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Australasian Plant Pathology, 49(5), pp. 515-524.

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<https://doi.org/10.1007/s13313-020-00722-8>



Characterisation of the *Pseudomonas savastanoi* pv. *phaseolicola* population found in Eastern Australia associated with halo blight disease in *Vigna radiata*

Thomas J. Noble¹ · Anthony J. Young² · Lisa A. Kelly³ · Roberto A. Barrero⁴ · Colin A. Douglas⁵ · Hao Long¹ · Brett Williams¹ · Sagadevan Mundree¹

Received: 11 February 2020 / Accepted: 29 May 2020
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Abstract

This study analysed the phenotypic and genotypic variation among 511 *Pseudomonas savastanoi* pv. *phaseolicola* (Psp) isolates, causing halo blight in mungbeans. Collected from symptomatic mungbean (*Vigna radiata*) crops throughout Australia between 2005 and 2018, a total of 352 Psp isolates were phenotypically screened. Our *in planta* screening against a set of four mungbean cultivars with known susceptible and resistant reactions revealed five distinctive pathotypes. Isolates belonging to pathotype 2 were the most prevalent at 84% and were found to be highly pathogenic towards all tested mungbean genotypes. Genomic variation was investigated for 205 isolates using DNA fingerprints, splitting the halo blight pathogen population into two broad genetic lineages. Further genetic testing for two known avirulence genes, *avrPphE* and *avrPphF*, identified the *avrPphE* gene in all the tested isolates and *avrPphF* present in all but two. To identify candidate avirulence genes unique to Psp isolates infecting mungbean in Australia, a comparative genomics study was undertaken on the whole-genome sequences of two epidemiologically important Psp isolates, T11544 and K4287. The information presented in this study has the potential to dramatically improve mungbean disease resistance now and into the future.

Keywords Mungbean · Pathotype · Avirulence · BOX · ERIC · IS50

Introduction

Mungbean (*Vigna radiata* L. Wilczek var. *radiata*) is a grain legume that provides a vital source of nutrition for many

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13313-020-00722-8>) contains supplementary material, which is available to authorized users.

✉ Thomas J. Noble
t2.noble@qut.edu.au

¹ Centre for Agriculture and the Bioeconomy (CAB), Queensland University of Technology, Brisbane City, QLD 4000, Australia

² School of Agriculture and Food Sciences, The University of Queensland, Gatton, QLD 4343, Australia

³ Department of Agriculture and Fisheries (DAF), Toowoomba, QLD 4350, Australia

⁴ eResearch Office, Queensland University of Technology, Brisbane City, QLD 4000, Australia

⁵ Department of Agriculture and Fisheries (DAF), Hermitage Research Facility, Warwick, QLD 4370, Australia

countries and contributes significantly to Australian agricultural exports (Noble et al. 2019; Shanmugasundaram et al. 2009). Severely limiting the production of commercial mungbean crops in Australia is the seed-borne bacterial disease halo blight, caused by *Pseudomonas savastanoi* pv. *phaseolicola*. An emerging threat in 1980, halo blight is now responsible for large-scale annual losses in mungbean crops and threatens the sustainability of the industry (Noble et al. 2019). Knowledge of genetic variation within the pathogen population and pathogenicity towards elite germplasm is crucial to developing multifaceted management options that will secure the future sustainability of mungbean production.

Taylor et al. (1996a), used a global study of 859 isolates from 303 disease occurrences from the common bean differential set to assign nine races of *P. savastanoi* pv. *phaseolicola*. Arnold et al. (2011) further refined the *P. savastanoi* pv. *phaseolicola* race structure by calculating the frequency of resistance (R) and avirulence (avr) genes among the host race typing set and global pathogen population, finding that a higher R gene frequency in the host led to a lower matching avr gene frequency in the pathogen

population. This suggests that when an R gene is extensively deployed, races of the pathogen carrying the corresponding *avr* gene are suppressed (Arnold et al. 2011). The common bean differential set has since been used to assess the race diversity of 30 *P. savastanoi* pv. *phaseolicola* strains isolated from Australian mungbeans. Race 7, as described by Taylor et al. (1996b), was identified as well as variants that did not coincide with the race structure reported for common bean (Ryley et al. 2010; Taylor et al. 1996b).

This study characterises 511 *P. savastanoi* pv. *phaseolicola* isolates sampled from the naturally occurring population in Eastern Australia, focusing primarily on the South East Queensland region. In doing so, the isolates were categorised into five pathotypes and two broad genetic lineages. Two isolates with markedly different pathologies were further investigated using whole genome sequences to compare virulence factors. The findings presented here will improve basic research and applied outcomes for mungbean researchers, breeders and industry.

Materials and methods

P. savastanoi pv. *phaseolicola* bacterial strains isolated from *Vigna radiata*

A total of 511 *P. savastanoi* pv. *phaseolicola* isolates were isolated from symptomatic mungbean leaf tissue, stored at either the Queensland Department of Agriculture and Fisheries (DAF) or the Queensland University of Technology (QUT) (Table S1). Symptomatic leaves were surface-sterilized by spraying with 70% ethanol and rubbing with sterile lint-free tissues several times. Lesions were dissected from the leaf and bisected with one piece observed for ooze using a compound microscope and the other placed in a drop of sterile water for bacterial elution. Following confirmation of oozing, a sterile loop was used to streak the water suspension onto King's B (KB) medium (King et al. 1954). The plates were incubated at 28 °C for 24 h. Single colonies were twice sub-cultured to obtain pure cultures. A loop of the pure culture suspended in a 500 µL aliquot of sterile water and used directly as the template in molecular assays. Bacterial suspensions were stored at -20 °C, and for long-term storage, suspended duplicates of each isolate were held in 50% glycerol at -80 °C.

Host-pathogen interactions based on the phenotypic assessment of disease reactions on four *Vigna radiata* genotypes

In consultation with the Australian National Mungbean Breeding Improvement Program (NMIP), four host genotypes of mungbean (*V. radiata*) were selected based on disease

reactions observed in artificially inoculated glasshouse and field disease screening experiments. Two seeds of each member of the differential set were sown into commercial potting mix (Rocky Point Mulching™) in seedling trays consisting of 42 wells 4 cm³ in volume. For each genotype, six wells were used and thinned to one seedling per well after emergence. Seedlings were maintained in a growth cabinet at 22±5 °C under a 12 h light/12 h dark regime and watered regularly. Before the emergence of the first leaves, pure cultures of each isolate were spread on KB medium and incubated at 28 °C for 72 h. A sterile needle dipped into a single colony was pierced through the leaves. As a control treatment, six seedlings per tray were inoculated with a sterile needle dipped in sterile water. After inoculation, seedlings were sprayed briefly with a handheld spray bottle containing sterile distilled water and covered with a plastic bag. After 48 h the plastic bags were removed. Inoculated leaves were assessed for the presence of a chlorotic halo at the point of inoculation and small, circular, dark brown water-soaked lesions fourteen days after inoculation. Genotypes displaying characteristic symptoms were recorded as susceptible, while those with only a necrotic lesion at the point of inoculation were considered resistant. Isolations were made from the symptomatic tissues of randomly selected genotypes using the methods outlined previously. Variation in pathogenicity was assessed for a total of 352 *P. savastanoi* pv. *phaseolicola* isolates.

Genomic profiling and amplification of avirulence genes

The presence or absence of avirulence genes and DNA fingerprints were generated for 205 isolates of *P. savastanoi* pv. *phaseolicola* (primers listed in Table S2). PCR conditions for each of the primers were as described in the literature: ERIC (Versalovic et al. 1991), BOX (Versalovic et al. 1994), IS50 (Sundin et al. 1994), *avrPphE* (Stevens et al. 1998) and *avrPphF* (Tsiamis et al. 2000). In brief, 10 µL reactions contained 1 µL of bacterial cells at a concentration of $\sim 1 \times 10^6$ CFU mL⁻¹ as template, the final primer concentration in the reaction was 50 pM and PCR programs were as described in each reference. Thermal cycling was conducted using an Applied Biosystems, Life Technologies Proflex PCR system and products were separated by electrophoresis on 1% agarose gels, cast and run in 0.5x TAE buffer. Bands were visualised using SybrSafe stain and the G-Box Syngene gel documentation system.

Cloning and sequence analysis of the amplicon unique to the IS50-B genotype

To identify the DNA sequence and unique variations present in the PCR product of IS50-B, the 500 bp amplicon was excised and cloned into a pGEM-T Easy vector (Promega). A

total of eight sequences were obtained from three isolates T13733B, T13804A, and T14028 by Sanger sequencing (Macrogen, Seoul, Rep. of Korea) using the M13 forward primer. Sequencing results were checked using Vector NTI and BLAST analysis against the NCBI database and assembled genomes from the study of T11544 and K4287. Protein modelling of the sequence through Phyre2 considered hits with homology confidence of >99 to determine possible functions (Kelley et al. 2015).

Whole-genome comparison of two epidemiological significant *P. savastanoi* pv. *phaseolicola* isolates T11544 and K4287

Isolated from infected mungbean leaf tissue in 2005, isolate T11544 has been used to challenge germplasm and breeding lines in the Australian National Mungbean Improvement Program for over a decade. However, another strain present in Australia, K4287 isolated in 2013, is highly virulent and overcomes the defences of the germplasm used as resistance donors against the T11544 strain. Before high-throughput sequencing of *P. savastanoi* pv. *phaseolicola* strains K4287 and T11544, pathogenicity was confirmed on *V. radiata*. DNA was extracted from pure plate cultures of each isolate using a DNeasy Blood & Tissue Kit (QIAGEN). Library preparation and sequencing were performed by Macrogen, using the TruSeq Nano DNA Kit for library preparation (TruSeq Nano DNA Sample Preparation Guide, Part # 15041110 Rev. A) and high-throughput sequencing was conducted using an Illumina HiSeq 2500 System (User Guide Document # 15035786 v01HCS 2.2.70). In total, 26,385,408 and 27,767,198 paired-end reads were generated for T11544 and K4287, respectively.

Adapter sequences and low-quality sequences based on quality scores of Phred <30 and a minimum length of 50 bp were removed using Trim Galore (version 0.5.0) (Krueger 2015). After quality control, the Illumina reads were de novo assembled using the SPAdes (version 3.13.0) genome assembly algorithm (Bankevich et al. 2012). Completeness of the assembly was assessed using QUAST (Quality Assessment Tool for Genome Assemblies, version 5.0.2) (Gurevich et al. 2013). Assembled contigs were annotated using the RAST annotation pipeline (Aziz et al. 2008).

The de novo assembled K4287, and T11544 genomes were aligned and compared using Mauve (Darling et al. 2004). Genome-wide comparison of sequence similarity was conducted with BLASTN, filtering scaffolds with >= 50% coverage of the queried sequence. BLASTP compared protein-coding genes hits, filtering those with a coverage >= 95% and sequence similarity of >= 98%. Average Nucleotide Identity (ANI) was calculated between the K4287 and T11544 genome assemblies as described by Yoon et al. (2017).

The raw Illumina data has been deposited in the NCBI Short Read Archive database under SRA accession PRJNA603636 for the T11544 and K4287 genomes. The whole-genome shotgun (WGS) draft genome assemblies were deposited in GenBank under the accession numbers JAAFOY000000000 for *P. savastanoi* pv. *phaseolicola* T11544 strain and JAAFOZ000000000 for *P. savastanoi* pv. *phaseolicola* K4287 strain.

Results

Host-pathogen interactions reveal five pathotypes of *P. savastanoi* pv. *phaseolicola* infecting Australian mungbeans

The existence of five pathotypes among 352 *P. savastanoi* pv. *phaseolicola* isolates was revealed based on the pathogenicity of the isolates towards four *V. radiata* genotypes (Table 1). Of the five disease reactions observed, isolates belonging to pathotype 2 were the most pathogenic, producing symptoms on all genotypes. Isolated from all growing regions throughout Australia (Fig. 1), pathotype 2 comprised 84% of all isolates screened in this study (Table 1). In contrast, isolates from pathotype 1 are only pathogenic towards the commercial cultivar Crystal and accounted for 10% of the population (Table 1). The remaining pathotypes, 3, 4 and 5 combined comprise as little as 6% of the population and exhibit cultivar-specific virulence (Table 1). Based on prior knowledge and results from this study, isolates T11544 and K4287 were selected for further investigation. Isolate K4287 represents isolates from pathotype 2, identified to overcome known resistance in glasshouse screening (Table 1). Isolate T11544, used as the primary source of inoculum in resistance breeding, represents pathotype 1 as a direct comparison.

Table 1 Pathotype variation among 352 *Pseudomonas savastanoi* pv. *phaseolicola* isolated from *Vigna radiata*

Genotype	1	2	3	4	5
AusTRC321818	–	+	–	–	+
M773	–	+	–	+	–
OAEM58–62	–	+	+	–	–
Crystal	+	+	+	+	+
Totals	34	294	5	5	14
Frequency	10%	84%	1%	1%	4%

Isolates were inoculated on four *Vigna radiata* genotypes with known variation in resistance to halo blight disease (+ = inoculated plants displayed symptoms; – = no symptoms)

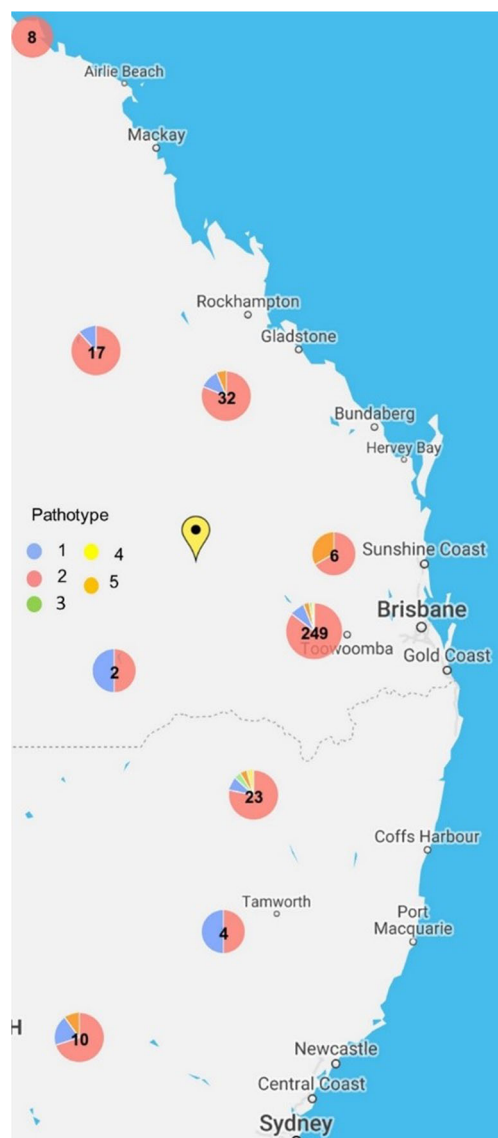


Fig. 1 Clustering and distribution of 352 *P. savastanoi* pv. *phaseolicola* isolates based on location isolated and assigned pathotype. 1: blue, 2: red, 3: green, 4: yellow, 5: orange

Genomic profiling of the *P. savastanoi* pv. *phaseolicola* population in Australia

Molecular markers reveal the population of Australian *P. savastanoi* pv. *phaseolicola* is highly conserved (Fig. 2) and splits into two broad genetic lineages (Fig. 3). The ERIC and BOX primers, previously used in genomic profiling to distinguish between bacterial strains within *P. savastanoi* pathovars (Weingart and Völksch 1997), revealed no differences among representatives from the five pathotypes analysed here. In contrast, the IS50-PCR DNA fingerprints distinguished two patterns, “A” and “B”, which were discernible by the presence or absence of a specific amplicon of approximately 500 bp (Fig. 3). A subset of 58 isolates from 2005 to 2016 were primarily categorised as sub-populations

IS50-A (75%) and IS50-B (25%). In comparison, the 148 *P. savastanoi* pv. *phaseolicola* isolates from 2017 and 2018 revealed a pronounced decrease in IS50-A to 55%, while IS50-B increased to 45% of all isolates (Fig. 4). This difference is statistically significant at the 1% confidence level (chi-square statistic with Yates correction = 6.9388, p value = 0.008435). Sub-population IS50-A concentrated in South East Queensland was revealed as decreasing in size over time, while sub-population IS50-B grew by 80% in 2017 and 2018, spreading north to Central Queensland and south to Northern New South Wales. Isolates T11544 and K4287 were categorised as IS50-A, suggesting other factors are affecting virulence.

The ~500 bp fragment cloned from IS50-B showed a 99.5% identity to a hypothetical protein encoded by “*Pseudomonas syringae* pv. *cerasicola* strain CFBP 6110 genome assembly, plasmid: PP2”. BLASTn returned no significant hits when using the K4287 and T11544 genome assemblies from this study as custom databases. Further to this, BLASTx did not return any hits or identify conserved domains. Analysis of the sequence using Phyre2 modelling software revealed multiple significant hits for kinase-related proteins.

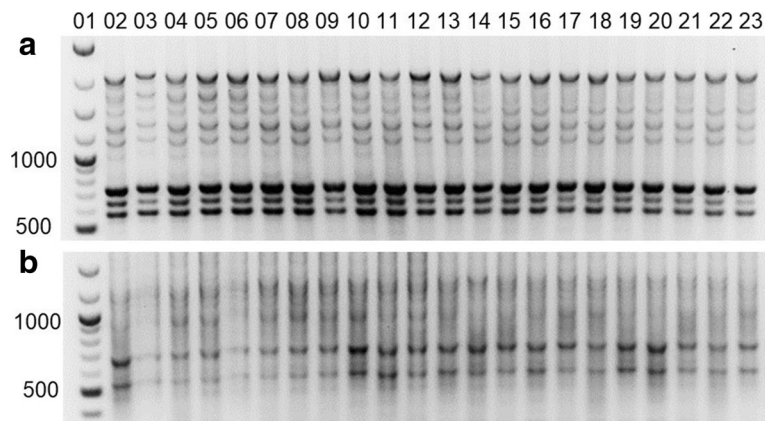
Presence/absence of cloned avirulence genes *avrPphE* and *avrPphF*

The *avrPphE* gene, cloned from race 4 but present in all races, is reported to affect cultivar-specific avirulence in common bean (Mansfield et al. 1994; Nimchuk et al. 2007; Stevens et al. 1998). The *avrPphF* gene, located within a pathogenicity island on the plasmid (pAV511) was cloned from race 5 and 7 of *P. savastanoi* pv. *phaseolicola* (Tsiamis et al. 2000). Of the 205 isolates analysed in this study, all carry avirulence gene *avrPphE*. Two isolates PSP023 (Fig. 4) and T13733B (pathotype 2), were identified as missing *avrPphF* but otherwise had the same genotypic grouping IS50-B (Table S1).

Whole-genome comparison of two epidemiological significant strains of *P. savastanoi* pv. *phaseolicola* T11544 and K4287

To identify candidate virulence genes unique to strains endemic to Australia, the whole-genome sequences of strains T11544 (pathotype 1) and K4287 (pathotype 2) were assembled de novo, annotated and comparatively analysed. Both had highly similar genomes with an average nucleotide identity of 99.98% (Table S3) (Yoon et al. 2017) comprising genome sizes of ~6 Mbp (Table 2, Table S4). A Benchmarking Universal Single-Copy Orthologs’s (BUSCO) (Simão et al. 2015) analysis determined that both genome assemblies were 99.32% complete. When comparing the two genomes, 49,330 additional base pairs were identified as unique to the T11544

Fig. 2 Examples of DNA fingerprints from *P. savastanoi* pv. *phaseolicola* (PSP) using **a** ERIC and **b** BOX A1R, PCR primers. Lane 01: 2-Log DNA Ladder (0.1–10.0 kb) (New England Biolabs); Lanes 02–23: PSP001-PSP022. Isolates were not distinguishable based on their ERIC/BOX A1R profiles



genome, of which ~40 kbp were located on the chromosome (Table S4). Strain K4287 had a smaller genome by approximately 50 kbp. The majority of the difference in genomic material between the strains was missing from the chromosome of K4287, while its plasmids A and B gained ~6 kbp and 4 kbp respectively (Table S4). The additional unique genetic material from both strains comprised a total of 29 annotated regions primarily associated with virulence (Table 3). Assessment of the repertoire of type III secretion proteins in both K4287 and T11544 genome assemblies identified the same 17 proteins conserved in both genomes with 100% nucleotide sequence similarity (Table S5).

Discussion

Known for over 90 years, halo blight disease of the Fabaceae family continues to threaten food production globally (Arnold et al. 2011; Burkholder 1926; Noble et al. 2019). However, limited research has explicitly focused on the interactions between the causal agent *P. savastanoi* pv. *phaseolicola* and *Vigna radiata* (Noble et al. 2019; Sun et al. 2017). This study provides both a broad and in-depth investigation of the phenotypic and genotypic variation that exists within the

population of *P. savastanoi* pv. *phaseolicola* infecting Australian mungbeans. In addition to the identification of characterised and unique virulence factors, the categorisation of five pathotypes provides vital information to inform future breeding practices and research.

In Australia, races 2 and 7 of the nine global races were first identified from *Macroptilium atropurpureum* and *Neonotonia wightii* (Taylor et al. 1996a). In 2010, race 7 was reported as the cause of halo blight disease in Australian mungbeans with variations acknowledged that did not match any of the nine global races (Ryley et al. 2010). Four mungbean accessions with known reactions to halo blight disease were selected to dissect how those variations affect the pathogenicity towards mungbean in Australia. Of the five pathotypes described in this study, those assigned to highly pathogenic pathotype 2 accounted for 84% of all the isolates screened (Table 1). Widely dispersed across farming land, the majority of isolates screened belonged to this pathotype (Fig. 1).

Pathogenicity of bacterial pathogens is highly regulated by pairs of corresponding avirulence (*avr*) and resistance (*R*) genes in the pathogen and host, respectively (Flor 1971). Effector proteins produced by avirulence genes are injected into the host via the type three secretion systems (T3SS) found in *P. savastanoi* pv. *phaseolicola*, catalysing the infection

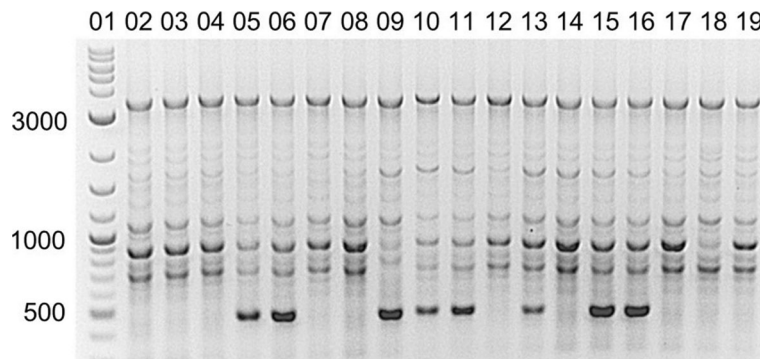
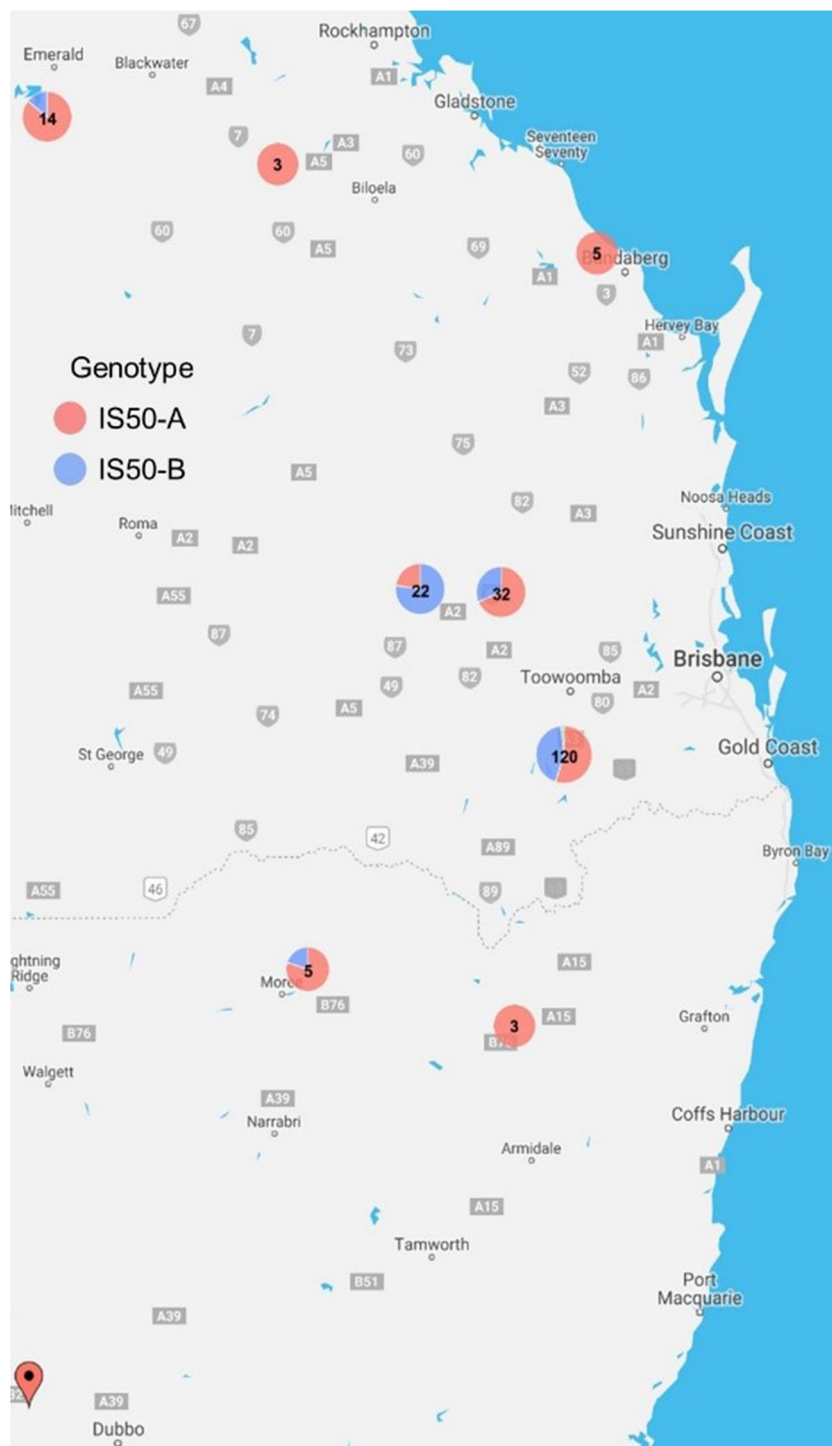


Fig. 3 Examples of DNA fingerprints from *P. savastanoi* pv. *phaseolicola* using IS50 PCR primers. Lane 01: 2-Log DNA Ladder (0.1–10.0 kb) (New England Biolabs); Lanes 02: T11544; 03: T14006; 04: K4287; 05: T14031; 06: T13913; 07: T13976; 08: T13994; 09:

T13733B; 10: T13804A; 11: T13805H; 12: T13992; 13: T13754B; 14: T13734A; 15: T13804F; 16: T13982; 17: T14007; 18: T13636C; 19: T13981

Fig. 4 A total of 205 *P. savastanoi* pv. *phaseolicola* isolates were geotagged and screened with IS50 PCR primers, dividing the population into two broad genotypes. The panel is a map of mungbean growing districts in Australia highlighting the distribution of the isolates throughout Southern and Central Queensland and Northern New South Wales. The two IS50 groups are designated red for IS50-A and blue for IS50-B



process or inducing a hypersensitive response if a matching R gene is present (Alfano and Collmer, 1997). Isolated and sequenced from race 4, strain 1302A of *P. savastanoi* pv. *phaseolicola*, *avrPphE* matches the R2 gene for resistance in *Phaseolus* cultivars (Mansfield et al. 1994). Family members of *avrPphE* are prevalent among a diverse background of pathogenic bacteria suggesting a conserved role in virulence (Lindeberg et al. 2005; Nimchuk et al. 2007). Consistent with

those reports, this study identified *avrPphE* in all the tested isolates, and *avrPphF* in all but two (Table S1). A loss of virulence towards susceptible *P. vulgaris* cultivars has been reported for strains of *P. savastanoi* pv. *phaseolicola* missing the pAV511 plasmid where *avrPphF* is located (Tsiamis et al. 2000). This was not the case for isolate T13733B which was missing *avrPphF* but retained its virulence against all four of the genotypes screened in this study. This suggests that other

Table 2 Comparison of the general genomic features of *P. savastanoi* pv. *phaseolicola* strains T11544 and K4287

Feature	<i>P. savastanoi</i> pv. <i>phaseolicola</i> T11544	<i>P. savastanoi</i> pv. <i>phaseolicola</i> K4287
Molecule	Chromosome + plasmids	Chromosome + plasmids
Size (bp)	6,080,137	6,030,807
Coding sequence (bp)	5,362,702	5,323,461
Contigs	221	218
Contigs N50	77,371	77,371
G + C %	57.8	57.8
Number of ORFs	5912	5862
Annotated ORFs	4539	4513
Hypothetical protein	1373	1349
tRNA	159	159
rRNA	12	12

Table 3 Unique genes encoded in T11544 and K4287 scaffolds not found in the respective genome

Scaffold ID	Start	End	Total length	Annotation description
K4287_000077	18,681	20,180	1499	Mobile element protein
K4287_000077	18,106	18,624	518	GCN5-related N-acetyltransferase
K4287_000078	20,269	20,604	335	ISPpu14%2C transposase Orf2
K4287_000137	156	758	602	Cysteine protease
K4287_000142	66	833	767	Phage integrase family protein
K4287_000153	1816	2415	599	Polygalacturonase (EC 3.2.1.15)
T11544_000074	6450	8588	2138	Bacteriocin/lantibiotic_efflux_ABC_transporter%2C_permease/ATP-binding_protein
T11544_000074	17,608	19,710	2102	Signal_transduction_histidine_kinase_CheA
T11544_000074	15,922	17,577	1655	Methyl-accepting_chemotaxis_sensor/transducer_protein
T11544_000074	10,041	11,657	1616	Sensor_histidine_kinase/response_regulator
T11544_000074	1874	3304	1430	ISPsy6%2C_transposase
T11544_000074	8600	9964	1364	Type_I_secretion_outer_membrane_protein%2C_TolC_family
T11544_000074	21,905	23,191	1286	NADH_dehydrogenase_(EC_1.6.99.3)
T11544_000074	20,449	21,666	1217	Methyl-accepting_chemotaxis_protein
T11544_000074	12,944	14,020	1076	Chemotaxis_response_regulator_protein-glutamate_methylesterase_CheB_(EC_3.1.1.61)
T11544_000074	4543	5529	986	Error-prone%2C_lesion_bypass_DNA_polymerase_V_(UmuC)
T11544_000074	14,513	15,322	809	Chemotaxis_protein_methyltransferase_CheR_(EC_2.1.1.80)
T11544_000074	15,332	15,871	539	Positive_regulator_of_CheA_protein_activity_(CheW)
T11544_000074	13,983	14,516	533	Chemotaxis_protein_CheD
T11544_000074	23,409	23,942	533	MOSC_domain_protein
T11544_000074	286	786	500	Putative_cytoplasmic_protein_USSDB7A
T11544_000105	3603	4844	1241	Membrane-bound lytic murein transglycosylase B
T11544_000105	2874	3362	488	Probable integral membrane protein Cj0014c
T11544_000105	1899	2375	476	Thiol-disulfide isomerase and thioredoxins
T11544_000105	7533	7778	245	Resolvase%2C putative
T11544_000105	6998	7201	203	RNA polymerase-binding transcription factor DksA
T11544_000105	6785	6973	188	RNA polymerase-binding transcription factor DksA
T11544_000128	3454	5565	2111	Virulence protein
T11544_000128	384	1799	1415	Type II restriction-modification system methylation subunit

Assembled genomic scaffolds (≥ 1 kbp) were subjected to QUAST sequence comparison to identify unaligned contigs unique to the T11544 and K4287 genome. Annotation of genomic scaffolds was performed using the RASTtk pipeline. Hypothetical proteins were not included

virulence factors are responsible for cultivar specific avirulence, or that the relevant genes have been transferred to the chromosome.

Genomic profiling further revealed a highly conserved genome at the population level (Fig. 2), delineating into two subpopulations: IS50-A and IS50-B (Fig. 3). Analysis of the amplicon unique to IS50B was limited to the identification of a hypothetical protein homolog on a plasmid from *Pseudomonas syringae* pv. *cerasicola* when interrogating the NCBI database. Protein modelling based on the sequence suggests the translated product belongs to the kinase family. The characterisation of a kinase protein linked to pathogenicity may represent an advance in understanding the mechanisms of bacterial infection. The eukaryote-like Serine Threonine Protein Kinase (STPK) family discovered in pathogenic bacteria from the genus *Yersinia* (Håkansson et al. 1996) was reported to play a role in sabotaging specific host defence via the G protein pathway, which is universal in plants and animals (Canova and Molle 2014; Håkansson et al. 1996). Further studies will be required to confirm the role of the uncharacterised protein, to identify if it belongs to the type III effector protein family, and whether it can be translocated to plant cells.

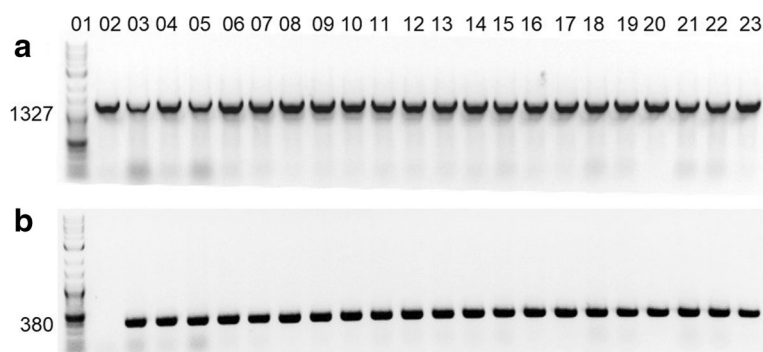
The majority (84%) of isolates categorised as pathotype 2 infected all four of the mungbean genotypes tested (Table 1). Further to this, all isolates contained the *avrPphE* gene, and all but two amplified the *avrPphF* gene (Fig. 5, Table S1). This suggests the matching R genes are absent from the four *V. radiata* genotypes tested. If for example, commercial mungbean varieties contained the R genes associated with *avrPphE* or *avrPphF*, it would be expected that strains carrying those genes would be suppressed (Arnold et al. 2011; Taylor et al. 1996a). Thus, it is likely that current varieties are particularly vulnerable to halo blight disease because they do not contain the gene-for-gene resistance that results from interactions between *avr* and R genes. Celera II–AU, released in 2015, is an exception and represents the gold standard for resistance to halo blight disease in Australian commercial mungbeans. However, its small seed makes it suitable for a limited number of markets, so it accounts for less than 10% of total Australian production (AMA 2015). Released in 2013,

Jade-AU gained a 12% yield advantage over its predecessor Crystal but does not contain the resistance found in Celera II–AU (AMA 2015). The wide-release and adoption of new varieties bred for resistance against T11544 represents a selection pressure that has likely influenced the population of *P. savastanoi* pv. *phaseolicola* (Table 1, Fig. 4). Therefore, research, breeding and industry practices will need to adapt to stay ahead of the evolution of new pathogenic strains.

The identification of *avrPphE* and *avrPphF* throughout the pathogen population provides new targets that could significantly decrease the incidence of halo blight disease. Primary resistance could be integrated into commercial mungbean through the introgression of the R genes associated with *avrPphE* (Stevens et al. 1998) and *avrPphF* (Tsiamis et al. 2000). Screening and analysis of large germplasm resources have identified dominant resistance genes and assisted in the efficient integration of disease resistance in *Phaseolus vulgaris* (Tock et al. 2017). Large germplasm sets representing the global diversity of *V. radiata* have been sequenced and characterised to conduct similar studies investigating *V. radiata* disease resistance (Breria et al. 2019; Noble et al. 2017). Monitoring the pathogen population as it adapts to the release of major R gene resistance will be required as gene-for-gene resistance can break down. To preserve the longevity of newly deployed resistance, rigid integrated disease management practices, must be adhered to, such as seed screening, phytosanitation and rotation of crops.

Comparative analysis between *P. savastanoi* pv. *phaseolicola* and *P. savastanoi* pv. *tomato* DC3000 has previously revealed a high degree of conservation at the gene and genome level, with variation among genes involved in virulence and transposition (Joardar et al. 2005). A near perfect sequence similarity was observed (99.98%) in a fraction of 84% of the whole-genome sequences with orthologous relationships between isolates T11544 and K4287 from pathotypes 1 and 2 (Supplementary Table S4). Both genomes were ~6 Mbp and highly conserved except the less virulent T11544 strain which contained an extra ~50 kbp in sequence primarily related to virulence proteins. The coding sequences were located close together on three contigs T11544_000105 and T11544_000074 and T11544_000128 (Table 3).

Fig. 5 Gel image showing the amplification of *P. savastanoi* pv. *phaseolicola* avirulence genes **a** *avrPphE* and **b** *avrPphF*. Lane 01: 2-Log DNA Ladder (0.1–10.0 kb) (New England Biolabs); Lane 02: PSP023; Lanes 03–23: PSP066-P086. Note that *avrPphF* was not present in lane 02, isolate PSP023



Commercial varieties and breeding material have been selected for their defences against the virulence proteins found in T11544 due to its use as inoculum for field resistance screening. This has potentially allowed a highly virulent population of *P. savastanoi* pv. *phaseolicola* to thrive unknown until now (Table 1). The information presented here provides clear direction and targets for breeders and researchers to reduce the risk of halo blight on mungbean crops.

Acknowledgments This work was supported by the Australian Mungbean Association PhD scholarship program (2017000885) and the Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Qld. The Grains and Research and Development Corporation (GRDC) and The Department of Agriculture and Fisheries of Queensland, Australia investments in grains pathology project (DAQ00186) and the National Mungbean Improvement Program (DAQ00210). We acknowledge the Office of eResearch at QUT for providing access to compute infrastructure and bioinformatics pipelines for this study. We also thank the industry as a whole for their support in providing samples from across Australia. (Burkholder 1926).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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