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Review

Diagnosis and management of halo blight in Australian mungbeans: a review

Thomas J. Noble[A], *Anthony J. Young*[B], *Colin A. Douglas*[C], *Brett Williams*[A],
and Sagadevan Mundree[A,D]

[A]Centre for Tropical Crops and Bio Commodities (CTCB), Queensland University of Technology, Brisbane City, Qld 4000, Australia.
[B]School of Agriculture and Food Sciences, The University of Queensland, Gatton, Qld 4343, Australia.
[C]Department of Agriculture and Fisheries (DAF), Hermitage Research Facility, Warwick, Qld 4370, Australia.
[D]Corresponding author. Email: sagadevan.mundree@qut.edu.au

Abstract. Mungbean (*Vigna radiata* L. Wilczek var. *radiata*) is an important food crop cultivated on over 6 Mha throughout the world. Its short duration of 55–70 days, capacity to fix atmospheric nitrogen, and exceptional grain nutritional profile makes the crop a staple for smallholder and subsistence farmers. In Australia, mungbean is grown as a high-value export crop and established as a main summer rotation for dryland farmers. A major threat to the integrity of the industry is halo blight, a bacterial disease leading to necrotic lesions surrounded by a chlorotic halo that stunts and ultimately kills the plant. Caused by *Pseudomonas savastanoi* pv. *phaseolicola*, this seed-borne disease is extremely difficult to control, resulting in significant yield loss and production volatility. The challenge of managing halo blight is exacerbated by a wide host range that includes many legume and weed species, and the presence of multiple epidemiologically significant strains. Molecular technologies could play a pivotal role in addressing these issues. This review synthesises current and emerging technologies to develop improved management strategies for the control of halo blight in mungbean.

Additional keywords: bacterial pathogen, disease management, host–pathogen interactions, molecular characterisation.

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Introduction

Mungbean (*Vigna radiata* L. Wilczek var. *radiata*) is a vital component of global nutrition and food security (Day 2013). With the capacity to fix atmospheric nitrogen and short duration of 55–70 days, mungbean is a leading choice for double and intercropping farming systems, particularly between cereals (Senaratne and Gunasekera 1994; Yaqub *et al*. 2010). Consumption of mungbean worldwide has increased 60% over the last three decades with over 6 Mha of farming land being used for cultivation, concentrated mainly in Southeast Asia (Kim *et al*. 2015). This growing demand for mungbean requires innovative approaches to stabilise and increase production of the crop.

One of the foremost risk factors to the stability of the Australian mungbean industry is the seed-borne bacterial disease halo blight (Ryley and Tatnell 2011; AMA 2015). Identified in 1931 on French bean (common bean, *Phaseolus vulgaris* L.) in Queensland, Australia, it was not until the mid-1980s that halo blight was found infecting mungbeans (Ryley *et al*. 2010). At the same time, during 1983–84, the first recorded study of mungbeans affected by halo blight was reported in Pakistan (Akhtar 1988). Recently halo blight has been found in mungbeans in China, the world's second highest producer (Sun *et al*. 2017). Given its broad host range (Table 1) and known geographical distribution, halo blight is suspected to be present in many mungbean-growing areas, but has not yet been documented.

Significance of halo blight to mungbean

Halo blight is caused by a group of bacterial strains belonging to *Pseudomonas savastanoi* pv. *phaseolicola*, closely related to a range of fluorescent pseudomonads that cause disease in a broad range of plant hosts (Arnold *et al*. 2011). Characterised by Burkholder (1926) as *Phytomonas medicaginis* variant *phaseolicola*, it was revised to *Pseudomonas medicaginis* variant *phaseolicola* by Dowson (1943) and then later to *P. syringae* pathovar *phaseolicola* (Young *et al*. 1978). Most recently *P. syringae* was regrouped into nine discrete genomospecies with *P. savastanoi* including the pathovars *phaseolicola*, *savastanoi*, *glycinea* and *tabaci* (Gardan *et al*. 1992). While it is still common to see these pathovars referred to as *Pseudomonas syringae*, the nomenclature *P. savastanoi* pv. *phaseolicola* is used throughout this review in light of the most recent taxonomic work.

Although the mechanisms of infection are yet to be fully elucidated, the key pathogenicity determinants have been identified. One of the main factors is the production of phaseolotoxin by some strains, which are referred to as toxigenic (Prosen *et al*. 1993). Characteristic symptoms of toxigenic strains

Table 1. Host range of *Pseudomonas savastanoi* pv. *phaseolicola* causal agent of halo blight

Scientific name	Common name	Reference
Cajanus cajan	Pigeon pea	Taylor *et al*. 1996
Desmodium sp.	Tick clover	Taylor *et al*. 1996
Fumaria sp.	Fumitory	Fernández-Sanz *et al*. 2016
Lablab purpureus	Hyacinth bean	Sherf and MacNab 1986
Macroptilium atropurpureum	Purple bean	Taylor *et al*. 1996
Mercurialis annua	Annual mercury	Fernández-Sanz *et al*. 2016
Neonotonia wightii	Perennial soybean	Taylor *et al*. 1996
Phaseolus coccineus	Runner bean	Taylor *et al*. 1996
Phaseolus lunatus	Lima bean	Sherf and MacNab 1986
Phaseolus vulgaris	Common bean	Sherf and MacNab 1986
Pisum sativum	Pea	Arnold *et al*. 2001
Pueraria montana var. *lobata*	Kudzu vine	Sherf and MacNab 1986
Sonchus oleraceus	Sowthistle	Fernández-Sanz *et al*. 2016
Vigna angularis	Adzuki bean	Taylor *et al*. 1996
Vigna radiata	Mungbean	Taylor *et al*. 1996

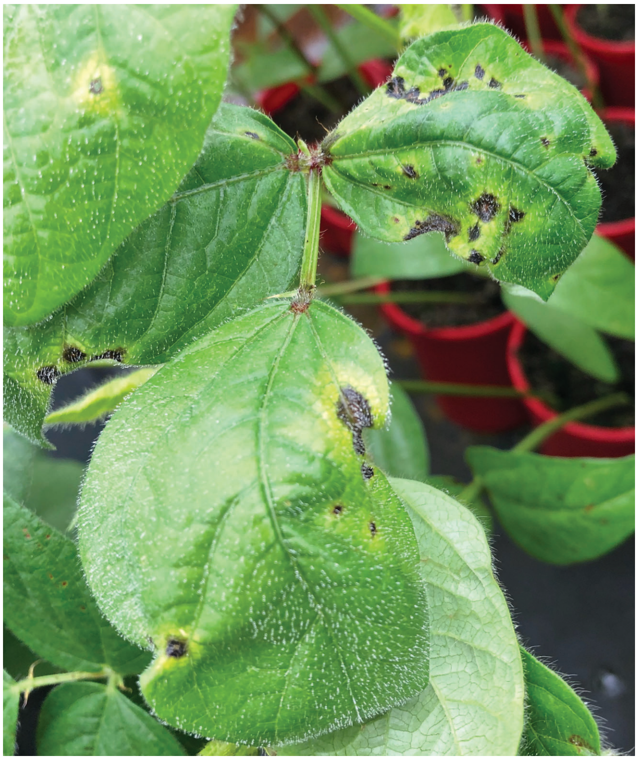

Fig. 1. Glasshouse-inoculated mungbean plant displaying characteristic symptoms of halo blight. Dark-brown, water-soaked lesions surrounded by chlorotic yellow halos indicate the bacteria have released phaseolotoxin.

are round, water-soaked lesions surrounded by a chlorotic halo of greenish yellow (Fig. 1) (Taylor *et al*. 1979*a*). Symptoms are most evident on leaf tissue, where a bacterial ooze can be seen exuding from the infection site. A slightly different symptom is observed when non-toxigenic strains are involved in infection. These strains proliferate within the plant, causing water-soaked lesions without the characteristic chlorotic halos (Patil *et al*. 1974; González *et al*. 2003). Yield loss directly attributable to halo blight is inherently problematic to determine through controlled experiments because it is difficult to exclude the pathogen from control plots as well as achieve consistent inoculation and symptom expression of trial plots. Taking into consideration these factors, a field study in Australia of mungbeans inoculated with a mixture of *P. savastanoi* pv. *phaseolicola* isolates found yield losses up to 75% and severe stunting (Ryley *et al*. 2010). A survey of mungbean fields throughout China between 2009 and 2014 reported similar yield reductions of 30–50% and total crop failure in extremely infected fields (Sun *et al*. 2017).

Environmental factors of disease development

Epidemics of halo blight are facilitated by cool (18–23°C), wet and windy conditions, which facilitate bacterial dispersal and infection (Taylor *et al*. 1979*b*). One study showed that the bacteria could infect plants situated 26 m away (Walker and Patel 1964). Marques and Samson (2016*b*) investigated the epiphytic life cycle of *P. savastanoi* pv. *phaseolicola*, confirming that asymptomatic dispersal precedes symptoms and that infection is dependent on weather conditions, developmental phase of the crop and strain of bacterium. This has large implications for crop management.

Growth of *P. savastanoi* pv. *phaseolicola* and production of phaseolotoxin are tightly regulated by temperature (Aguilera *et al*. 2017). Therefore, environmental effects such as location, sowing time and growing conditions can have significant impacts on establishment and severity of disease symptoms. The ideal temperature range for growth of *P. savastanoi.* pv. *phaseolicola* is 25–28°C and temperatures >49°C are lethal (Burkholder 1926; Nüske and Fritsche 1989; Aguilera *et al*. 2017), whereas phaseolotoxin production is highest at 18–20°C and ceases at temperatures >28°C (Nüske and Fritsche 1989; Aguilera *et al*. 2017). Temperatures of 18–28°C coincide with the ideal conditions for mungbean plants growing in warm and humid subtropical regions. Thus, in Australia, the most severe infections are generally found in southern Queensland and northern New South Wales. Disease epidemics are substantially less prevalent and severity of infection is lower in the central Queensland region, located in the semi-arid tropics. From a management perspective, this makes central Queensland a good option for clean seed production, which is a crucial factor in the control of a seed-borne disease.

Epidemiology of *Pseudomonas savastanoi* pv. *phaseolicola*

Pseudomonas savastanoi pv. *phaseolicola* comprises a broad array of distinct strains that vary in their epidemiology. By using disease reactions among a differential set of eight genotypes of common bean, nine distinct races were identified within a broad international collection of strains isolated from multiple legume species (Taylor *et al*. 1996). These differential reactions were attributed to the interactions of five pairs of avirulence (*avr*) and resistance (*R*) genes (Taylor *et al*. 1996). Races 2 and 7 were identified in Australia, isolated from purple bean (*Macroptilium atropurpureum* (DC.) Urb.) and perennial soybean (*Neonotonia wightii* (Wight&Arn.) J.A.Lackey) (Taylor *et al*. 1996). Race 7 has been further characterised in Australia infecting mungbeans, based on 30 isolates inoculated on the common bean differential set (Taylor *et al*. 1996; Ryley *et al*. 2010). Although currently there is no definitive mungbean differential set for halo blight, differences in pathogenicity among those isolates have been observed (Ryley *et al*. 2010). Twelve putative races have been identified, of which two strains are the most prevalent. These are referred to as the 'T' and 'K' strains, designated by the origin of the isolates initially characterised: T11544, isolated from mungbeans in Toowoomba in 2005; and K4287, isolated from mungbeans in Kingaroy in 2013. T11544 has been used in conventional resistance breeding, with some mungbean genotypes expressing moderate resistance (Ryley *et al*. 2010), whereas K4287 apparently overcomes all known resistance (Kelly 2016).

Seed-borne infections are recognised as the primary source of inoculum and play a major role in the long-distance dispersal of *P. savastanoi* pv. *phaseolicola* (Grogan and Kimble 1967; Taylor *et al*. 1979*a*). Seedlots with as little as 0.01% infected seed can lead to outbreaks of halo blight (Taylor 1970), alongside the capacity to transmit disease for up to 4 years in uncontrolled storage, and 6–10 years in controlled grain-storage facilities (7–10°C, 45–50% relative humidity) (Taylor *et al*. 1979*a*). The persistence of infection in seeds makes halo blight particularly difficult to manage (Taylor 1970). The provision of disease-free seed stocks and the use of sensitive diagnostic techniques are imperative to eliminating infected seed and reducing transmission.

Host–pathogen interactions

Although seed-borne inoculum is the main source of epidemics, bacteria readily enter through natural openings such as stomata and wounds (Taylor *et al*. 1979*b*). The bacteria then move into and proliferate in the apoplast (Melotto *et al*. 2008; Rufián *et al*. 2017). At this juncture, plants launch their first line of molecular defence, the innate immunity of each plant cell. Specialised receptors on the plant cell surface recognise molecular structures essential for a pathogen survival, such as flagellin, peptidoglycan, elongation factor and lipopolysaccharide. These are known as microbe-associated molecular patterns (MAMPs) (Jones and Dangl 2006; Newman *et al*. 2013). Surprisingly, little is known about MAMPs, their range and diversity, although molecular tools are rapidly advancing our knowledge. Further research into this area has been suggested with the aim to reveal novel antimicrobial agents through identification of MAMPs and their signalling pathways (Vidaver and Lambrecht 2004; McCann *et al*. 2012).

Many Gram-negative bacteria use quorum-sensing signalling molecules to overcome host defences. Using *N*-acyl homoserine lactones (AHLs) as signalling molecules, individual cells can sense population density before entering through external surfaces and releasing virulence factors (Cha *et al*. 1998). This has been shown to be a key virulence factor allowing bacteria to coordinate attacks and maximise the likelihood of success (von Bodman *et al*. 2003). A novel transcriptional regulator $aefR_{NPS3121}$ identified in a mutant *P. savastanoi* pv. *phaseolicola* strain has been shown to regulate the synthesis of AHL and the induction of type III secretion system (T3SS) genes in response to cell density (Deng *et al*. 2009). The T3SS is an essential protein complex that facilitates transport of pathogenicity determinants such as pectinases, cellulases and proteases from the bacterium into the host. Thus, the quorum-sensing associated signalling and effector molecules directly affect the ability of the bacterium to infect its host.

Early genetic work on *P. savastanoi* pv. *phaseolicola* demonstrated many basic mechanisms of virulence. Transposon mutagenesis helped to define the role of *hrp* (hypersensitive reaction and pathogenicity) genes (Lindgren *et al*. 1986), which have since been extensively characterised as major contributors to the T3SS (Alfano and Collmer 1997). The capacity for *P. savastanoi* pv. *phaseolicola* to gain entry to its host and interfere with plant defences is dependent on the use of bacterial T3SS translocating effector proteins to the host cells (Vencato *et al*. 2006; Cunnac *et al*. 2009). Susceptibly in a host is caused by pathogen effectors mimicking hormones that induce the host to cease defence too early or produce the incorrect defence signals (Gimenez-Ibanez *et al*. 2014; Ma *et al*. 2015). Effector-triggered immunity results when a host plant recognises a bacterial effector through resistance (*R*) genes, commonly stimulating a rapid and amplified defence mechanism leading to localised cell death, known as the 'hypersensitive response' (Dangl and Jones 2001). A minor change in an *avr* gene producing the effectors, or the *R* genes recognising them, can have a profound effect on the interaction between a particular bacterial strain and plant species (Flor 1971; Collinge and Slusarenko 1987; Jackson and Taylor 1996). Identifying the *avr* genes present in the *P. savastanoi* pv. *phaseolicola* population infecting mungbeans and their associated *R* genes will be a valuable step towards controlling halo blight.

Plant pathogenic bacteria have a remarkable ability to manipulate their genomes to avoid host defence systems. They move DNA within and between bacterial genomes by means of mobile genetic elements such as plasmids, bacteriophages, integrons and transposons (Frost *et al*. 2005). Non-pathogenic strains of *P. savastanoi* pv. *phaseolicola* can gain virulence capabilities through persistent contact with pathogenic strains, as revealed by confocal microscopy (Rufián *et al*. 2017). Thus, continual monitoring of changes in the pathogen's repertoire of effectors, and mining new sources for resistance, will be necessary to stay ahead of the rapidly evolving pathogen population.

Diagnosis of the halo blight pathogen

In Australia, mungbean seed-crop production currently relies heavily on the absence of observed symptoms in the field. The use of diagnostic assays to screen diseased mungbean material and seed for pathogenic bacteria has not yet been implemented. Investigating molecular technologies to detect *P. savastanoi* pv. *phaseolicola* in mungbean seed samples will have a major economic benefit to the industry, providing cleaner seed and stability in production.

In the later stages of infection, halo blight disease symptoms can be particularly difficult to identify on a visual basis. Lesions become indistinguishable from other necrotic leaf spots or natural senescence as the water-soaked lesions turn a dry papery brown and the yellow halo dissipates (Burkholder 1930). Serology, plate culture, microscopy and molecular diagnostics are used globally throughout the bean industry and overcome the disadvantages of visual identification (Guthrie *et al*. 1965; Vuurde *et al*. 1991; Prosen *et al*. 1993;

Félix-Gastélum *et al*. 2016). The differing characteristics of the strains of *P. savastanoi* pv. *phaseolicola* offer several targets for specific diagnostic tools.

The primary target for molecular diagnostics is PCR amplification of the phaseolotoxin gene cluster (Prosen *et al*. 1993). However, phaseolotoxin-negative strains, such as are present in Spain, are undetectable by PCR targeting the phaseolotoxin gene cluster (Rico *et al*. 2003). Two genetic lineages, those with the *tox* gene cluster (*tox*+) and those without (*tox*–), have been definitively separated (Oguiza *et al*. 2004), and a multiplex PCR now capable of detecting both *tox*– and *tox*+ strains was developed (Rico *et al*. 2006).

Quantitative detection of plant pathogens through qPCR is a powerful technique using highly specific primers and fluorescent probes (Schaad and Frederick 2002). A hydrolysis-probe-based qPCR assay was designed to amplify both *tox*– and *tox*+ by targeting the cytochrome o ubiquinol oxidase subunit II gene. This had a reported detection limit of 4.5×10^3 colony forming units (CFU) mL^{-1} (Xu and Tambong 2011). Because this is a single-copy gene, it is equal to four and a half cells per reaction when 1 μL template is used. A similar assay, targeting a site-specific recombinase gene, reported a detection limit of 7 CFU per reaction (Seok Cho *et al*. 2010).

An alternative diagnostic assay that can be deployed in the field is loop-mediated isothermal amplification (LAMP). By using a combination of six specially designed primers, amplification takes place under isothermal conditions (Notomi *et al*. 2000). A LAMP protocol has been established to detect *P. savastanoi* pv. *phaseolicola* and could serve as a rapid protocol for identification and detection of the bacteria in samples away from the laboratory (Li *et al*. 2009).

Digital droplet PCR (ddPCR) is a recent technology that provides both detection and quantification. Unlike other methods, ddPCR does not require inclusion of standards of known concentration to achieve quantification (Huggett and Whale 2013). The technology is also reported to deal better with environmental samples and inhibitors than qPCR, making it an ideal choice for seed testing (Dingle *et al*. 2013). Although there are no reported ddPCR primers for *P. savastanoi* pv. *phaseolicola*, the technology has been used to detect and provide quantification of other important Gram-negative plant bacterial pathogens such as *Erwinia amylovora* and *Ralstonia solanacearum* (Dreo *et al*. 2014). Current qPCR primers specific to *P. savastanoi* pv. *phaseolicola* are expected to be compatible with the ddPCR platform. A comprehensive set of reported PCR primers specific to *P. savastanoi*. pv. *phaseolicola* (Table 2) could be trialled to test their usefulness in identifying strains infecting Australian mungbeans.

Management strategies

Bacterial seed-borne diseases continue to have outbreaks causing significant economic loss due to limited management options. It is extremely difficult to control bacterial pathogens such as *P. savastanoi*. pv. *phaseolicola* that have a wide host range encompassing the majority of the Fabaceae family, including weed species (Table 1). To develop an effective strategy to manage bacterial disease, an integrated approach is essential, taking into consideration conducive conditions, cultural practices, chemical options, seed source and crop susceptibility. Although halo blight inoculum is primarily introduced though infected seed, cultural practices greatly influence the development and spread of the disease. Crop rotation, removal of crop debris and volunteers, weed control, tilling, restricting movement though paddocks especially during wet conditions, as well as thoroughly washing and disinfecting machinery play important roles in controlling disease outbreaks (Hall and Nasser 1996). Movement through paddocks should be carefully considered because crops that appear symptomless may harbour large populations of epiphytically infected plants, after which mechanical damage will allow these epiphytes to enter freely and cause disease (Marques and Samson 2016*a*).

Streptomycin, kanamycin and copper oxychloride have previously been used as foliar sprays and seed treatments by the American bean industry, but are not a viable option in Australia owing to regulations prohibiting their use on plant crops, poor efficacy and uneconomical application regimes (Taylor 1972; Taylor and Dudley 1977; Sundin *et al*. 1994). Thermotherapy using hot air or water to kill pathogens is an unexplored area in Australia; however, reported premature germination and reduced shelf life of planting seed are adverse effects to be considered (Grondeau *et al*. 2011). Effective management of halo blight will involve a greater emphasis on cultural practices, a better understanding of infection pathways from alternative hosts, production and maintenance of certified disease-free seed and ultimately production of varieties that have increased resistance to the disease (Taylor 1970; Taylor *et al*. 1979*b*; Bastas and Sahin 2017).

Strict seed-production protocols are the first line of defence against seed-borne bacterial diseases. Seed crops should be grown in climates and locations non-conducive to pathogens, under drip irrigation to limit the amount of free moisture, and far removed from commercial crops (Grogan and Kimble 1967; Webster *et al*. 1983; Gitaitis and Walcott 2007). Confirming the presence of pathogens in planting seed by using diagnostic assays such as serology, culturing and PCR further reduces the risk of epidemics (Vuurde *et al*. 1991; Prosen *et al*. 1993; Marques *et al*. 2000; Rico *et al*. 2006; Xu and Tambong 2011). Ultimately, managing the transmission of seed-borne diseases relies on precise identification of the target pathogens in planting seed and the development of targeted resistance to the pathogens within the host species (Bastas and Sahin 2017). Ensuring that seed is of the highest quality and free of disease through a rigorously upheld seed scheme is a key factor to reducing the impact of bacterial disease (Gitaitis and Walcott 2007).

A seed scheme implemented in 1998 by the Australian Mungbean Association requires all seed crops to be visually inspected for disease by a third-party seed inspector (AMA 2015). However, it is unlikely that the current implementation of this strategy is having the desired impact on disease prevention, with severe epidemics seen in Australia on a yearly basis (Ryley *et al*. 2010). Sole reliance on visual field inspections to produce clean seed can lead to epidemics, owing to fundamental characteristics of the halo blight pathogen. As an asymptomatic epiphyte able to survive on the outer surface of a plant, *P. savastanoi*. pv. *phaseolicola* becomes pathogenic only under favourable environmental conditions (Grogan and Kimble

Table 2. PCR primers used to identify and diagnose the presence of *Pseudomonas savastanoi* pv. *phaseolicola*, causal agent of halo blight

Assay	Primer name	Sequence 5′–3′	Target locus	Amplicon size (bp)	Reference
Conventional PCR	HM6	CGTGTCCTGGGATAAAAGC	Phaseolotoxin gene cluster	1900	Prosen *et al*. 1993
	HM13	GTTGAATTTCACTACCCG			
	HB14F	CAACTCCGACACCAGCGACCGAGC	Phaseolotoxin gene cluster	1400	Audy *et al*. 1996
	HB14R	CCGGTCTGCTCGACATCGTGCCAC			
	PHA19	CGTCTGTAACCAGTTGATCC	*amtA* gene	480	Marques *et al*. 2000
	PHA95	GAATCCTTGAATGCGAAGGC			
	PphE-F	GCGTTCGATCATAACGTTGA	*hopX1* (*avrPphE*)	1400	Stevens *et al*. 1998
	PphE-R	TCATTGGCAGAGCGATGAGT			
	PphB-F	TGGATCCACCATGAAAATAGGTACGC	*hopAR1* (*avrPphB*)	850	
	PphB-R	TTCGCACTCGAGTGGTAAATATTGCCG			
	PphF-F	ATGAAGAATTCGTTCGACCG	*hopF1* (*avrPphF*)	1400	Tsiamis *et al*. 2000
	PphF-R	TCAGACCGAACTCTCAGACA			
	P3004 L	CTGTCTGGCAGCCACTACAAAG	GenBank acc no. AJ568001	240	Rico *et al*. 2006
	P3004R	GGCTGCAAATTGTGGGATTT			
Nested external	P5.1	AGCTTCTCCTCAAAACACC	Phaseolotoxin gene cluster	502	Schaad *et al*. 1995
	P3.1	TGTTCGCCAGAGGCAGTCATG			
Nested internal	P5.2	TCGAACATCAATCTGCCAGCCA		450	
	P3.2	GGCTTTTATTATTGCCGTGGGC			
REP	ERIC1R	ATGTAAGCTCCTGGGGATTCAC	Random	250–5000	Versalovic *et al*. 1991
	ERIC2	AAGTAAGTGACTGGGGTGAGCG			
	BOXA1R	CTACGGCAAGGCGACGCTGACG	Random	500–2000	Versalovic *et al*. 1994
	REP1R-I	I I I ICGICGICATCIGGC	Random	250–5000	Versalovic *et al*. 1991
	REP2-I	ICGICTTATCIGGCCTAC			
qPCR-TaqMan	Psy_cyoII-pb	GCCAAGTACACGCCGGACTGGTC	Cytochrome o ubiquinol oxidase subunit II	176	Xu and Tambong 2011
	Psy-cyoII-F	TCGAGCAGCGGAACCTGATC			
	Psy-cyoII-R	TGGGTACGCCCCAGACTGCGA			
	SSRP_F	GACGTCCCGCGAATAGCAATAATC	Site-specific recombinase gene	183	Seok Cho *et al*. 2010
	SSRP_R	CAACGCCGGCGCAATGTCG			
	SSRP_P	TGACGTGACACTCGCCGAGCTGCA			
	PsF-tox 188_F	GGGGTGGGACGTGTTAT	*tox-argK* chromosomal cluster		Schaad *et al*. 2007
	PsR-tox 557_R	CTTGTAGTTAGACGGTCGG			
	PsF-tox 286_P	ACCATCCGAATGCCAGTAATGCC			
LAMP	BIP	GCAAATTATCTGCCGCCATGCTAAAAGCCGGAATAACTGCTCAGG	Polyketide synthase (PKS) gene		Li *et al*. 2009
	FIP	TCGGGCCTCATACCACGCTCAAAACAAAATGTTGGCTGACACGG			
	B3	GAAACGCAGAGGTCGCTG			
	F3-Outer	TGCTACTGGCGGTGAAAC			
	LF	ACTATGAAGCCTTGTTGGCC			
	LB	GGCGACGGAGACGGATACAC			

1967; Legard and Schwartz 1987; Niknejad Kazempour 2002; Marques and Samson 2016*b*). Rainfall volume and intensity is thought to induce disease symptoms by driving the bacterium into the apoplast of the leaves where it has the optimum environment and nutrients available for growth (Marques and Samson 2016*b*). This potential for latent infections severely compromises the efficacy of certification schemes based only on visual symptoms.

For most of its cultivated history, mungbean has been grown by subsistence agriculture relying on conventional plant-breeding techniques (Fernandez *et al*. 1988). These techniques are still employed today in both developing and developed countries, although the transition towards molecular-based breeding is moving at a fast pace as technologies mature and costs fall (Chen *et al*. 2013; Schafleitner *et al*. 2015; Noble *et al*. 2018). With the increased adoption of genomic technologies to characterise and select germplasm, researchers are discovering untapped resources of genetically diverse material that may contain unique alleles for disease resistance (Lawn and Rebetzke 2006; Schafleitner *et al*. 2015; Noble *et al*. 2018).

The gene-for-gene interactions between *P. savastanoi.* pv. *phaseolicola* and its hosts make it ideal for marker-assisted selection, because resistance is commonly conferred by a single, large-effect quantitative trait locus (QTL) (Jenner *et al*. 1991; Stevens *et al*. 1998; Tsiamis *et al*. 2000). Establishing these marker associations in mungbean will be accomplished through genome-wide association studies comprising large, diverse mungbean mapping populations representative of worldwide germplasm (Schafleitner *et al*. 2015; Noble *et al*. 2018). To limit the effect of major *R* genes breaking down over time, implementation of genomic selection in breeding programs will help to ensure that small-effect genes are also incorporated into new cultivars (Jannink *et al*. 2010). This will accelerate the breeding cycle by replacing lengthy phenotypic evaluation with models able to predict the breeding value of lines by incorporating phenotypic data and high-density markers (Nair *et al*. 2012; Chen *et al*. 2013; Dhole and Reddy 2013).

Conclusions and recommendations

Halo blight is a destructive disease in mungbean crops throughout Australia and is emerging globally in other production areas. Eliminating infected seed and developing resistant commercial cultivars are the key strategies required to control halo blight. Adoption of the methods discussed in this review will have a substantial impact on reducing the incidence and severity of halo blight in mungbean-cropping regions.

In the short term, *P. savastanoi* pv. *phaseolicola* isolated from mungbean plants across a broad range of Australian growing regions should be screened against the PCR assays listed in Table 2. This would provide foundational molecular tools to identify and screen for the disease. Development of a diagnostic assay for seedlots would have a beneficial effect on monitoring and controlling the spread of halo blight. Whole-genome sequencing of unique strains will reveal unique targets to develop diagnostic assays able to differentiate between strains endemic to a particular region. These assays would provide further sensitivity in identification, and surveillance of population dynamics. Understanding of which strains are present on particular mungbean genotypes in particular regions and years, and under which specific environmental conditions, will inform and direct pathology research.

Development and implementation of genomic tools will be required to support sustainable resistance breeding in the long term. Genome-wide association studies will identify regions of the mungbean genome related to disease resistance. Once these associations have been made, the identified markers could be used to guide introgression of disease resistance into genetically favourable backgrounds. Having the means to edit genomes directly with breakthrough technologies such as CRISPR will further reduce the timeframe from gene discovery to cultivars possessing traits of interest (Dangl *et al*. 2013). Sequencing collections of mungbean accessions in combination with genome-editing tools could see stable resistance introduced into mungbean with greater accuracy and speed.

No single strategy will be the answer. An integrated approach that continues to address all of these areas will be needed to overcome halo blight in mungbeans.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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