detection of live cells of *A. citrulli*, where seed extracts were spiked with 3×10^6, 3×10^7 and 3×10^8 CFU/mL, treated with EMA at 0, 1, 2, 3 and 4 μg/mL concentration, incubated at room temperature and at 75 °C for three minutes in order to kill the cells. Results from the experiment revealed that heat-treated suspensions containing dead cells of *A. citrulli* at a level of 3×10^6 CFU/mL were PCR negative when pre-treated with 2 mg/L or greater EMA concentration at 10 min or more of time exposures (Jian-Jun et al. 2008).

Tian et al. (2016) encountered a novel method for detection of *A. citrulli* using DNA-intercalating agent propidium monoazide (PMA). In this method, viable cells of *A. citrulli* were treated with DNA-binding PMA dye, followed by real-time fluorescent PCR amplification for detection and differentiation from dead cells using novel primers and tagged with a TaqMan probes. A TaqMan probe-based assays in general, is more expensive than the SYBR Green-based assays. Thus, it is significant to generate a simple, rapid, convenient method for the detection of *A. citrulli* strains. Cho et al. (2015) developed a novel SYBR Green-based real-time PCR assay targeting a YD-repeat protein gene of *A. citrulli*. The YD-repeat protein comprised of six structurally distinct lineages within the Enterobacteriaceae family that exhibits significant intergenomic variation in the YD repertoire (Jackson et al. 2009). This method is a highly sensitive and reliable tool without using selective media, biochemical tests or DNA extraction but requires an expensive specialized portable thermocycler to complete the reaction (Cho et al. 2015).

Immunological techniques

Immunological techniques are based on specific binding and recognition of the antibody to the corresponding antigen (Zeng et al. 2016). Anti-*A. citrulli* antibody is immobilised on microtiter plate wells, followed by incubation of cell suspension in the plate well to form an antigen-antibody complexes. A segment of known DNA is biotinylated to serve as a marker, and it can be specifically attached to antigen-antibody complexes immobilised on a microtiter plate wells by using streptavidin-protein A complex (Sano et al. 1991). The plate wells were later washed used for PCR amplification. Several immuno-capture PCR assays have been evaluated for detection of *A. citrulli* in bacterial cell suspension and melon seeds using a set of primer pair WFB1/WFB2 (Zhao et al. 2006; Rui et al. 2009). Compared to direct PCR, this assays exhibited a higher sensitivity with detection up to 50–100 target CFU/mL, while lowering the effects of PCR inhibitors in watermelon or melon seeds. This technique however, requires at least eight hours to complete (Zeng et al. 2016).

Immunomagnetic separation (IMS)

The IMS is a combination of serology method and PCR. The IMS-PCR uses small, super-paramagnetic beads coated with antibodies specific to the pathogen of interest to separate it from heterogeneous mixtures (Olsvik et al. 1994). Walcott & Gitaitis (2000) developed an IMS PCR-based assay in which antibodies were covalently joined to magnetic beads, and bound the target cell by specific antibody-antigen interaction during the incubation period. These immunomagnetic bead (IMS)-bacteria complexes were held by a magnetic force, while non-target cell and inhibitor compounds were washed off. It has been reported that the detection sensitivity of IMS method in *A. citrulli* from watermelon seed-wash was 10 CFU/mL and seed lots with 0.1% infestation when the IMB-bound target cells were amplified using PCR with primers WFB1/WFB2. This method also eradicates inhibitory effects of watermelon seed-wash, increases detection sensitivity and effectiveness of the diagnosis. On the contrary, direct PCR without IMS failed to detect *A. citrulli* cells caused by PCR inhibitors in the seed wash. Therefore, IMS-PCR eradicated the inhibitory effects of watermelon seed-wash and boosted the detection sensitivity and effectiveness of the diagnosis. Walcott et al. (2006) stated that IMS-PCR provided 25.2 and 87% of detection frequencies for watermelon seed lots (10 000 seed/lot) with 0.01 and 0.1% *A. citrulli*-infested seed, respectively. The main drawback of this protocol is it requires specific

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antibodies that are commonly unavailable (Walcott & Gitaitis 2000).

BIO-PCR Assay

BIO-PCR is a technique involves of plating samples onto agar media for enriching the target bacterium before being subjected to PCR amplification (Schaad et al. 1995). It is designed to enhance the detection sensitivity by increasing the target cell population, while eradicating the PCR inhibitors. The BIO-PCR is highly influenced on the timing of enhancement to obtain specific size target colonies, where the minimum number of cells required for PCR detection is 1 000 cells in 1 µL samples. Target colonies were grown, followed by plates wash off which results in much larger colonies. Meanwhile, non-target saprobes were grown for inhibition of PCR (Zhao et al. 2009). Thus, this technique is highly sensitive due to enrichment of the target bacterium and eradication of PCR (Schaad et al. 1995). However, the main pitfall of BIO-PCR is that false-negative results may occur if there was deficiency on transmission of the pathogen from seeds to seedlings, and failure of primers to counter with certain strains of the pathogen. Although microbial enrichment can be combined with real-time PCR (real time BIO-PCR) to improve detection sensitivity to 1 to 2 CFU/mL of *A. citrulli* (Zhao et al. 2009), it is not recommended if the test duration is more crucial than the sensitivity e.g. in some cases related to entry-exit port quarantines (Feng et al. 2013).

Pulse field gel electrophoresis (PFGE)

The PFGE has proven to be one of the powerful discriminatory tools and widely used for investigating the genetic diversity of *A. citrulli* strains between different hosts (Walcott et al. 2000; Burdman et al. 2005; Yan et al. 2013). PFGE use for the separation of big DNA molecules after digesting it with unique restriction enzymes and applying to a gel matrix under the electric field that periodically changes direction (Sharma-Kuinkel et al. 2016). According to Yan et al. (2013), the strains from infected watermelon, pumpkin and cantaloupe were compared using standard methods used with *E. coli* (Ribot et al. 2006) and *SpeI*-digestive enzyme. The PFGE electrophoresis was carried out using 0.5 × TBE buffer at 14 °C for 17 h under two states of electrophoretic state with voltage gradient of 6 V/cm, electric field angle of 140° and switching time of 20 ~ 80 seconds. Afterward, the gels were stained with a solution of 0.5 µg/mL of ethidium bromide for 30 min and the digital images were captured using gel imaging system, BIO-RAD Gel Doc. The result showed unique DNA fragments and distinct haplotypes between the strains. However, PFGE method is unable to determine the genetic relationships among and between strains at deeper phylogenetic levels (Noller et al. 2003).

Fatty acid methyl ester analysis (FAME)

The FAME has been acknowledged as beneficial biochemical markers for classification and characterization of bacteria (Welch 1991). Walcott et al. (2000) was performed FAME method to identify the genotype and discriminate among *A. citrulli* strains. The strains were grown on tryptic soy broth agar (Difco Laboratories) at 28 °C for 24 hours. Extraction and analysis of FAMEs were conducted on each strain according to Gitaitis and Beaver (1990). The strains were assigned to haplotype based on their DNA banding pattern and proceed with cluster analysis using Kodak 1D Digital Sciences gel imaging software (version 3.0). Similarity between haplotypes was determined using Dice's coefficient (Dice 1945). Dissimilarity or distance indices were calculated from the SIs and the distance matrix data were used for cluster analysis by the unweighted pair group method with arithmetic means (UPGMA) algorithm using the NEIGHBOR program of the PHYLIP phylogenetic software package. The robustness of the groups established by cluster analysis were assessed by bootstrap analysis using the Winboot computer program and the trees were reconstructed 2 000 times.

Repetitive sequence based polymerase chain reaction (rep-PCR)

The rep-PCR method has been recommended for characterization of bacteria and rapid method for distinguishing closely related species, subspecies, strains, serotypes, and others (Rampadarath et al. 2015; Besler & Little 2017). Numerous studies of have shown the application of rep-PCR using three specific primers, ERIC, BOX and REP to distinguish the strains of *A. citrulli* from different hosts (Walcott et al. 2004; Burdman et al. 2005; Choi et al. 2015; Song et al. 2018). The primers used were BOXA1R primer (5'-CTA CGG CAA GGC GAC GCT GAC G-3') (Walcott et al. 2004; Song et al. 2018), ERIC1R (5'-ATGT AAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGT AAGTGACTGGGGTGAGCG-3') (Choi et al. 2015; Song et al. 2018),

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REP (REP1R-I (5'-IIIICGICGI CATCIGGC-3') and REP2-I (5'-ICGICTTATCIGGCCTAC3') (Walcott et al. 2004). PCR amplification contained of 25 µL mixtures of 20 ng DNA template, pure water, 0.2 µM of each primer, 2.75 mM bovine serum albumin, 2.5 µL 10 × buffer PCR buffer, 1 U of Red *Taq* polymerase, 2.5 µL of 25 mM $MgCl_2$ and 2 mM of dNTP. The PCR amplification protocol was perfomed included initial denaturation at 95 °C for 7 min followed by 30 cycles of PCR consisting of denaturation at 95 °C for 1 min, annealing at 53 °C for 1 min, and extension at 65 °C for 8 min. PCR results were determined by gel electrophoresis 6V for 4 h on a 1.5% agarose gel. The gel was strained with ethium bromide solution and photographed with transmitted UV light at 295 nm. Three independent experiments were performed for each primer run.

Multilocus sequence typing (MLST)

The MLST has proven to be a useful tool for identifying genetic structure and fingerprinting strains of bacteria (Maiden 2006; Scally et al. 2006) based on the principles of multilocus enzyme electrophoresis (MLEE) (Selander et al. 1986). MLST is based directly on nucleotide sequence differences in 5 to 10 genes. Feng et al. (2009) developed MLST protocol for distinguishing the *A. citrulli* strains using seven genes (*gmc*, *ugpB*, *pilT*, *lepA*, *trpB*, *gltA* and *phaC*). Each PCR amplification consisted of 25 µL reaction volume including 5 to 20 ng/µL of DNA template, 1 × buffer, 0.2 mM deoxynucleotide triphosphates mixture, 1 µM each primer, and 0.04 U of *Taq* polymerase. PCR amplification protocol included initial denaturation step at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, annealing at 60 °C for 30 s, and extension period at 72 °C for 30 seconds. The final step was an extension period at 72 °C for 5 minutes. Then, proceed to the purification and direct sequencing of the PCR product. For sequence analysis, the sequences for the seven loci were visualized and aligned using BIOEDIT (version 7.2.5) and ClustalX (version 2.1). The eBURST program use for indicating the clonal complex.

Multilocus sequence analysis (MLSA)

The MLSA is a powerful tool and widely used to elucidate the BIOEDIT genetic relationship and phylogenetic analysis of closely related strains (Peeters et al 2016; Whatmore et al. 2016). MLSA method uses several nucleotide sequences and concatenated sequences of fragments of housekeeping genes to determine genus-wide phylogenetic relationships. Song et al. (2018) analyzed the relationship between *A. citrulli* strains using six housekeeping genes (*adk*, *gltA*, *glyA*, *pilT*, *ugpB* and *phaC*). Each PCR amplification by mixture under PCR condition described by Kang et al. (2002). The PCR products were sequenced, visualized and aligned using MEGA5 (version 5) (Tamura et al. 2011).

BFB disease management

Innumerable management approaches have been implemented to prevent *A. citrulli* infection including chemical controls, seed treatments, pathogen exclusion, resistance cultivar, seed health testing and field applications of biological control agents (BCA) (Figure 3). However, all these methods are insufficient and limited in combating It has reported that level of *A. citrulli* infection influences the epidemic of BFB disease (Dutta et al. 2012). Hence, the exclusion of *A. citrulli* from the seed and transplant is critical for effective BFB disease

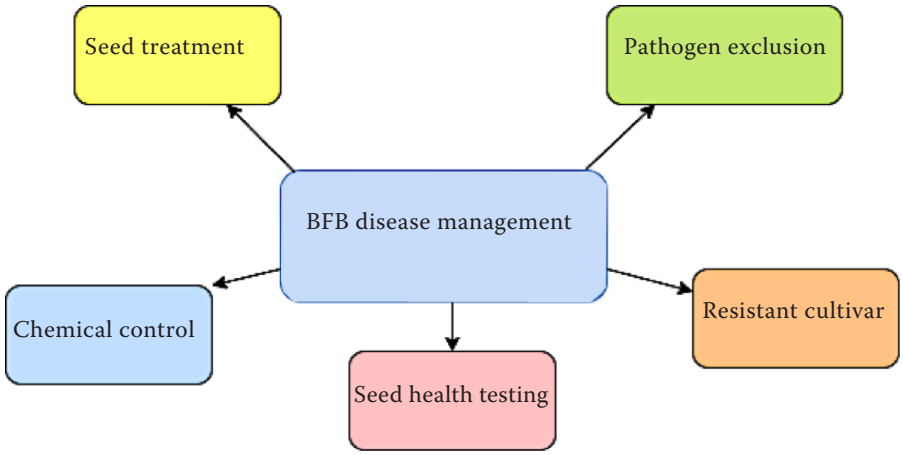

Figure 3. Integration of different management approaches of BFB disease for controlling *Acidovorax citrulli* infection

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management (Walcott 2008). In this case, application of pathogen-free seeds is the best method to prevent the introduction of *A. citrulli* in the field, and can be done by routine seed lots testing for detection of bacterial pathogens (Rahimi-Midani et al. 2018). On the other hand, seeds can be grown under dry and cool climates as an effort to reduce the risk of BFB infection (Rahimi-Midani et al. 2018).

Chemical control

Chemical control of BFB disease encompasses copper-containing bactericide or fungicide application on infested fields (Hopkins 1992), but it is quite inefficient since it is useful only under suitable environmental conditions (Dutta 2011). The use of copper-containing fungicide on fruit helps to reduce occurrence of fruit blotch symptoms. Conventionally, Kocide was applied as precaution treatment against BFB (Hopkins 1991); however, it can be phytotoxic to watermelon and other cucurbit seedlings and can develop copper tolerance by *A. citrulli*. The use of copper-containing fungicide on fruit helps to reduce occurrence of fruit blotch symptoms. At least two to three copper applications are required for an extensive foliage coverage (Cheng et al. 2000). Applications of fungicide should be applied before flowering, or earlier, and continues until the fruits mature (Traore 2014). Streptomycin and kasugamycin are use as protective sprays to 2-week-old melon seedling to avoid the transmission of *A. citrulli* under greenhouse conditions (Shimizu et al. 2008). Unfortunately, antibiotics are not reliable and less effective in prevention BFB disease.

Resistant cultivars

The host range of BFB disease is restricted to only Cucurbitaceae family (Latin & Hopkins 1995; Burdman & Walcott 2012). Previously, Hopkins et al. (1993) reported that watermelon cultivars with dark-colored rinds were more resistant than light-colored cultivars. However, they can be infected under BFB favorable condition. Furthermore, only two PIs (PI 482279 and PI 494817 from Zimbabwe and Zambia, respectively) displayed resistant to BFB (Hopkins & Thompson 2002a). These cultivars could reduce the inoculum produced on leaves and thereby reduce the risk of BFB development on fruits. Bahar et al. (2009) screened commercially available and wild melon (*C. melo*) lines for BFB resistance using seed-to-seedling transmission and seedling-inoculation assays. The authors unable to identify resistant melon cultivars/lines but they identified a commercial cultivar, ADIR339 displayed a high level of tolerance to BFB. Moreover, the level of BFB tolerance of melon cultivars/lines revealed varying depending on the screening assay used. Thus, resistant cultivars were insufficient to manage this disease due to tremendous genetic diversity of *A. citrulli* (Yan et al. 2013). Thus, resolving genetic relationship between the host and geographical origin of the pathogen could provide a strong basis for screening resistance cultivar to warfare of this disease.

Seed treatment

Seed treatment and seed health testing are the most widely applied management tools for battling *A. citrulli* (Dutta et al. 2012). Yet, none of these methods were entirely effective against BFB disease. Seed treatments including thermotherapy and NaOCl (Hopkins et al. 2003), fermentation (Hopkins & Thompson 2002b), HCl, peroxyacetic acid (Hopkins et al. 2003), 9 h of exposure to chlorine gas (Stephens et al. 2008), cupric sulfate and acidic electrolyzed water (AEW) (Feng et al. 2009) were proven to significantly reduced *A. citrulli* transmission. Seeds that were treated with HCl (1%) or $CaOCl_2$ (1%) for 15 min (Hopkins et al. 1996) and treated at 85 °C for 3–5 days effectively removed the pathogen, but can reduce the germination of seeds (Kubota et al. 2012). Treatment with 0.5–1% CaOCl or NaOCl for 15–20 min able to reduce *A. citrulli* transmission yet failed to eradicate the pathogen from the seed (Hopkins et al. 1996; Hopkins et al. 2003). Hopkins et al. (1996) documented that fermentation of seeds in watermelon juice for 24–48 h, followed by treatment with 1% HCl for 15 min, prevent the BFB seedling transmission. However, this can adversely affect seed quality parameters of certain hybrids (Hopkins et al. 1996). Additionally, wet seed treatment with 1 600 µg of peroxyacetic acid per liter of water for 30 min followed by drying at 40 °C for 48 h effectively eradicated BFB seedling transmission (Hopkins et al. 2003). However, these treatments are not 100% effective as BFB outbreaks continue to occur. Only AEW successful eliminate *A. citrulli* from infested seeds without affect seed germination or seedling establishment (Feng et al. 2009). Recently, some biochemical, in particular, chitosan A at 0.40 mg/mL significantly inhibit the growth of *Acidovorax citrulli* and has potential in controlling BFB disease (Li et al. 2013).

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Seed health testing assay

Seed health testing is the most frequently used method for excluding *A. citrulli*. Some of the seed health testing assays were performed on semi-selective media, sweat-box text, seedling grow-out (greenhouse), PCR and ELISA. However, these methods were limited by inadequacy of sensitivity and specificity, time constraint and large greenhouse space (Ghedini & Fiore 1995; Gitaitis & Walcott 2007; Dutta et al. 2012). Also, there are some factors which influence the effectiveness and accuracy of seed health testing for *A. citrulli* e.g. sampling method and size, efficiency of pathogen extraction and seed inoculum threshold (Dutta et al. 2012).

Biological control agent (BCA)

Biological control via microbial antagonists has been previously explored as an alternative to substitute chemical methods in managing and controlling the plant diseases including the BFB. This method provides a non-toxic to nature and reduces the environmental impacts (Zhan et al. 2012; Jiang et al. 2015). Fessehaie and Walcott (2005) reported that *A. avenae* subsp. *avenae* AAA99-2, isolated from maize could have reduced infection by BFB disease seeds to the seedling. In addition, *A. citrulli* seed infestation and transmission to seedling was decreased by 67 and 96%, respectively when the bacterium was applied as a protectant female watermelon blossoms (Fessehaie & Walcott 2005). Jiang et al. (2015) successfully proved that *Bacillus amyloliquefaciens* (*B. amyloliquefaciens)* was able to control BFB disease by portraying as plant growth promoting rhizobacteria (PGPR) that increase the available nitrogen, phosphorus, potassium contents in plant soil and the leaf chlorophyll. Moreover, *B. amyloliquefaciens* significantly control BFB disease by accumulation of hydrogen peroxide and increasing the expression level of defense-related gene PR1 of the host plant.

Likewise, the application of *Pseudomonas fluorescens* Migula A506 strain was reported to suppress the growth of the pathogen and reduced infestation in the seeds of fruits (Fessehaie & Walcott 2005). In 2016, *Paenibacillus polymyxa* (SN-22 strains) and *Sinomonas atrocyanea* (NSB-27 strains) were proved as effective biocontrol-plant growth-promoting rhizobacteria on watermelon where both of these bacteria were able to reduce BFB infection, while enhancing the growth of watermelon fruit (Adhikari et al. 2017). Recently, Horuz & Aysan (2018) documented the potential bacterial antagonist *Curtobacterium flaccumfaciens*, *Microbacterium oxydans*, *Pseudomonas oryzihabitans*, and *Pseudomonas fluorescens* as seed treatments in the biological control of Ac-induced BFB as these bacteria exhibited low disease severity and disease incidence on watermelon seedling.

CONCLUSION

Acidovorax citrulli associated with BFB in cucurbits posed a serious threat in cucurbits global industry, leading to critical yield loss in many countries worldwide. A better understanding of the disease history, etiology and symptoms, epidemiology, diagnostic tools, as well as various disease management strategies that have been employed to control this BFB disease will help to improve and develop more effective and powerful disease control approaches against this emergent and wide-spreading bacterium to global cucurbits production.

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