MOLECULAR CHARACTERISATION AND IDENTIFICATION OF *PSEUDOMONAS SAVASTANOI* PV. *PHASEOLICOLA*, INFECTING MUNGBEANS IN AUSTRALIA

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Keywords

Mungbean, *Vigna radiata*, halo blight, *Pseudomonas savastanoi* pv. *phaseolicola*, Australia, genetic diversity

Abstract

Mungbean (*Vigna radiata* L. Wilczek var. *radiata*) is an important grain legume crop grown on over 6 million hectares globally in tropical and subtropical climates. Its short duration of 55-90 days, capacity to fix atmospheric nitrogen and exceptional grain nutritional profile make the crop a staple for smallholder and subsistence farmers throughout southern and south-eastern Asia. In Australia, mungbean is a high-value export crop and a primary summer rotation for dryland farmers. A significant threat to the integrity of the industry in Australia and an emerging global issue is halo blight, a bacterial disease caused by the phytopathogen *Pseudomonas savastanoi* pv. *phaseolicola*. Infected tissue develops necrotic lesions surrounded by a yellow halo. Plants become stunted and ultimately die. The research for this PhD describes the investigation of the population genetics for the *Pseudomonas savastanoi* pv. *phaseolicola* and *Vigna radiata* populations in Australia.

Pseudomonas savastanoi pv. *phaseolicola* isolates cultured from mungbeans leaf samples were collected throughout Australia, primarily from Northern New South Wales and Queensland's cropping regions. A collection of 511 isolates were characterised using phenotypic and genotypic methods. Five pathotypes were revealed as well as two broad genetic lineages and several candidate pathogenicity related genes. Pathotype 2, accounting for 84% of all isolates, was identified as the isolate with the highest virulence. Isolates belonging to pathotype 2 were able to overcome the resistance revealed against the remainaing four pathotypes. Proteins associated with pathogenicity, unique to isolates T11544 (pathotype 1) and K4287 (pathotype 2) were identified through a comparative analysis of their respective whole-genome sequences. The genes related to the identified proteins provide new targets for molecular resistance breeding. Overall the population was found to be highly conserved with minor variation among vital pathogenicity related genes.

The Australian mungbean diversity panel consisting of 466 individuals associated with 22,000 highly polymorphic SNP markers were characterised to provide a platform for trait dissection at the genome level. Analysis of the markers and population investigated the genetic diversity, population structure and linkage disequilibrium (LD) of mungbean, resulting in the characterisation of four distinct subgroups associated with the geographic origin and morphological characteristics. A

pilot genome-wide association study (GWAS) demonstrated the capacity of this population and its associated markers to dissect out genes controlling important agronomical traits. Phenotyping the panel with isolates from the most pathogenic and widely dispersed pathotypes will provide critical data to infer molecular markers associated with resistance to the disease. In summary, this study details the use of genomic markers, next generation sequencing and phenotypic analysis of halo blight isolates and broad mungbean breeding populations to present foundational insights into the genetics underlying the host-pathogen relationship between mungbean and the halo blight pathogen.

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1. **Thomas J. Noble**, Yongfu Tao, Emma S. Mace, Brett Williams, David R. Jordan, Colin A. Douglas and Sagadevan G. Mundree (2018) Characterization of Linkage Disequilibrium and Population Structure in a Mungbean Diversity Panel. Frontiers in Plant Science, doi.org/10.3389/fpls.2017.02102

2. **Thomas J. Noble**, Anthony J. Young, Colin A. Douglas, Brett Williams and Sagadevan Mundree (2019) Diagnosis and management of halo blight in Australian mungbeans: a review, Crop and Pasture Science 70(3) 195-203, doi.org/10.1071/CP18541

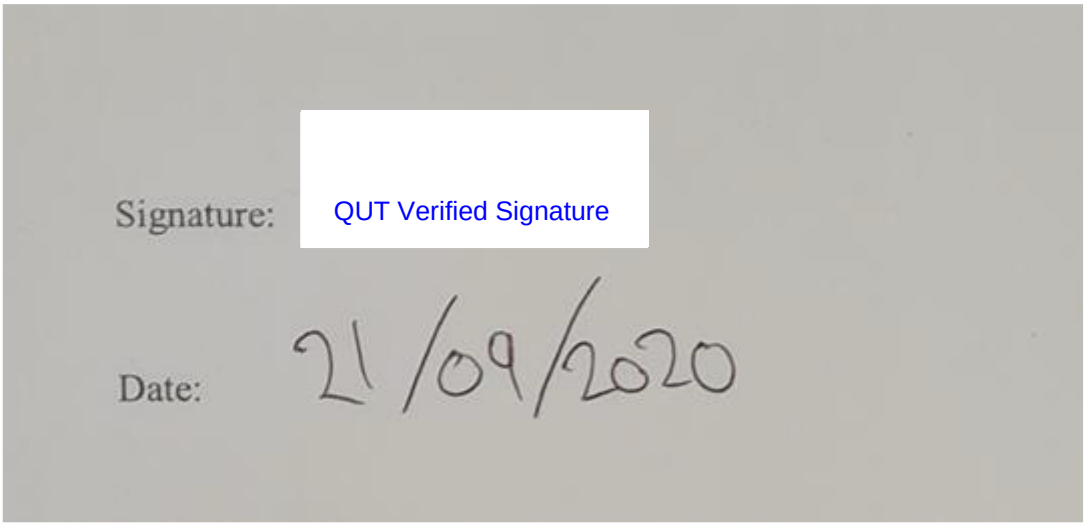
3. **Noble, T.J.,** *et al.* Characterisation of the *Pseudomonas savastanoi* pv. *phaseolicola* population found in Eastern Australia associated with halo blight disease in *Vigna radiata*. *Australasian Plant Pathol.* 49, 515–524 (2020). https://doi.org/10.1007/s13313-020-00722-8

List of Abbreviations

AMA	Australian Mungbean Association
Avr	Avirulence
bp	base pair
CARF	Central Analytics Research Facility
CTCB	Centre for Tropical Crops and Biocommodities
DAF	Queensland Department of Agriculture and Fisheries
ERIC	Extragenic repetitive consensus
ETI	Effector triggered immunity
GI	Genomic Island
HB	Halo Blight
HGT	Horizontal gene transfer
HR	Hypersensitivity Response
NMIP	National Mungbean Improvement Program
NGS	Next Generation Sequencing
QUT	Queensland University of Technology
REP	Repetitive Extragenic Palindrome
SEF	Science and Engineering Faculty
T3SS	Type three secretion system

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.



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Chapter 1: Introduction

This thesis is presented in 'Thesis by Publication' style containing a literature review (Chapter 2) published in the journal Crop and Pasture Science, followed by two results chapters (Chapters 3 and 4) and a general discussion chapter (Chapter 5). Of the two results chapters, Chapter 3 is published in the journal Frontiers in Science and Chapter 4 has been submitted for publication to Australasian Plant Pathology and is currently under review. Therefore, the presentation of the results chapters follows the formatting style of the target journals.

1.1 DESCRIPTION OF THE SCIENTIFIC PROBLEM INVESTIGATED

Mungbean (*Vigna radiata*) provides a vital source of food nutrition and security globally while contributing substantially to Australia's farming export industry. Threatening the supply of mungbeans in Australia and globally is the seed-borne bacterial disease halo blight. A lack of knowledge of host-pathogen interactions, particularly at the molecular level, is severely limiting production and stability. Genotyping and phenotyping both the pathogen (*Pseudomonas savastanoi* pv. *phaseolicola*) and host (*Vigna radiata*) collections will assist future endeavours to develop a resilient mungbean crop resulting in stable and reliable yields. Field studies by the National Mungbean Improvement Program (NMIP) recognised the scale of the problem that halo blight presents as well as to inform initial breeding efforts. However, the lack of molecular tools utilised to identify and study the relationship between *P. savastanoi* pv. *phaseolicola* and *Vigna radiata* has left a significant knowledge gap.

1.2 GENERAL OBJECTIVES OF THE STUDY

This study aimed to characterise the genetic and phenotypic diversity of the mungbean halo blight pathogen *P. savastanoi* pv *phaseolicola* to provide new knowledge and tools to control disease outbreaks.

The specific aims of the study were to 1) Conduct a review of how the Australian industry diagnoses and manages halo blight disease 2) Characterise the *P. savastanoi* pv. *phaseolicola* isolate collection infecting Australian mungbeans, defining the most

pathogenic populations and their associated genetic variants controlling virulence 3) Characterisation of the Australian mungbean diversity panel, determining the population structure, linkage disequilibrium and genome-wide association capacity.

1.3 ACCOUNT OF SCIENTIFIC PROGRESS LINKING THE SCIENTIFIC PAPERS

Diagnosis and management of mungbean in Australia: a review highlighted the critical need for in-depth molecular characterisation of the pathogen and host populations, to reveal genetic variations that are impacting pathogenicity. *Molecular characterisation of P. savastanoi* pv. *phaseolicola causal agent of halo blight in mungbeans* provided insights into critical avirulence genes present among the pathogen population, and broad genomic changes delineated the population in two. Of the two avirulence (avr) genes identified in Australian *P. savastanoi* pv. *phaseolicola* isoaltes, all contained *avrPphE*, while two were missing *avrPphF*. Pathogenicity screening of 352 *P. savastanoi* pv. *phaseolicola* isolates against four *V. radaiata* host genotypes resulted in five pathotypes of which "pathotype 2" was found to infect all the tested mungbean genotypes and accounted for 84% of all isolates screened.

Characterization of Linkage Disequilibrium and Population Structure in a Mungbean Diversity Panel defined the linkage disequilibrium (LD) of wild and cultivated mungbean revealing cultivated mungbean has an LD twice as high as wild accessions. The high LD in cultivated mungbean has resulted from selection pressure during domestication, causing a tightly bound genome responsible for linkage drag that limits the effectiveness of conventional breeding approaches. The population structure further highlighted the conserved nature of the breeding germplasm with some genetic outliers that could be more readily used. Introducing a higher degree of diversity from wild species and genetic outliers will increase the resilience of inbreeding elite breeding material.

The three manuscripts presented here provide insights into the genetic diversity among the host and pathogens populations, how that genetic diversity affects host-pathogen interactions and methods to continue research in these areas. This study provides significant new knowledge to both the scientific community and industry to implement innovative approaches to manage current and emerging diseases in mungbeans.

Chapter 2: Diagnosis and management of halo blight in Australian mungbeans: a review

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

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Thomas Noble (Student Author) Date: 27/4/19	Conceived the project idea, compiled the relevant literature, assessed the how that literature adds value to the review article, critically appraised research articles and wrote the manuscript QUT Verified Signature
Anthony Young	Aided in conceiving the project and editing the manuscript QUT Verified Signature 27/4/19
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Sagadevan Mundree	Contributed to the project idea, Aided experimental design, reviewed the final manuscript

Principal Supervisor Confirmation

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2.1 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

2.2 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.

2.3 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 are round, water-soaked lesions surrounded by a chlorotic halo of greenish yellow (Fig. 1) (Taylor et al. 1979a). 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).

Epidemics of halo blight are facilitated by cool (18–23°C), wet and windy conditions, which facilitate bacterial dispersal and infection (Taylor et al. 1979b). One study showed that the bacteria could infect plants situated 26 m away (Walker and Patel 1964). Marques and Samson (2016b) 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.

2.4 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. 1979a). 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. 1979a). 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.

2.5 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. 1979b). 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 aefRNPS3121 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.

2.6 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 × 103 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.

2.7 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 2016a).

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. 1979b; 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 1967; Legard and Schwartz 1987; Niknejad Kazempour 2002; Marques and Samson 2016b). 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 2016b). 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).

2.8 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.

2.9 FIGURES

Figure 1: Chapter 2, 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.

2.10 TABLES

Table 1: Chapter 2, 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)
Phaseolous coccineus	Runner bean	Taylor *et al.* (1996)
Phaseolus lunatus	Lima bean	Sherf and MacNab (1986)
Phaseolus vulgaris	Common bean	Sherf and MacNab (1986)
Pueraria montana var. lobata	Kudzu vine	Fernández-Sanz *et al.* (2016)
Vigna angularis	Adzuki bean	Taylor *et al.* (1996)
Vigna radiata	Mungbean	Taylor *et al.* (1996)

Table 2: Chatper 2, 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	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	(A 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			
	P3004L	CTGTCTGGCAGCCACTACAAAG	GenBank acc no. AJ568001	240	(Rico et al., 2006)
	P3004R	GGCTGCAAATTGTGGGATTT			
Nested External	P5.1	AGCTTCTCCTCAAAACACC	Phaseolotoxin gene cluster	502	(N W 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 et al., 2011)
	Psy-cyoII-F	TCGAGCAGCGGAACCTGATC			
	Psy-cyoII-R	TGGGTACGCCCCAGACTGCGA			
	SSRP_F	GACGTCCCGCGAATAGCAATAATC	Site-specific recombinase gene	183	(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			

2.11 CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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Chapter 3: Characterisation of the *Pseudomonas savastanoi* pv. *phaseolicola* population found in Eastern Australia associated with halo blight disease in *Vigna Radiata*

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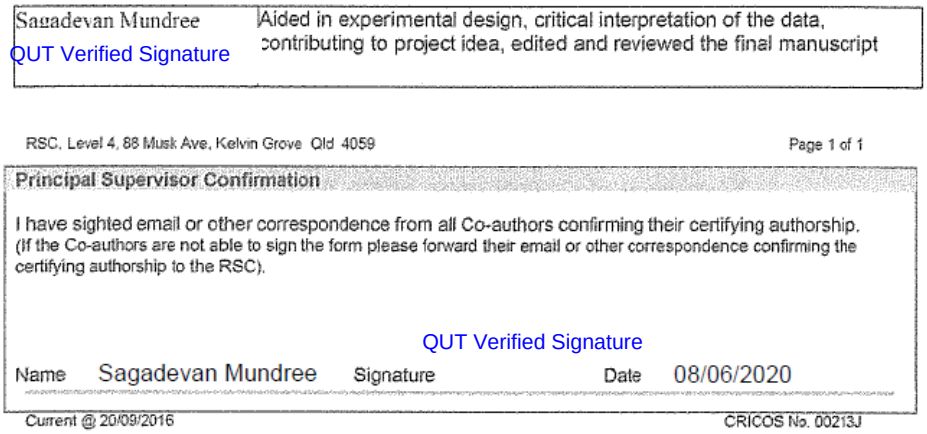

3.1 ABSTRACT

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

3.2 INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek var. *radiata*) is a grain legume that provides a vital source of nutrition for many countries and contributes significantly to Australian agricultural exports (Thomas J. Noble, Young, Douglas, Williams, & Mundree, 2019; Shanmugasundaram, Keatinge, & Hughes, 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 (Thomas J. 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, Teverson, Allen, and Pastor-Corrales (1996), 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, Lovell, Jackson, and Mansfield (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, Teverson, Allen, and Pastor Corrales (1996), was identified as well as variants that did not coincide with the race structure reported for common bean (Ryley et al., 2010; Taylor, Teverson, Allen, & Pastor Corrales, 1996).

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 linages. 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.

3.3 MATERIALS AND METHODS

3.3.1 *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, Ward, & Raney, 1954). The plates were incubated at 28°C for 24 hrs. 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.

3.3.2 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^{TM}) in seedling trays consisting of 42 wells 4 cm^3 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 ºC 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 ºC for 72 hr. 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 hrs 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.

3.3.3 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, Koeuth, & Lupski, 1991), BOX (Versalovic, Schneider, Bruijn, & Lupski, 1994), IS50 (Sundin, Demezas, & Bender, 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 ~$1x10^6$ 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.

3.3.4 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, Mezulis, Yates, Wass, & Sternberg, 2015).

3.3.5 Whole-genome comparison of two epidemiological significant P. savastanoi pv. phaseolicola epidemiological significant 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, Saveliev, Vyahhi, & Tesler, 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, Mau, Blattner, & Perna, 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, Ha, Lim, Kwon, and Chun (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.

3.4 RESULTS

3.4.1 Host-pathogen interactions reveals 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.

3.4.2 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 & 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 2005-2016 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 T115544 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.

3.4.3 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, Jenner, Hockenhull, Bennett, & Stewart, 1994; Nimchuk, Fisher, Desveaux, Chang, & Dangl, 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).

3.4.4 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, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 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 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).

3.5 DISCCUSION

Known for over 90 years, halo blight disease of the Fabaceae family continues to threaten food production globally (Arnold et al., 2011; Burkholder, 1926; Thomas J. Noble et al., 2019). However, limited research has explicitly focused on the interactions between the causal agent *P. savastanoi* pv. *phaseolicola* and *Vigna radiata* (Thomas J. 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, Teverson, Allen, & Pastor-Corrales, 1996). 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 virulence genes are injected into the host via the type three secretion systems (T3SS) found in *P. savastanoi* pv. *phaseolicola*, catalysing the infection process or inducing a hypersensitive response if a matching R gene is present (Alfano & Collmer, 1997). Isolated and sequenced from race 4, strain 1302A of *P. savastanoi* pv. *phaseolicola*, a*vrPphE* matches the R2 gene for resistance in *Phaseolus* cultivars (Mansfield et al., 1994). Family members of a*vrPphE* 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 a*vrPphF* 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 a*vrPphF* is located (Tsiamis et al., 2000). This was not the case for isolate T13733B which was missing a*vrPphF* but retained its virulence against all four of the genotypes screened in this study. This suggests that other 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, Galyov, Rosqvist, & Wolf-Watz, 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 & 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 a*vrPphF* 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 a*vrPphF*, it would be expected that strains carrying those genes would be suppressed (Arnold et al., 2011; Taylor, Teverson, Allen, & Pastor-Corrales, 1996). 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; T. J. 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).

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.

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3.7 CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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3.9 TABLES

Table 3: Chapter 3, Table 1. Race variation among 352 *Pseudomonas savastanoi* pv. *phaseolicola* isolated from *Vigna radiata.* Isolates were inoculated on four *Vigna radiata* genotypes with known variation in resistance to halo blight disease (+ = inoculated plants displayed symptoms; - = no symptoms).

Genotype	**1**	**2**	**3**	**4**	**5**
AusTRC 321818	-	+	-	-	+
M773	-	+	-	+	-
OAEM58-62	-	+	+	-	-
Crystal	+	+	+	+	+
Totals	34	294	5	5	14
Frequency	10%	84%	1%	1%	4%

Table 4: Chapter 3, Table 2. Comparison of the general genomic features of *P. savastanoi* pv. p*haseolicola* strains T11544 and K4287

Feature	***P. savastanoi* pv. *phaseolicola* T11544**	***P. savastanoi* pv. *phaseolicola* 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	5,912	5,862
Annotated ORFs	4,539	4,513
Hypothetical protein	1,373	1,349
tRNAs	159	159
RNA genes	12	12

Table 5: Chapter 3, Table 3. Unique genes encoded in T11544 and K4287 scaffolds not found in the respective genome. Assembled genomic scaffolds (>= 1kbp) were subjected to QUAST sequence comparison to idenify unaligned contigs unique to the T11544 and K4287 genome. Annotation of genomic scaffolds was performed using the RASTtk pipeline. Hypothetical proteins were not included

Scaffold ID	Start	End	Total Length	Annotation Description
K4287_000077	18681	20180	1499	Mobile element protein
K4287_000077	18106	18624	518	GCN5-related N-acetyltransferase
K4287_000078	20269	20604	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	17608	19710	2102	Signal_transduction_histidine_kinase_CheA
T11544_000074	15922	17577	1655	Methyl-accepting_chemotaxis_sensor/transducer_protein
T11544_000074	10041	11657	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	21905	23191	1286	NADH_dehydrogenase_(EC_1.6.99.3)
T11544_000074	20449	21666	1217	Methyl-accepting_chemotaxis_protein
T11544_000074	12944	14020	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	14513	15322	809	Chemotaxis_protein_methyltransferase_CheR_(EC_2.1.1.80)
T11544_000074	15332	15871	539	Positive_regulator_of_CheA_protein_activity_(CheW)
T11544_000074	13983	14516	533	Chemotaxis_protein_CheD

T11544_000074	23409	23942	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

3.10 FIGURES

Figure 2: Chapter 3, 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.

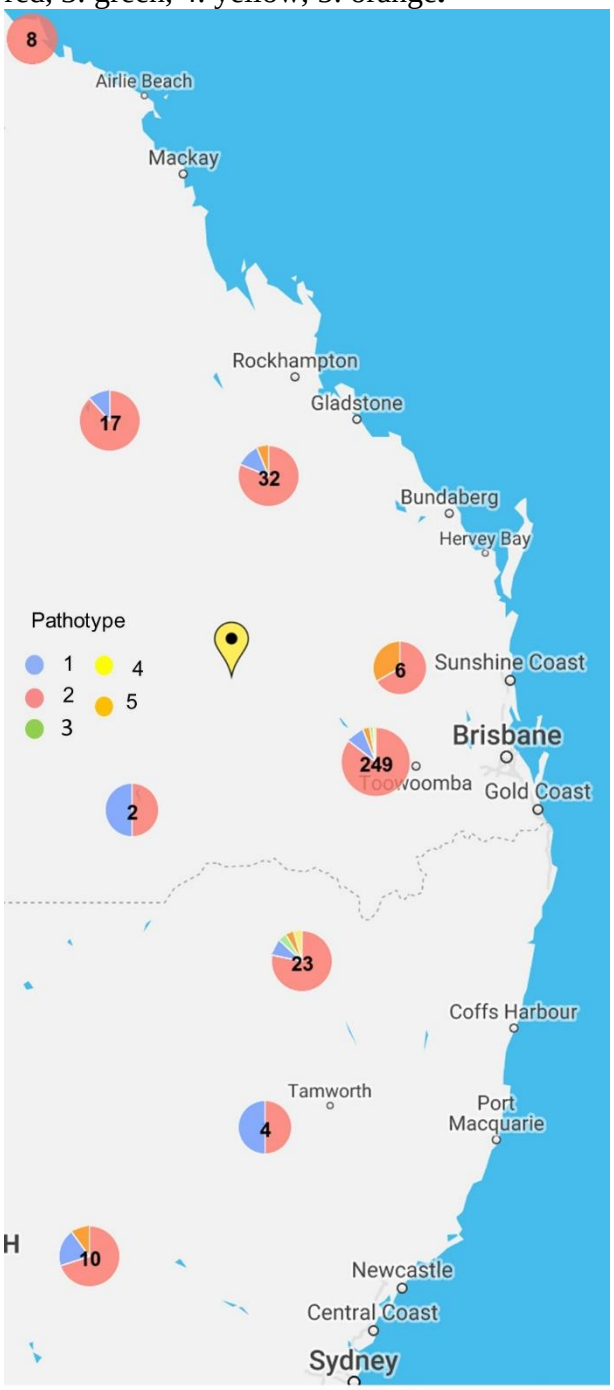

Fig. 1. Clustering and distribution of 352 *P. savastanoi* pv. *phaseolicola* isolates based on location isolated and assigned pathotype. 1: blue, 2: red, 3: green, 4: yellow, 5: orange.

Figure 3: Chapter 3, 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 profile.

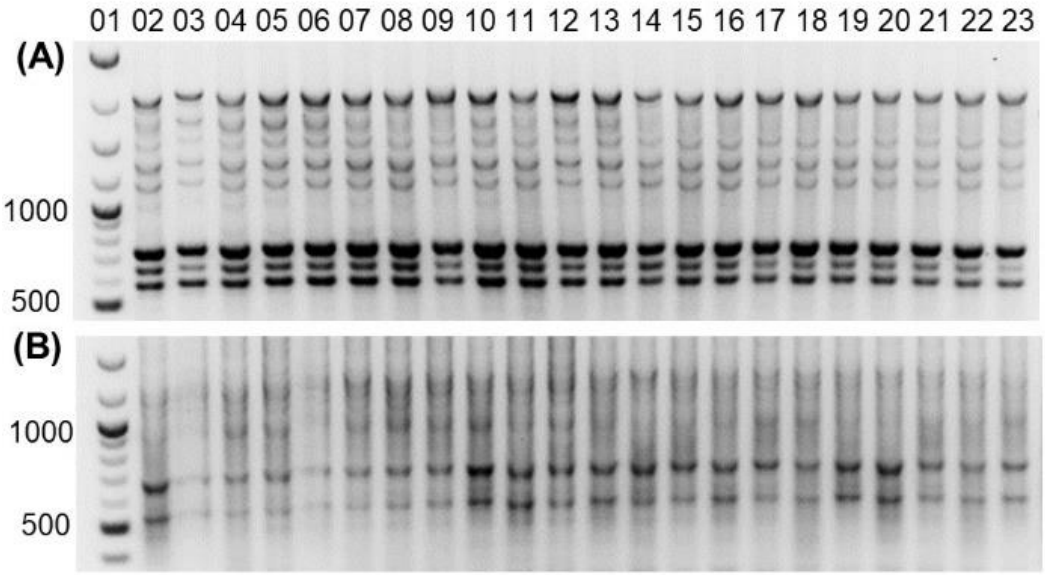

Fig. 2. Examples of DNA fingerprints from *P. savastanoi* pv. *Phaseolicola* (PSP) using (A) ERIC and (B) BOX A1R, PCR primers. Lane 01: 2-Log DNA Ladder (0.1 – 10.0 kb) (New England Biolabs); Lanes 02-23: PSP001-PSP022. Isolates were not distinguishable based on their ERIC/BOX A1R profiles.

Figure 4: Chapter 3, 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.

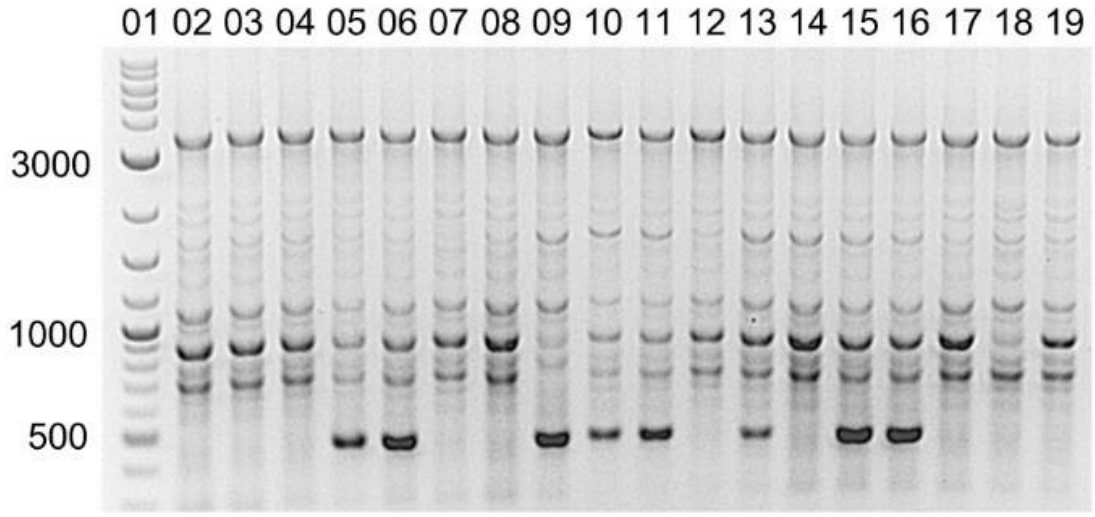

Fig. 3. Examples of DNA fingerprints from *P. savastanoi* pv. *phaseolicola* using IS50 PCR primers. Lane 01: 2-Log DNA Ladder (0.1 – 10.0 kb) (New England Biolabs); Lanes 02: T11544; 03: T14006; 04: K4287; 05: T14031; 06: T13913; 07: T13976; 08: T13994; 09: T13733B; 10: T13804A; 11: T13805H; 12: T13992; 13: T13754B; 14: T13734A; 15: T13804F ; 16: T13982; 17: T14007; 18: T13636C; 19: T13981.

Figure 5: Chapter 3, 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.

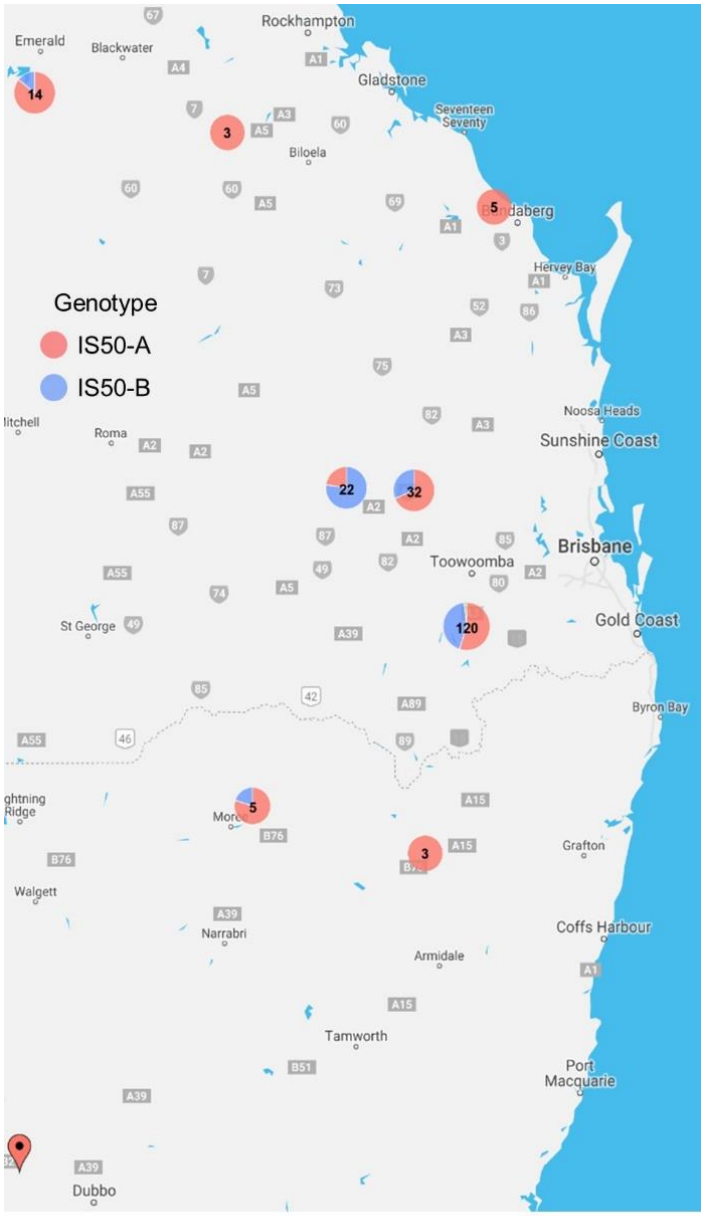

Fig. 4. A total of 205 *P. savastanoi* pv. *phaseolicola* isolates were geotagged and screened with IS50 PCR primers, dividing the population into two broad genotypes. The panel is a map of mungbean growing districts in Australia highlighting the distribution of the isolates throughout Southern and Central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

Figure 6: Chapter 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.

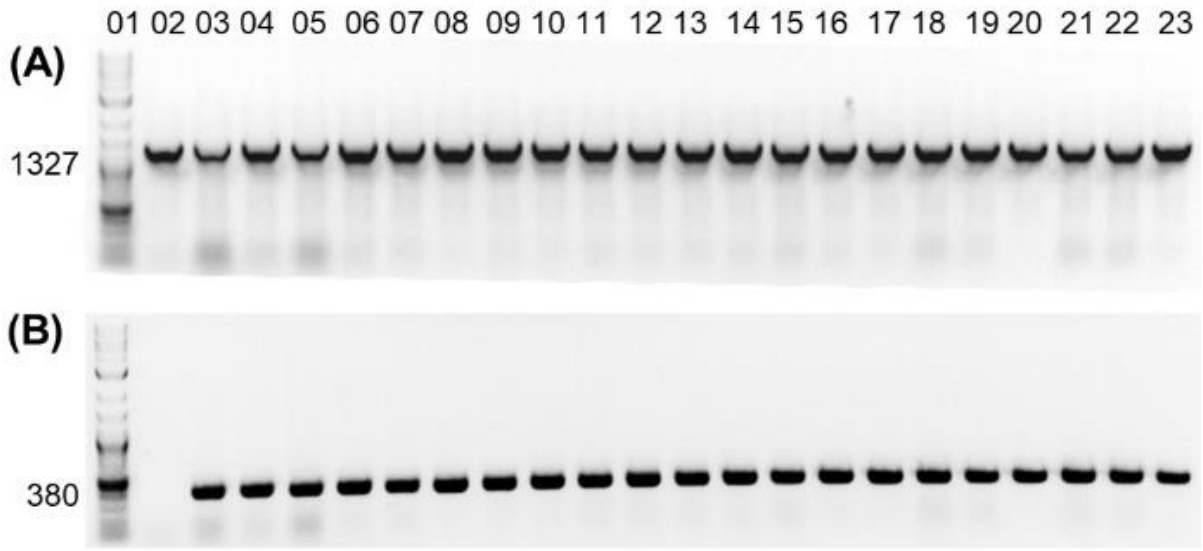

Fig. 5. Gel image showing the amplification of *P. savastanoi pv. phaseolicola* avirulence genes (A) avrPphE and (B) avrPphF. Lane 01: 2-Log DNA Ladder (0.1 – 10.0 kb) (New England Biolabs); Lane 02: PSP023; Lanes 03-23: PSP066-P086. Note that avrPphF was not present in lane 02, isolate PSP023.

3.11 SUPPORTING INFORMATION (APPENDIX A)

Table S1. Metadata and analysis results of 511 isolates of *Pseudomonas savastano*i pv. p*haseolicola* isolated from Australian mungbean leaf tissue.

Table S2 List of PCR primers used in this study

Table S3. Average nucleotide identity (ANI) between the K4287 and T11544 genome assemblies

Table S4. General features of the T11544 and K4287, *P. savastanoi* pv. phaseolicola genomes separated by chromosome and plasmid

Table S5. Type III secretion proteins in K4287 and T11544 genome assemblies. Genomic scaffolds were annotated using RAST and secreted Type III proteins were identified in each genome. Annotation as well as nucleotide and protein sequences are provided.

Chapter 4: Characterization of Linkage Disequilibrium and Population Structure in a Mungbean Diversity Panel

Characterization of linkage disequilibrium and population structure in a mungbean diversity panel

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Thomas Noble (Student Author) Signature: Date: 24/4/19	Conceived the project idea and experimental design, conducted laboratory experiments, analysed and interpreted results and wrote the manuscript QUT Verified Signature
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4.1 ABSTRACT

Mungbean [Vigna radiata (L.) R. Wilczek var. radiata] is an important grain legume globally, providing a high-quality plant protein source largely produced and consumed in South and East Asia. This study aimed to characterize a mungbean diversity panel consisting of 466 cultivated accessions and demonstrate its utility by conducting a pilot genome-wide association study of seed coat color. In addition 16 wild accessions were genotyped for comparison and in total over 22,000 polymorphic genome-wide SNPs were identified and used to analyze the genetic diversity, population structure, linkage disequilibrium (LD) of mungbean. Polymorphism was lower in the cultivated accessions in comparison to the wild accessions, with average polymorphism information content values 0.174, versus 0.305 in wild mungbean. LD decayed in ~100 kb in cultivated lines, a distance higher than the linkage decay of ~60 kb estimated in wild mungbean. Four distinct subgroups were identified within the cultivated lines, which broadly corresponded to geographic origin and seed characteristics. In a pilot genome-wide association mapping study of seed coat color, five genomic regions associated were identified, two of which were close to seed coat color genes in other species. This mungbean diversity panel constitutes a valuable resource for genetic dissection of important agronomical traits to accelerate mungbean breeding.

4.2 INTRODUCTION

Mungbean [Vigna radiata (L.) R. Wilczek var. radiata] is a grain legume originating from South Asia. It belongs to subgenus Ceratotropis of the Fabaceae family which comprises 23 species including the closely related wild mungbean [Vigna radiata var. sublobata (Roxb) Verdcourt]. As a summer legume species with a short growth duration (55–70 days from sowing to maturity) and the ability to fix atmospheric nitrogen, mungbean delivers economic, farming systems, and environmental benefits. The crop is vital to smallholder farmers in Asia with an annual production of 3.5–4.0 million tons where it represents an excellent cheap source of carbohydrates, high quality protein (dry seeds 27% protein), folate and iron (Day, 2013). Over the last three decades, the global consumption of mungbean has increased by 60% with a corresponding growth in production area up to 6 million hectares,

concentrated mainly in South, East, and Southeast Asia (Kim et al., 2015). As an orphan crop of subsistence agriculture with limited genetic information available, mungbean improvement has relied on traditional plant breeding methodologies for most of its cultivated history (Fernandez et al., 1988; Humphry et al., 2002). In fact investment to-date in the development of new mungbean varieties has been low, resulting in a narrow genetic base of the crop leaving the crop vulnerable to many abiotic and biotic stresses. Additionally, progress in developing genomics tools to support molecular breeding activities for developing improved mungbean varieties has been very limited. However, the recent release of a reference genome for mungbean (Kang et al., 2014) provides new opportunities for mungbean genomic research (Kim et al., 2015).

Mungbean is a self-pollinated diploid species with a chromosome number of 2n = 22 with an estimated genome size of 543 mega bases (Mb) (Parida et al., 1990; Kang et al., 2014). The relatively small genome size makes it an attractive and valuable model for advancing the understanding of diversity and evolution of legume genomes. The domestication of mungbean is considered to have taken place approximately 3500 years ago (Kim et al., 2015) from a small number of elite founders. The domestication process has resulted in significant genetic bottlenecks in the cultivated mungbean genome (Lakhanpaul et al., 2000). Previous genetic diversity studies of cultivated and wild mungbean germplasm, using both morphological and molecular markers, have highlighted low levels of genetic diversity in cultivated mungbean compared to the broader diversity found in wild mungbean (Santalla et al., 1998; Saravanakumar et al., 2004; Sangiri et al., 2007).

Wild mungbean relatives are of critical importance to broaden the genetic diversity of cultivated mungbean given the important biotic and abiotic stress resistance genes they may contain, which have not yet been incorporated into cultivated material by breeding programs.

In fact the use of wild relatives to increase productivity has been successfully demonstrated in many other crops including sorghum (Jordan et al., 2011) and barley

(Ellis et al., 2000). Previous studies of genetic variation in wild mungbean germplasm have reported valuable genes affecting important agronomical traits such as phenology (Rebetzke and Lawn, 2006a); growth, biomass, and seed yield (Rebetzke and Lawn, 2006b); root and shoot attributes (Rebetzke and Lawn, 2006c). These genes could be introgressed into cultivated mungbeans to increase the resilience of cultivated material and broaden the overall genetic base (Pandiyan et al., 2008). Introgression of traits from closely related wild relatives into cultivated mungbean has been reported to produce fertile progeny with favorable morphological characteristics (Lambrides et al., 1999). By contrast, Vigna mungo [V. mungo (L.) Hepper], or black gram, produces sterile progeny when hybridized to mungbean (Lambrides et al., 1999).

Dissecting the genetic basis of important agronomic traits, such as seed coat color, grain size, flowering time and disease resistance, are critical to manipulating and introgressing these traits precisely to achieve breeding goals. Traditionally, linkage mapping is the primary tool to identify genetic loci underlying traits of interest. A limited number of genetic linkage maps have been developed in mungbean (Lambrides et al., 2000; Humphry et al., 2002; Isemura et al., 2012), however, despite great efforts a comprehensive and saturated genetic linkage map of all 11 chromosomes has not been generated (Kim et al., 2015). Instead, high density maps developed from whole genome sequences (Kang et al., 2014) enable further advancement in alternative approaches to trait dissection, such as association mapping, also known as linkage disequilibrium (LD) mapping (Gupta et al., 2005; Abdurakhmonov and Abdukarimov, 2008). LD mapping takes advantage of historical recombination events in a diverse set of lines to identify the genetic basis of traits at a higher resolution than traditional genetic linkage mapping. The resolution of association mapping relies upon the extent of LD. The degree of LD has yet to be accurately determined in mungbean. In cultivated material of the closely related species soybean, high levels of LD have been reported (~150 kb), potenitally caused by inbreeding, adaptation and human selection (Lam et al., 2010). By contrast LD in wild soybean species was considerably lower (~75 kb), indicating wild germplasm could play a major role in influencing the dynamics of genome change.

This study aims to assess the genetic diversity, population structure, LD and mapping capabilities of a large and diverse mungbean germplasm panel using a high-

throughput SNP genotyping platform. This study provides a unique genomic resource for genetic dissection of important agronomical traits to accelerate mungbean breeding.

4.3 MATERIALS AND METHODS

4.3.1 Plant Materials

The mungbean diversity panel consists of 466 accessions representing the cultivated mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*] germplasm held in Australia by the National Mungbean Improvement Program (Queensland, DAF). The material originated from various sources worldwide and represents the widest range of phenotypic traits observed and characterized by the mungbean breeding team over the past 50 years. These included seed coat color, seed size and weight, days to flower, days to maturity, plant habit, plant height, and reaction to key foliar diseases. Sixteen wild accessions [*Vigna radiata* var. *sublobata* (Roxb Verdcourt)] originating from Australia were also included in the study as a comparison to the diversty panel of cultivated mungbeans.

4.3.2 Genotyping

Total genomic DNA was extracted from bulked young leaves of a single plant per accession as described by DArT P/L (DArT[1]). The samples were genotyped following an integrated DArT and genotyping-by-sequencing (GBS) methodology involving complexity reduction of the genomic DNA to remove repetitive sequences using methylation sensitive restrictive enzymes prior to sequencing on next generation sequencing platforms (DArT[2]). The sequence data generated were then aligned to the mungbean reference genome sequence, Vradi_ver6 (Kang et al., 2014), to identify single nucleotide polymorphisms (SNPs) markers.

4.3.3 Phenotypic Data Collection

All 482 mungbean accessions were planted at Hermitage Research Facility, Warwick, QLD, Australia (28°12′ S, 152°5′ E), over the summer of 2015. The field trial design was unreplicated single field plots for each accession, 4.5 m2 in size containing an average of 130 plants. Seed coat color was qualitatively recorded based on five categories (green, black, brown, yellow, and speckled).

4.3.4 Analysis of Genetic Diversity

The polymorphism information content (PIC) of each DArTseq SNP marker was determined using the following formula: PIC = 1-Σ(Pi2)/n where Pi is the frequency of the i th allele and n is the total number of genotypes (Weir, 1990).

4.3.5 Estimation of Linkage Disequilibrium

The pairwise LD between SNPs genome-wide across the wild and cultivated mungbean genotypes was calculated based on the allele frequency correlations (r2) using the TASSEL program (v5.1.0) (Bradbury et al., 2007; Tao et al., 2013). Only r2 for SNPs with pairwise distance less than 500 kb of each chromosome were used to draw the average LD decay figure. The LD decay graph was drawn by fitting a smooth spline of averaged r2 over physical distance in R v3.3.1. The LD decay was calculated when the squared correlations of allele frequencies r2 decreased to half of its maximum value.

4.3.6 Analysis of Population Structure

Population structure of the 466 cultivated accessions was analyzed using a Structure-like Population Genetic Analyses in R package LEA (Falush et al., 2007). The number of subpopulations is determined using a cross-entropy criterion. The cross-entropy criterion is based on the prediction of a fraction of masked genotypes (matrix completion), and on the cross-validation approach. Smaller values of the cross-entropy criterion usually mean better runs. We perform runs for 9 values of K (K = 2:10), and choose the value of K for which the cross-entropy curve exhibits a plateau (K = 4).

Results of structure analysis were used to identify accessions representing the four sub-populations. An individual accession with more than 90% identity from a single sub-population was classified as representative of that sub-population. Genetic differentiation (Fixation index, FST) among the four sub-populations was calculated using R package PopGenome (Pfeifer et al., 2014).

Genetic relationships among cultivated accessions were also analyzed using principal coordinate analysis (PCA) performed in the software package DARwin v6.0 (Perrier and Jacquemoud-Collet, 2006).

4.3.7 Genome-Wide Association Mapping of Seed Coat Color

Association mapping was conducted using mixed linear model (MLM) controlling for Q and kinship (K) as fixed and random effects respectively in TASSEL 5.1.0. Minor allele frequency (MAF) > 0.01 was used to filter SNPs prior to analysis. Q was extracted from results of previous population structure analysis, which detected four sub-populations. K was calculated using Scaled IBS method implemented in TASSEL 5.1.0. Bonferroni correction was applied to set thresholds for P-value significant (0.05/n); n represented the number of SNPs used in trait-marker association analysis. The Manhattan plot was generated using the R package qqman (Turner, 2014).

4.4 RESULTS

4.4.1 Germplasm Diversity Analysis

A total of 22,230 SNP markers were identified in the cultivated and wild mungbean populations, of those 16,462 were physically mapped across the 11 mungbean chromosomes (Supplementary Table S1). An average of 1,497 SNPs were identified per chromosome (from 903 SNPs on chromosome 3 to 2,306 on chromosome 7) with an average marker density of 57.81 SNPs/Mb (Table 1 and Figure 1). A total of 7,675 SNPs segregated within the cultivated population, with an average PIC value of 0.174. In contrast, 6,174 SNPs segregated within the wild population with an average PIC value of 0.305 (Supplementary Figure S1).

4.4.2 Estimation of Linkage Disequilibrium

Linkage disequilibrium was estimated between all SNP markers over the 466 cultivated and 16 wild mungbean accessions. The squared correlations of allele frequencies r2 of the cultivated mungbean population decreased to half of its maximum value at approximately 100 kb physical distance compared to the wild mungbean population which had largely decayed by 60 kb (Figure 2). Additionally, individual chromosomes of the cultivated population were estimated, no substantial differences were observed (Supplementary Figure S2).

4.4.3 Population Structure of the Cultivated Population

Structure-like population genetic analysis was used to analyze the structure of the cultivated population (Figure 3). A range of sub-populations (K = 2:10) were tested and a K-value of 4 was determined to best capture the structure of the cultivated population based on minimal cross-entropy (Supplementary Figure S3). Representative accessions from the four sub-populations defined as being > 90% relationship to a single sub-population were further characterized using phenotypic data (Supplementary Table S2).

Sub-population 1 was made up of 25 accessions, there was limited passport data as the majority of lines are Australian commercial lines (Crystal, Jade-AU) or Australian breeding lines (e.g., M11122). Those accessions with passport data were mostly from Southern Asia, Taiwan, Thailand, Philippines, and Vietnam with uniformly green seed coats.

Sub-population 2 was comprised of 40 accessions and had the widest range of passport data, with the accessions predominantly from Middle Eastern countries including Afghanistan, Uzbekistan, Tajikistan, Kyrgyzstan and Iran, with single accessions from China, India, Netherlands, and Turkey. This sub-population also had the widest range of seed coat colors, although largely green with a number of speckled, a single yellow accession and additionally this was the only sub-population to contain brown seed coats.

Sub-population 3 contained the largest number of accessions (59) mainly derived from Southern Asian countries, Cambodia, Philippines and Thailand with single accessions from China, Hong Kong, India, Indonesia, and Vietnam. This sub-population also contained the highest number of accessions from Iran (6). Of the 59 accessions, 19 displayed yellow seed coats while the remaining accessions were green in color with two speckled seed coats.

Sub-population 4 contained the lowest number of accessions (22), and the majority of the accessions did not have passport data; those that did were from India and Pakistan with a single line from Taiwan. Seed coat colors were primarily green with three accessions displaying speckled seed coats.

Principal coordinate analysis was also used to visualize the relationships amongst the cultivated accessions in the panel. When the four sub-populations were plotted they clustered toward the extremities of the plot based on their genetic differences (Figure 4). The first two principal coordinates accounted for approximately 34.04% of the genotypic variance with coordinates one (x-axis) and two (y-axis) explaining 18.18 and 15.86%, respectively.

4.4.4 Genetic Diversity between Sub-populations

The genome-wide genetic differentiation between the four contrasting mungbean sub-populations identified in structure were calculated using fixation index (FST) using PopGenome. Sub-populations 1 and 3 were the most closely related with an overall FST value of 0.42, while sub-populations 1 and 2 show the highest degree of differentiation, with an FST value of 0.57 (Table 2). Sub-population 1 had uniform green seed coats, in contrast to sub-population 2 which had a wide variation of seed coat colors. Sub-populations 1 and 3 however, shared similar characteristics with many accessions originating from Southern Asia and uniform seed color.

4.4.5 Genome-Wide Association Study of Seed Coat Color QTL

TASSEL v5.1.0 was used to conduct a pilot genome-wide association mapping (GWAS) for seed coat color to demonstrate the effectiveness of the diversity panel for trait dissection. Seed coat color was chosen because it's an oligogenic trait which does not change based on the environment the plant is grown and is economically important to the mungbean industry. Using the MLM model, nine SNPs were identified as significantly associated with seed color based on P-value < 9.66E-06 (Bonferroni correction). These SNPs were located in five distinct genomic regions distributed across chromosomes 3, 4, 5, and 7 (Supplementary Figure S5 and Supplementary Table S3). Five significant SNPs, including the most highly significant SNP overall, clustered within an interval of 22 kb on chromosome 4 between positions 17,668,384 and 17,690,573. Identified within this region VrMYB113 is the homolog of the Arabidopsis gene MYB113 involved in anthocyanin biosynthesis (Gonzalez et al., 2008). Vrsf3′h1 is the homolog of a previously identified gene (sf3′h1) controlling seed coat color through flavonoid 3′-hydroxylase in soybean (Toda et al., 2012) associated with a significant SNP on chromosome 5 at position 32413093. The most significant marker (6160927|F| 0–16:A > G-16:A > G) on chromosome 4 position

17679978 had a 97% association with lighter colored seed coats (green/yellow) on allele “1,” while allele “0” had a 91% association with darker pigmentation (black/speckled) (Supplementary Table S4).

4.5 DISCUSSION

The replacement of traditional and local crops with elite varieties harboring superior agronomic traits has led to increased yields but also the gradual erosion of genetic diversity of cultivated varieties. Globally, plant breeders are addressing the threat to cultivated crops with narrow genetic diversity by establishing large collections of genetically diverse germplasm. In this study, we investigated the application of genome-wide SNP markers to analyze the genetic diversity of a large, diverse mungbean germplasm panel. The markers generated from this germplasm panel provide a new resource to conduct high-resolution analysis of genetic diversity, population structure, LD and the capacity to identify genes controlling important agronomic traits. Additionally, this is the first high-resolution quantification of LD decay in mungbeans, defining the extent of LD within and between cultivated and wild mungbeans. The genetic diversity analysis, population structure and LD analysis provide the foundation that can be used to broaden the genetic base of mungbean breeding material. The genome-wide association study provides an example of how the data can be used to identify genomic regions responsible for phenotypic traits. SNP markers provide a level of resolution to breeding programs far beyond traditionally used methods which relied solely on passport data such as geographical origin and pedigrees (Brown, 1989) or genetic markers and quantitative phenotypic data to conduct cluster analysis of core collections (Bretting and Widrlechner, 1995). Developing effective, informed strategies for the utilization and management of germplasm collections is critical to mine the ready available diversity in mungbean (Fernandez et al., 1988). Application of this resource as a breeding tool has the potential to transform the improvement of this globally significant yet orphan crop through informed trait introgression from exotic germplasm and wild species using marker assisted breeding.

4.5.1 Genetic Diversity of Cultivated Mungbean

The domestication syndrome, resulting from restricting the number of elite varieties used in breeding under selection for key traits such as larger grain size, has

come at the expense of diminishing overall genetic diversity (Gepts, 2010). Low genetic diversity is impeding the rate of genetic gain that can be made in breeding programs through limiting the number of alleles available in breeding populations, severely restricting the ability of breeders to continue developing improved resistance and tolerance to biotic and abiotic stressors. Broadening the genetic base of mungbean and incorporating exotic alleles is essential to developing agile breeding programs that can respond to new threats and challenges faced by the crop (Nair et al., 2012). Over the last decade, limited research has been conducted to investigate the degree of genetic diversity within and between cultivated and wild mungbean. The first large-scale analysis of cultivated and wild mungbean germplasm using molecular markers was conducted by Sangiri et al. (2007) who analyzed the domestication of mungbean. Our study represents the first extensive mungbean germplasm diversity analysis using high-density SNP markers.

Highly informative PIC values as defined by Botstein et al. (1980) are values equal to or greater than 0.5 when considering multi-allelic markers whose values range from 0 to 1. PIC values for bi-allelic SNP markers range from 0 to 0.5, therefore we have regarded PIC values greater than or equal to 0.25 as highly informative. Within the cultivated population 34% of the SNPs had a PIC value greater than or equal to 0.25 compared to the wild population, which had 56%. The high PIC value derived from the wild population is consistent with our expectations that we would see a greater proportion of highly polymorphic markers in the wild population due to the selective breeding seen in the cultivated population.

The clear differentiation between cultivated and wild mungbean gene pools, as demonstrated in the PCA analysis (Supplementary Figure S4), was also reported by Sangiri et al. (2007), who noted close to double the gene diversity in the wild population in comparison to cultivated mungbeans and clear genetic differentiation among the cultivated accessions. Sangiri et al. (2007) observed that approximately 50% of the genetic variation present in the wild mungbean gene pool was found in the cultivated germplasm. In contrast, other cultivated cereals have been observed to contain only ∼30% of the genetic diversity present in wild species, indicating that

mungbeans have experienced a weaker genetic bottle neck than that of the cereals, reflecting the difference in the domestication process of legumes compared with cereals (Buckler et al., 2001; Zhu et al., 2007).

4.5.2 LD in Mungbean Is Comparable to Closely Related Species

The number and density of markers required for an association mapping analysis is determined by the distance over which LD decays. The LD patterns of mungbean reflect its long history of domestication (Fuller, 2007) and as a self-pollinated species the extent of genome-wide recombination is expected to be less than that observed in cross pollinated species (Flint-Garcia et al., 2003). The r2 of cultivated mungbean decreased to half its maximum value at ∼100 kb compared to that of the wild species which decreased by ∼60 kb (Figure 2). Wild mungbean has retained a higher degree of allelic diversity providing an important source of material for increasing the genetic diversity of the cultivated gene pool.

Studies in closely related species report that LD largely decays by ∼150 kb in cultivated soybean; ∼75 kb in wild soybean (Lam et al., 2010) and in chickpea between 450 and 550 kb (Bajaj et al., 2015). In contrast, in Medicago truncatula, a leguminous plant used in genomic studies the LD decayed rapidly, dropping too approximately half of its initial value within ∼3 kb (Branca et al., 2011) similar to the model speices Arabidopsis thaliana: 3–4 kb (Kim et al., 2007). Cultivated and wild rice reported the highest variation between the highly cultivated varieties O. japonica at ∼200 kb and O. indica ∼65 kb in contrast to wild varieties O. rufipogon and O. nivara which exhibit rapid decay by ∼10 kb (Xu et al., 2011), similar to sorghum: improved inbreds ∼19.7 kb and land races ∼10.3 kb (Mace et al., 2013). These studies show the major influence domestication has had on changing genome dynamics and combined with PIC data, highlights the importance wild accessions and genetically diverse populations will have on providing access to new exotic alleles.

4.5.3 High-Resolution Association Mapping Pilot Study

QTL (quantitative trait loci) are identified through the establishment of association between genomic marker data and traits of interest. Previous studies revealed the genetic factors controlling important agronomic traits in mungbean using

biparental populations such as, major QTLs for hard-seededness and seed weight (Fatokun et al., 1992; Maughan et al., 1996; Humphry et al., 2005); powdery mildew resistance (Young et al., 1993; Chaitieng et al., 2002; Humphry et al., 2003; Kasettranan et al., 2010); yellow mosaic Indian virus resistance (Chen et al., 2012; Kitsanachandee et al., 2013); bruchid resistance (Young et al., 1992; Mei et al., 2009); Cercospora leaf spot resistance (Chankaew et al., 2011). Due to the limited number of recombination events (Balasubramanian et al., 2009), QTLs were commonly mapped to wide-ranging intervals extending over a number of centiMorgans (cM), usually megabases (Mb) long in physical distance, potentially containing 100s of candidate genes. Thus, the understanding of specific genes controlling agronomic traits is still restricted and further fine-mapping is often required to identify the underlying genes involved.

The structure of our population, which captured vast historical recombination accumulated during mungbean cultivation, combined with the high density of markers genome-wide overcome barriers seen in previous studies. The GWAS performed in this study (Figure 5) is an example of how the features of this data set can provide high resolution mapping opportunities. A Nested Association Mapping (NAM) population in mungbean, currently being developed by the Queensland mungbean improvement team, will provide a powerful complementary population to the diversity panel described here. The structure of the mungbean NAM population encompasses 26 of the most genetically and phenotypically diverse accessions, backcrossed to one elite line adapted to the local production (Supplementary Figure S5). environment. Robust precise phenotyping and genotying of the NAM population will push mungbean breeding years ahead of its time accelerating genome research and molecular breeding.

4.6 FIGURES

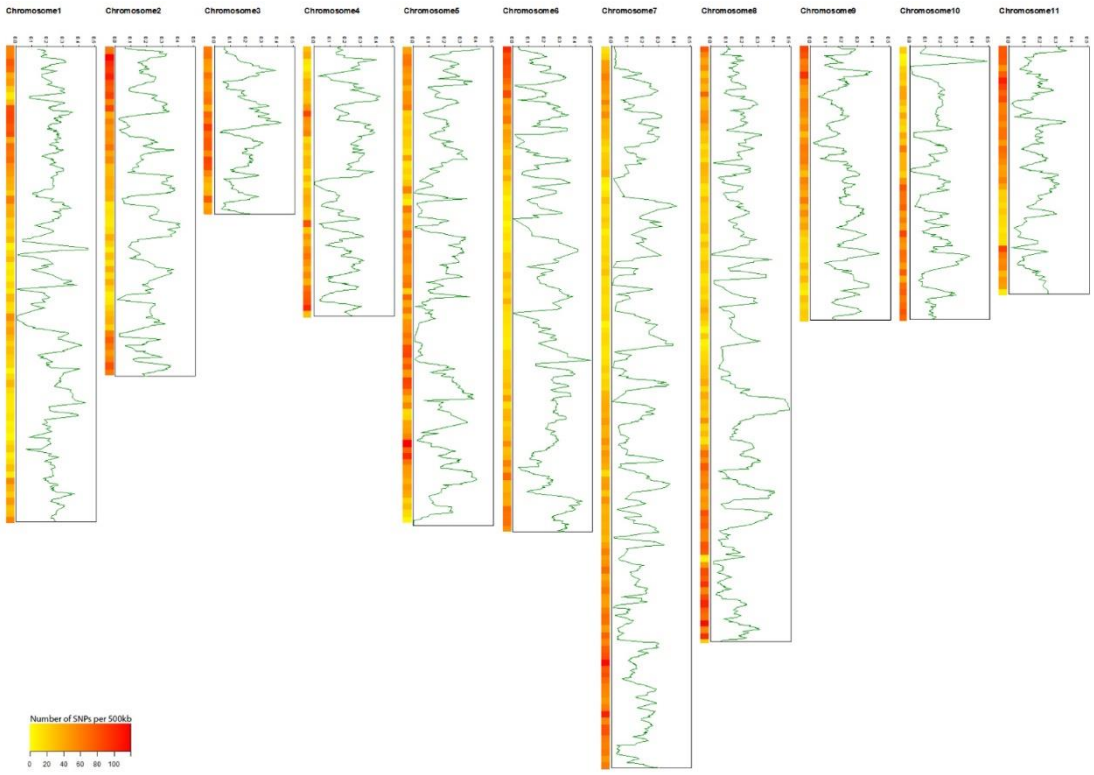

Figure 7: Chapter 4, FIGURE 1. The distribution of polymorphism information content (PIC) genome-wide of 466 cultivated mungbean accessions. Heat maps on each chromosome represent SNP densities within a window of 500 kb.

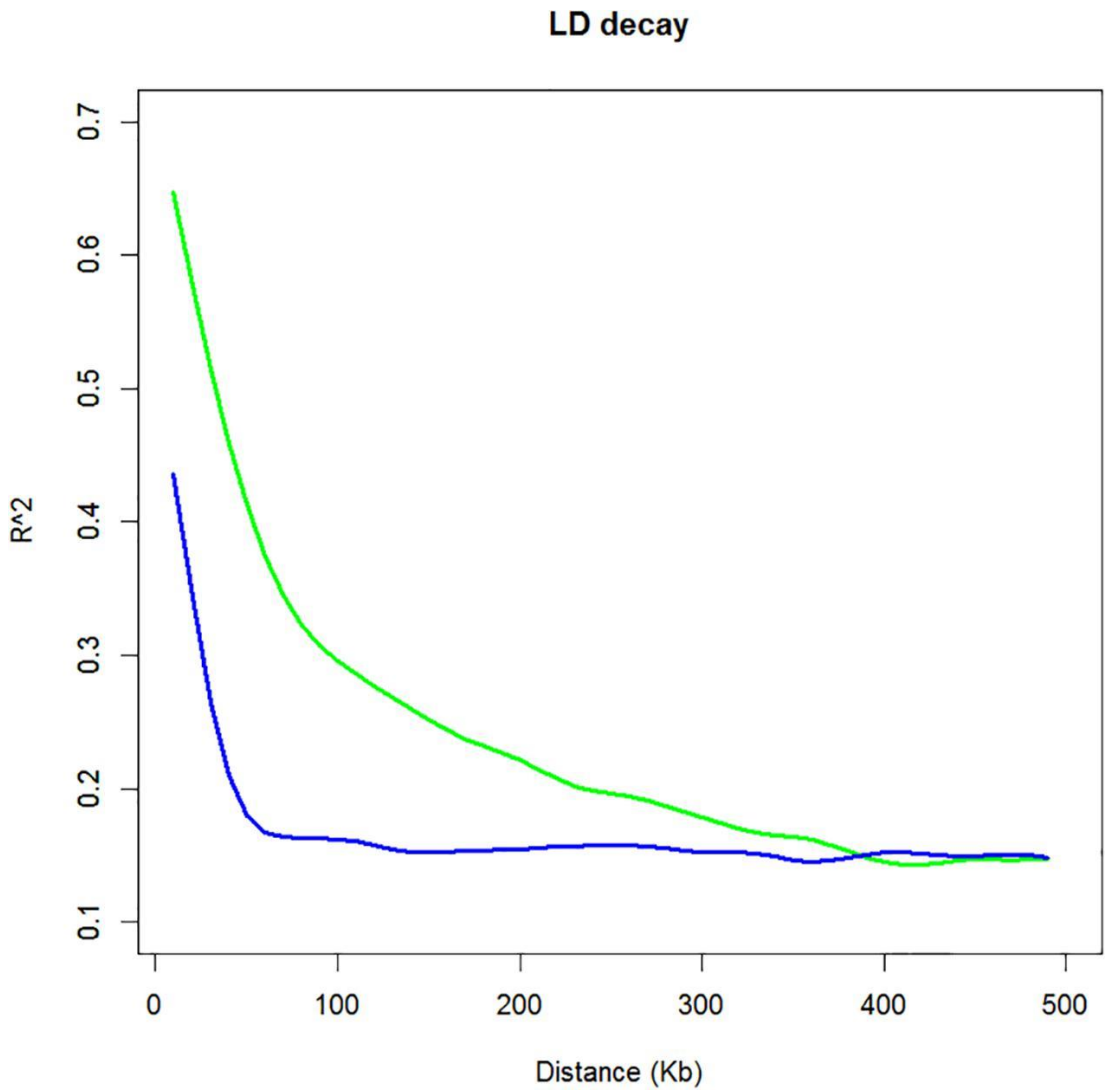

Figure 8 Chapter 4, FIGURE 2. Linkage disequilibrium (LD) graph of cultivated and wild mungbean. LD is determined by squared correlations of allele frequencies (r2) against distance between polymorphic sites, color-coded as follows: cultivated accessions (green) and wild accessions (blue).

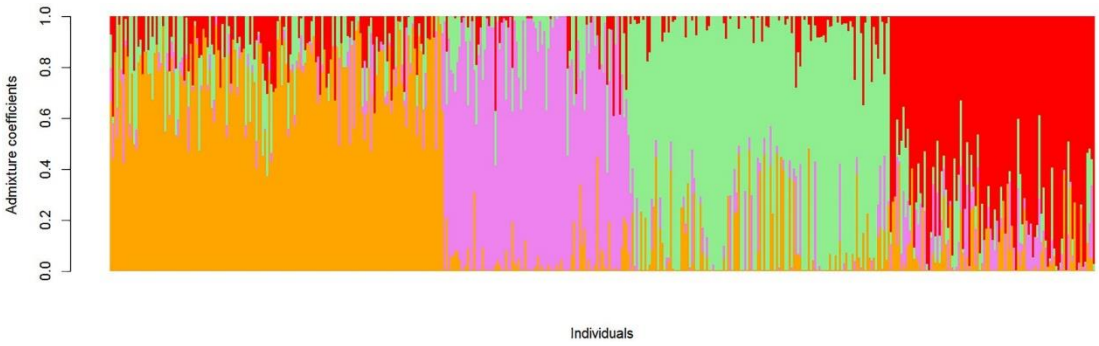

Figure 9: Chapter 4, FIGURE 3. Population structure of 466 cultivated mungbean accessions at K = 4. Each vertical bar represents a single accession, the length of each bar represents the proportion contributed by each sub-population. Sub-population 1 (color-coded orange), sub-population 2 (color-coded pink), sub-population 3 (color-coded green), and sub-population 4 (color-coded red).

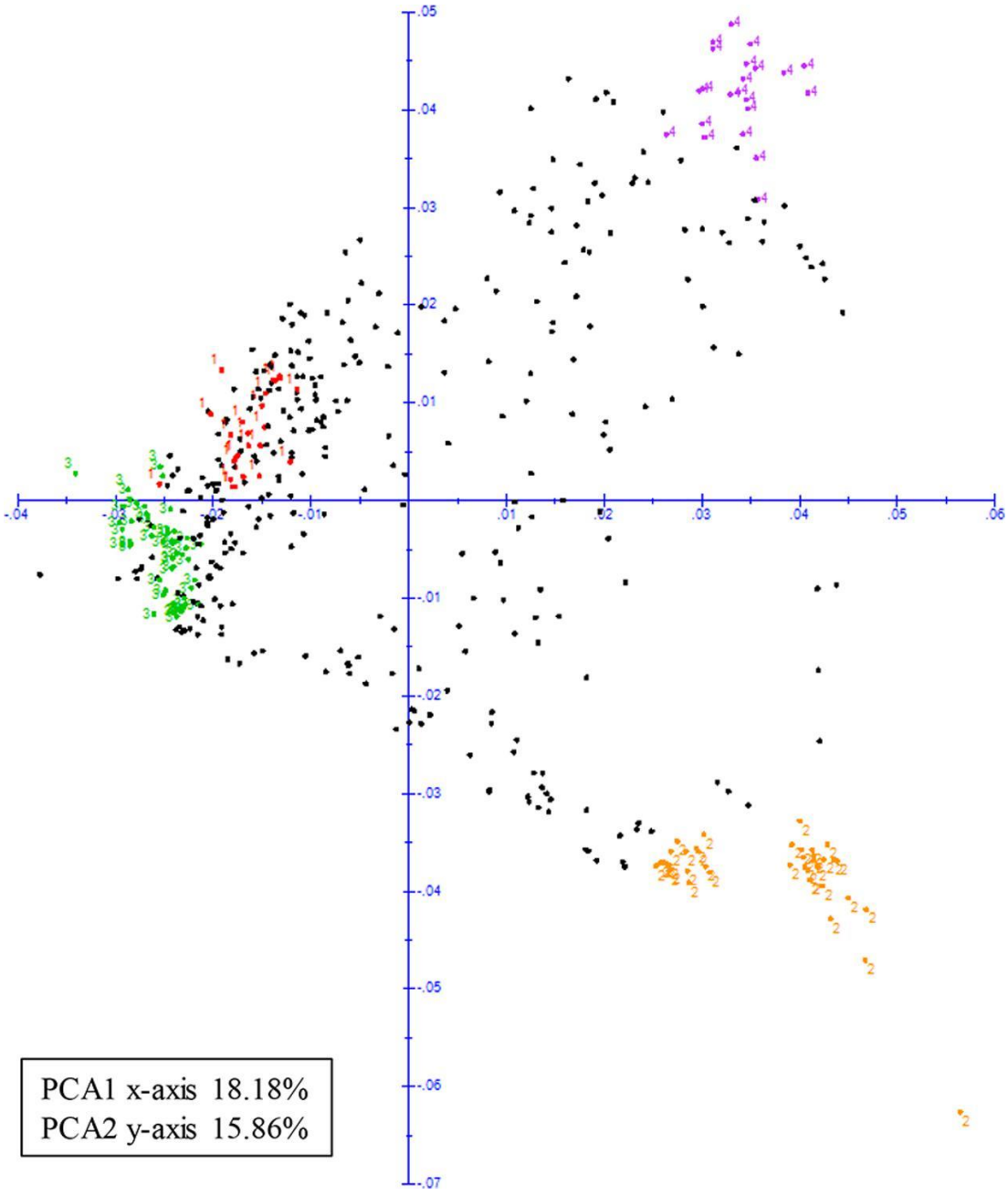

Figure 10: Chapter 4, FIGURE 4. Principal coordinate analysis (PCA) of 466 cultivated mungbean genotypes. Color-coded according to membership (based on >90% identity) to sub-populations identified from structure analysis; sub-population 1 (color-coded red), sub-population 2 (color-coded yellow), sub-population 3 (color-coded green), and sub-population 4 (color-coded purple).

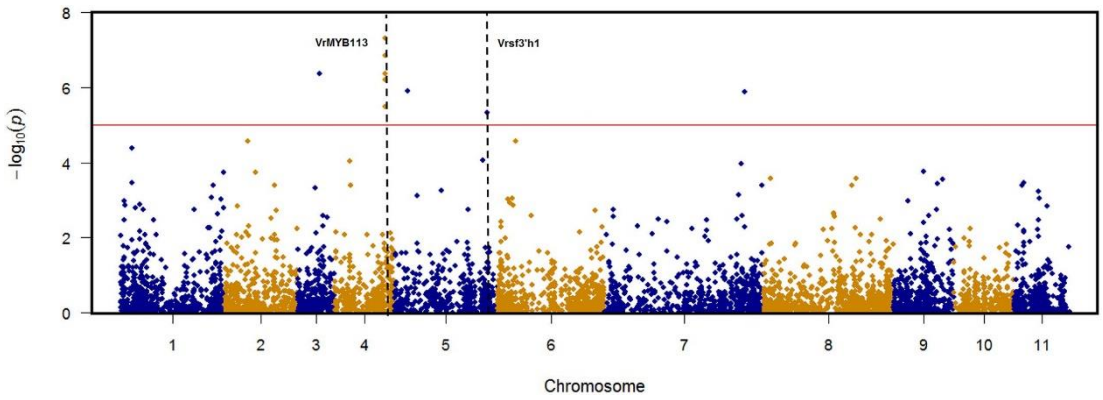

Figure 11: Chapter 4, FIGURE 5. Genome-wide association mapping (GWAS) results for seed coat color in mungbean. Nine SNPs identified as significantly associated with seed color located in five distinct genomic regions distributed across chromosomes 3, 4, 5, and 7. VrMYB113 is the homolog of Arabidopsis gene MYB113 located on chromosome 4 between position 17,668,384 and 17,690,573. Vrsf3′h1 is the homolog of soybean gene sf3′h1 located on chromosome 5 position 32,413,093.

4.7 TABLES

Table 6: Chapter 4, TABLE 1. Genomic distribution of 22,230 single nucleotide polymorphisms (SNPs) physically mapped on 11 cultivated and wild mungbean chromosomes/unanchored scaffolds.

Chromosome	Size of chromosome (Mb)	Total no. of SNPs	% of total SNPs	Avg. no. of markers per (Mb)	No. of cultivated SNPs	No. of wild SNPs
1	36.49	1643	7.39%	45.03	835	267
2	25.34	1332	5.99%	52.56	538	340
3	12.93	903	4.06%	69.85	457	171
4	20.78	1044	4.70%	50.23	512	219
5	37.09	1896	8.53%	51.12	769	483
6	37.41	1666	7.49%	44.53	851	278
7	55.45	2306	10.37%	41.59	979	498
8	45.72	2204	9.91%	48.21	1072	405
9	20.97	1208	5.43%	57.60	638	181
10	20.99	1099	4.94%	52.36	454	282
11	19.67	1161	5.22%	59.03	570	239
Total	332.84	16462	74.05%	NA	7675	3363
Average	30.26	1497	NA	52.01	698	306
Unanchored	NA	5768	25.95%	NA	2423	1180

Table 7: Chapter 4, TABLE 2. Genome-wide genetic differentiation (F_{ST}) between cultivated mungbean sub-populations.

	Pop2	Pop3	Pop4
Pop1	0.57	0.42	0.48
Pop2		0.56	0.52
Pop3	0.56		0.53

4.8 AUTHOR CONTRIBUTIONS

CD, DJ, and EM conceived and designed the experiments. TN collected the data. TN, EM, and YT analyzed data. TN, EM, and YT wrote the manuscript. SM, BW, DJ, and EM revised the manuscript. All authors read and approved the final manuscript.

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4.10 CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4.12 SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2017.02102/full#supplementary-material

4.13 FOOTNOTES

1. ^http://www.diversityarrays.com
2. ^http://www.diversityarrays.com

4.14 REFERENCES

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Chapter 5: General Discussion

The stability of agricultural production is crucial to food security and economic growth worldwide (Wharton, 2017). Mungbean, grown on over 6 million hectares of farming land worldwide, contributes significantly to the livelihoods of smallholder farmers throughout South and South East Asia (Kim et al. 2015). In the past, mungbean has been an opportunistic cash crop for Australian farmers due to limited agronomic knowledge and varietal options. The changing perception mungbean in Australia over the past decade is the result of extensive industry engagement through the Australian Mungbean Association (AMA) and broadly adapted varieties developed through conventional breeding in public sector programs. Together these have significantly improved yields and grower confidence. However, securing those yields to ensure a consistent supply has proven difficult. Limited management options for the seed-borne bacterial diseases are a significant risk to the mungbean industry in Australia and globally. Although the causal agent *Pseudomonas savastanoi* pv. *phaseolicola* has been studied at length in relation to *Phaseolus vulgaris* (common bean) (Arnold et al., 2011), minimal research beyond basic agronomic effects have investigated the relationship between *P. savastanoi pv. phaseolicola* and its disease-causing interactions with *Vigna radiata* (mungbean). Before this project, genomic profiles of the pathogen, distribution of pathotypes, and possible candidate genes associated with pathogenicity were largely untested.

Several mungbean genotypes have shown varying levels of resistance to *P. savastanoi* pv. *phaseolicola* strain T11544 when artificially inoculated in controlled environments and field nurseries (Ryley et al., 2010). Selection and breeding with those genotypes resulted in the release of a small-seeded commercial variety in 2014 with significant resistance to halo blight (Celera II-AU). However, due to market constraints, small-seeded varieties account for a small fraction of production in Australia. Since then, the resistance found in Celera II-AU has proven challenging to recombine into a large-seeded background, including the commercial varieties Crystal and Jade-AU, which account for ~95% of Australian plantings. A detailed review of the mungbean industry in Australia, and disease management practices of similar crops

in other countries, highlighted the need for molecular characterisation of both the pathogen and host populations (Thomas J. Noble et al., 2019).

Genomic profiling and screening for the presence of avirulence genes revealed a highly conserved halo blight pathogen population. The population broadly split into two linages with isolates categorised as IS50-A or IS50-B. A significant increase in the IS50-B genotype led to further analysis the amplicon unique to the IS50-B genotype. Cloning, sequencing and blasting of the amplicon sequence revealed it had a 99.5% identity to a hypothetical protein encoded by Pseudomonas syringae pv. cerasicola. Protein modelling produced multiple significant hits for kinase-related proteins that act as regulartors of pathogenicity related genes through sensing signals from plant defences and/or quorum sensignals from bacteria. To functionally characterise how the protein is affecting pathogencity, cloning and gene expression studies will identify the proteins targeted for phosphorylation and the pathways affected.

The categorisation of five pathotypes in this study revelaed a single highly destrudcive pathotype “pathotype 2” that could overcome the resistance identified against other pathotypes and represents 84% of all the isolates tested. Comprehensive sampling, testing and sequencing will be required annually to track the changes in the pathogen population. This will alert the industry to the regions that are currently most impacted by the disease and predict which areas are most at risk through modelling. A diverse set of isolates selected using the data presented in this study need to undergo whole-genome sequencing to generate a high-density phylogeny of the pathogen population, identify the range and variation in virulence genes and investigate how those changes affect pathogenicity.

This project was also the first to use an extensive collection of mungbean germplasm and dense SNP markers to characterise a mapping population. The population structure analysis revealed four sub-populations with similar country of origins passport data and seed morphology traits. Linkage disequilibrium (LD) in *Vigna radiata* (cultivated) and *Vigna radiata sublobata* (wild) revealed a higher degree of LD in the cultivated accessions, following sustained selection during breeding for traits such as large seed size, green seed coat colour and a shiny seed coat. Linkage Disequilibrium is an important factor that informs the density of markers required for association mapping, defining haplotype blocks and recombination patterns. Based on

the above results, a pilot genome-wide association study (GWAS) of seed coat colour was undertaken identifying homologous genes highly associated with seed coat colour.

Phenotyping the Australian mungbean diversity panel for a GWAS based on its reaction to isolates from pathotype 2 will reveal genetic loci associated with resistance to the most pervasive and destructive strains. The identified resistance genes could be incorporated into commercial mungbean cultivars such as Jade-AU through marker assisted selection (MAS) and conventional breeding practices. Further to this, genomic selection using the SNP markers will assist in protecting the longevity of major R gene resistance as breeding programs can ensure small-effect genes related to innate immunity are being incorporated. If however, the resistance loci proves difficult to transfer using convetional breeding approaches, genetic modificiation using CRISPR or a similar technology will dramatically speed up the time from gene discover to trait delivery. Whether conventional breeding with MAS or editing cultivars with CRISPR, knowing the location of genetic loci associated with agronomically important traits is critical.

A global whole-genome sequencing effort of elite mungbean cultivars and genetically diverse breeding material will provide a complete picture of the *Vigna radiata* genome. A pan-genome study of the sequence data would highlight the core genes found in all genotypes and dispensible genes. Found in small pools of genotypes, dispensible genes are often associated with adaptive traits controlling disease resistance and abiotic tolerance. Therefore, they make excellent tragets to narrow down the regions of the genome to consider during bioinformatic analysis. The sequences data would also assist in many other areas such as identiffing homologs of previously reported R genes in similar species such as common bean and soy bean, provide a genetic map to make accurate targets for CRISPR edits and imputing a dense SNP matrices for the current asscoation mapping panels.

To our knowledge, this study is the first to determine the incidence and distribution of *P. savastanoi* pv. *phaseolicola* infecting mungbeans from Australia and the first mungbean germplasm characterisation using SNP markers. These results considerably expand the knowledge of the pathogen and host populations in Australia. A greater understanding of the diversity of these populations will enable breeders and researchers to make informed decisions on the most effective strategies to enhance disease resistance and diagnostic protocols. Additionally, it has laid the groundwork for future studies on seed-borne bacterial diseases in mungbean. The outcomes from

this research project have been disseminated to the scientific community through publications and to other stakeholders directly. The Australia Mungbean Association and Queensland Department of Agriculture and Fisheries (DAF) mungbean breeding team are currently utilising the information to develop management strategies to reduce the risk halo blight diease in mungbeans.

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Appendices

Appendix A

Table S1. Metadata and analysis results of 511 isolates of *Pseudomonas savastano*i pv. p*haseolicola* isolated from Australian mungbean leaf tissue.

ID	Year	Town	State	Post Code	Race	avrPphE	avrPphF	IS50-B	ERIC	BOX
T14174	2016	Warra	QLD	4411	1	1	1	A	1	1
T14153	2016	Brookstead	QLD	4364	1	1	1	A	1	1
T14138	2016	Cambooya	QLD	4358	1	1	1	A	1	1
T14043	2015	Condamine	QLD	4416	1	1	1	A	1	1
T13975A	2015	Baralaba	QLD	4702	1	1	1	A	1	1
T14006	2015	Westbrook	QLD	4350	1	1	1	A	1	1
T11544	2005	Hermitage	QLD	4370	1	1	1	A	1	1
T14028	2015	Warra	QLD	4411	1	1	1	B	1	1
T13636A	2013	Dalby	QLD	4405	1	NA	NA	NA	NA	NA
T13912A	2015	Hermitage	QLD	4370	1	NA	NA	NA	NA	NA
T13912B	2015	Hermitage	QLD	4370	1	NA	NA	NA	NA	NA
T13912C	2015	Hermitage	QLD	4370	1	NA	NA	NA	NA	NA
T13917C	2015	Hermitage	QLD	4370	1	NA	NA	NA	NA	NA
T13918A	2015	Hermitage	QLD	4370	1	NA	NA	NA	NA	NA
T13954	2015	Dalby	QLD	4405	1	NA	NA	NA	NA	NA

T13964	2015	Bellata	NSW	2397	1	NA	NA	NA	NA	NA
T13971A	2015	Theodore	QLD	4719	1	NA	NA	NA	NA	NA
T13973	2015	Theodore	QLD	4719	1	NA	NA	NA	NA	NA
T14001	2015	St George	QLD	4487	1	NA	NA	NA	NA	NA
T14015A	2015	Hermitage	QLD	4370	1	NA	NA	NA	NA	NA
T14018	2015	Nobby	QLD	4360	1	NA	NA	NA	NA	NA
T14029	2015	Warra	QLD	4411	1	NA	NA	NA	NA	NA
T14036	2015	Hermitage	QLD	4370	1	NA	NA	NA	NA	NA
T14088C	2015	Banana	QLD	4702	1	NA	NA	NA	NA	NA
T14110A	2016	Miles	QLD	4415	1	NA	NA	NA	NA	NA
T14159	2016	Brookstead	QLD	4364	1	NA	NA	NA	NA	NA
T14244	2016	Jondaryan	QLD	4357	1	NA	NA	NA	NA	NA
T14250	2016	Wee Waa	NSW	2388	1	NA	NA	NA	NA	NA
T14252	2016	Spring Ridge	NSW	2343	1	NA	NA	NA	NA	NA
T14253	2016	Spring Ridge	NSW	2343	1	NA	NA	NA	NA	NA
T14259	2016	Narromine	NSW	2821	1	NA	NA	NA	NA	NA
T14261	2016	Trangie	NSW	2823	1	NA	NA	NA	NA	NA
T14529A	2017	Emerald	QLD	4720	1	NA	NA	NA	NA	NA
T14624	2018	Emerald	QLD	4720	1	NA	NA	NA	NA	NA
T14262	2016	Trangie	NSW	2823	2	1	1	A	1	1
T13934	2015	Moree	NSW	2400	2	1	1	A	1	1
T14004	2015	Inverell	NSW	2360	2	1	1	A	1	1
T13933	2015	Condamine	QLD	4416	2	1	1	A	1	1
T13939	2015	Crooble	NSW	2400	2	1	1	A	1	1
T13924B	2015	Ranger's Bridge	NSW	2370	2	1	1	A	1	1
T13911A	2015	Dalby	QLD	4405	2	1	1	A	1	1

T13915B	2015	Dalby	QLD	4405	2	1	1	A	1	1
T13931	2015	Moree	NSW	2400	2	1	1	A	1	1
T13929	2015	Moree	NSW	2400	2	1	1	A	1	1
T13903A	2014	Dalby	QLD	4405	2	1	1	A	1	1
T13691B	2014	Tuckerang	QLD	4411	2	1	1	A	1	1
T14007	2015	Kingaroy	QLD	4610	2	1	1	A	1	1
T13734A	2014	Warwick	QLD	4370	2	1	1	A	1	1
T13992	2015	Cambooya	QLD	4358	2	1	1	A	1	1
T13994	2015	Yandilla	QLD	4364	2	1	1	A	1	1
T13976	2015	Baralaba	QLD	4702	2	1	1	A	1	1
K4287	2013	Kingaroy	QLD	4610	2	1	1	A	1	1
T13735A	2014	Hermitage	QLD	4370	2	1	1	B	1	1
T13982	2015	Pampas	QLD	4352	2	1	1	B	1	1
T13804F	2014	Jimbour	QLD	4406	2	1	1	B	1	1
T13754B	2014	Warwick	QLD	4370	2	1	1	B	1	1
T13825B	2014	Warwick	QLD	4370	2	1	1	B	1	1
T13805H	2014	Kingsthorpe	QLD	4400	2	1	1	B	1	1
T13804A	2014	Jimbour	QLD	4406	2	1	1	B	1	1
T13733B	2014	Warwick	QLD	4370	2	1	0	B	1	1
T13913	2015	North Star	NSW	2408	2	1	1	B	1	1
T14031	2015	Warra	QLD	4411	2	1	1	B	1	1
T13636D	2013	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T13691A	2014	Tuckerang	QLD	4411	2	NA	NA	NA	NA	NA
T13691C	2014	Tuckerang	QLD	4411	2	NA	NA	NA	NA	NA
T13691D	2014	Tuckerang	QLD	4411	2	NA	NA	NA	NA	NA
T13691E	2014	Tuckerang	QLD	4411	2	NA	NA	NA	NA	NA

T13733A	2014	Warwick	QLD	4370	2	NA	NA	NA	NA	NA
T13750B	2014	Nobby	QLD	4360	2	NA	NA	NA	NA	NA
T13750C	2014	Nobby	QLD	4360	2	NA	NA	NA	NA	NA
T13800	2014	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13801A	2014	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13801B	2014	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13801C	2014	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13804B	2014	Jimbour	QLD	4406	2	NA	NA	NA	NA	NA
T13804C	2014	Jimbour	QLD	4406	2	NA	NA	NA	NA	NA
T13804D	2014	Jimbour	QLD	4406	2	NA	NA	NA	NA	NA
T13804E	2014	Jimbour	QLD	4406	2	NA	NA	NA	NA	NA
T13804F	2014	Jimbour	QLD	4406	2	NA	NA	NA	NA	NA
T13805A	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805B	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805C	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805D	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805E	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805F	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805G	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805I	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805J	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805K	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805L	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805M	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805N	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13805O	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA

T13825C	2014	Warwick	QLD	4370	2	NA	NA	NA	NA	NA
T13826A	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13826B	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13826C	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13826D	2014	Kingsthorpe	QLD	4400	2	NA	NA	NA	NA	NA
T13871	2014	Biloela	QLD	4715	2	NA	NA	NA	NA	NA
T13872	2014	Biloela	QLD	4715	2	NA	NA	NA	NA	NA
T13903D	2014	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T13906	2015	Brightview	QLD	4311	2	NA	NA	NA	NA	NA
T13911B	2015	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T13911C	2015	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T13911D	2015	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T13911F	2015	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T13912D	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13915A	2015	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T13915E	2015	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T13916A	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13916B	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13916C	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13916D	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13917B	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13917D	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13917E	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13918B	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13918C	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13918D	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA

T13918E	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T13923	2015	Ranger's Bridge	NSW	2370	2	NA	NA	NA	NA	NA
T13924A	2015	Ranger's Bridge	NSW	2370	2	NA	NA	NA	NA	NA
T13927A	2015	Nandi	QLD	4405	2	NA	NA	NA	NA	NA
T13927B	2015	Nandi	QLD	4405	2	NA	NA	NA	NA	NA
T13928A	2015	Tummaville	QLD	4352	2	NA	NA	NA	NA	NA
T13928B	2015	Tummaville	QLD	4352	2	NA	NA	NA	NA	NA
T13928C	2015	Tummaville	QLD	4352	2	NA	NA	NA	NA	NA
T13938	2015	Warra	QLD	4411	2	NA	NA	NA	NA	NA
T13956A	2015	Inverell	NSW	2360	2	NA	NA	NA	NA	NA
T13956B	2015	Inverell	NSW	2360	2	NA	NA	NA	NA	NA
T13957	2015	Inverell	NSW	2360	2	NA	NA	NA	NA	NA
T13970	2015	Cecil Plains	QLD	4407	2	NA	NA	NA	NA	NA
T13975B	2015	Baralaba	QLD	4702	2	NA	NA	NA	NA	NA
T13983	2015	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T13984	2015	Oakey	QLD	4401	2	NA	NA	NA	NA	NA
T13986	2015	Oakey	QLD	4401	2	NA	NA	NA	NA	NA
T13991	2015	Linthorpe	QLD	4356	2	NA	NA	NA	NA	NA
T13999	2015	St George	QLD	4487	2	NA	NA	NA	NA	NA
T14012	2015	North Star	NSW	2408	2	NA	NA	NA	NA	NA
T14015C	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14015D	2015	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14019	2015	Nobby	QLD	4360	2	NA	NA	NA	NA	NA
T14086A	2015	Monto	QLD	4630	2	NA	NA	NA	NA	NA
T14086B	2015	Monto	QLD	4630	2	NA	NA	NA	NA	NA
T14086C	2015	Monto	QLD	4630	2	NA	NA	NA	NA	NA

T14088A	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14088B	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14088D	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14088E	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14091A	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14091B	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14092A	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14092B	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14093	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14094	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14095A	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14095B	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14096A	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14096B	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14097A	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14097B	2015	Banana	QLD	4702	2	NA	NA	NA	NA	NA
T14098	2016	Biloela	QLD	4715	2	NA	NA	NA	NA	NA
T14099A	2016	Jandowae	QLD	4410	2	NA	NA	NA	NA	NA
T14099B	2016	Jandowae	QLD	4410	2	NA	NA	NA	NA	NA
T14099C	2016	Jandowae	QLD	4410	2	NA	NA	NA	NA	NA
T14099D	2016	Jandowae	QLD	4410	2	NA	NA	NA	NA	NA
T14099E	2016	Jandowae	QLD	4410	2	NA	NA	NA	NA	NA
T14100A	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14100B	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14100C	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14100D	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA

T14100E	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14100F	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14100G	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14100H	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14100I	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14100J	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14101A	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14101B	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14101C	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14101D	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14102A	2016	Wowan	QLD	4702	2	NA	NA	NA	NA	NA
T14102B	2016	Wowan	QLD	4702	2	NA	NA	NA	NA	NA
T14104A	2016	Home Hill	QLD	4806	2	NA	NA	NA	NA	NA
T14104B	2016	Home Hill	QLD	4806	2	NA	NA	NA	NA	NA
T14105A	2016	Home Hill	QLD	4806	2	NA	NA	NA	NA	NA
T14105B	2016	Home Hill	QLD	4806	2	NA	NA	NA	NA	NA
T14106A	2016	Home Hill	QLD	4806	2	NA	NA	NA	NA	NA
T14106B	2016	Home Hill	QLD	4806	2	NA	NA	NA	NA	NA
T14106C	2016	Home Hill	QLD	4806	2	NA	NA	NA	NA	NA
T14106D	2016	Home Hill	QLD	4806	2	NA	NA	NA	NA	NA
T14107A	2016	Rolleston	QLD	4702	2	NA	NA	NA	NA	NA
T14107B	2016	Rolleston	QLD	4702	2	NA	NA	NA	NA	NA
T14107C	2016	Rolleston	QLD	4702	2	NA	NA	NA	NA	NA
T14107D	2016	Rolleston	QLD	4702	2	NA	NA	NA	NA	NA
T14108A	2016	Rolleston	QLD	4702	2	NA	NA	NA	NA	NA
T14108B	2016	Rolleston	QLD	4702	2	NA	NA	NA	NA	NA

T14109A	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14109B	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14109C	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14109D	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14109E	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14110B	2016	Miles	QLD	4415	2	NA	NA	NA	NA	NA
T14110C	2016	Miles	QLD	4415	2	NA	NA	NA	NA	NA
T14115A	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14115B	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14116A	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14116B	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14116C	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14116D	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14116E	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14116F	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14118A	2016	Cambooya	QLD	4358	2	NA	NA	NA	NA	NA
T14118B	2016	Cambooya	QLD	4358	2	NA	NA	NA	NA	NA
T14118C	2016	Cambooya	QLD	4358	2	NA	NA	NA	NA	NA
T14118D	2016	Cambooya	QLD	4358	2	NA	NA	NA	NA	NA
T14118E	2016	Cambooya	QLD	4358	2	NA	NA	NA	NA	NA
T14118F	2016	Cambooya	QLD	4358	2	NA	NA	NA	NA	NA
T14119A	2016	Kincora	QLD	4356	2	NA	NA	NA	NA	NA
T14119B	2016	Kincora	QLD	4356	2	NA	NA	NA	NA	NA
T14119C	2016	Kincora	QLD	4356	2	NA	NA	NA	NA	NA
T14121	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14122A	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA

T14122B	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14125A	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14125B	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14125C	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14126	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14127A	2016	Parkes	NSW	2870	2	NA	NA	NA	NA	NA
T14127B	2016	Parkes	NSW	2870	2	NA	NA	NA	NA	NA
T14128A	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14128B	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14129A	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14129B	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14129C	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14132A	2016	Oakey	QLD	4401	2	NA	NA	NA	NA	NA
T14132B	2016	Oakey	QLD	4401	2	NA	NA	NA	NA	NA
T14133	2016	Oakey	QLD	4401	2	NA	NA	NA	NA	NA
T14134A	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14134B	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14134C	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14134D	2016	Hermitage	QLD	4370	2	NA	NA	NA	NA	NA
T14135	2016	Nobby	QLD	4360	2	NA	NA	NA	NA	NA
T14136	2016	Nobby	QLD	4360	2	NA	NA	NA	NA	NA
T14139	2016	Cambooya	QLD	4358	2	NA	NA	NA	NA	NA
T14140	2016	Jimbour	QLD	4406	2	NA	NA	NA	NA	NA
T14143	2016	Jimbour	QLD	4406	2	NA	NA	NA	NA	NA
T14144	2016	Jimbour	QLD	4406	2	NA	NA	NA	NA	NA
T14145A	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA

T14145B	2016	Chinchilla	QLD	4413	2	NA	NA	NA	NA	NA
T14146	2016	Clifton	QLD	4361	2	NA	NA	NA	NA	NA
T14148	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14149A	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14149B	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14150A	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14150B	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14155	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14156	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14157	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14158	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14160	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14161	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14162	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14163	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14164	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14166	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14167	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14168	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14169	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14170	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14171	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14172	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14176	2016	Brigalow	QLD	4412	2	NA	NA	NA	NA	NA
T14178	2016	Warra	QLD	4411	2	NA	NA	NA	NA	NA
T14179	2016	Warra	QLD	4411	2	NA	NA	NA	NA	NA

T14180	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14181	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14182	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14183	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14192	2016	Mondure	QLD	4611	2	NA	NA	NA	NA	NA
T14209	2016	Nobby	QLD	4360	2	NA	NA	NA	NA	NA
T14212	2016	Milmerran	QLD	4357	2	NA	NA	NA	NA	NA
T14213	2016	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14218	2016	Oakey	QLD	4401	2	NA	NA	NA	NA	NA
T14220	2016	Jondaryan	QLD	4357	2	NA	NA	NA	NA	NA
T14221	2016	Jondaryan	QLD	4357	2	NA	NA	NA	NA	NA
T14234	2016	Kingaroy	QLD	4610	2	NA	NA	NA	NA	NA
T14235	2016	Kingaroy	QLD	4610	2	NA	NA	NA	NA	NA
T14236	2016	Kingaroy	QLD	4610	2	NA	NA	NA	NA	NA
T14238	2016	North Star	NSW	2408	2	NA	NA	NA	NA	NA
T14239	2016	North Star	NSW	2408	2	NA	NA	NA	NA	NA
T14248	2016	Wee Waa	NSW	2388	2	NA	NA	NA	NA	NA
T14249	2016	Wee Waa	NSW	2388	2	NA	NA	NA	NA	NA
T14251	2016	Mullaley	NSW	2379	2	NA	NA	NA	NA	NA
T14254	2016	Spring Ridge	NSW	2343	2	NA	NA	NA	NA	NA
T14255	2016	Parkes	NSW	2870	2	NA	NA	NA	NA	NA
T14256	2016	Parkes	NSW	2870	2	NA	NA	NA	NA	NA
T14260	2016	Trangie	NSW	2823	2	NA	NA	NA	NA	NA
T14444	2017	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T14445	2017	Dalby	QLD	4405	2	NA	NA	NA	NA	NA
T14448	2017	Inverell	NSW	2360	2	NA	NA	NA	NA	NA

T14480	2017	Forbes	NSW	2871	2	NA	NA	NA	NA	NA
T14506	2017	Nobby	QLD	4360	2	NA	NA	NA	NA	NA
T14512	2017	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14515	2017	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14516	2017	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14523A	2017	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14523B	2017	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14524	2017	Brookstead	QLD	4364	2	NA	NA	NA	NA	NA
T14525	2017	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14527	2017	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14528	2017	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14529B	2017	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14531A	2017	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14531B	2017	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14551A	2017	Clifton	QLD	4361	2	NA	NA	NA	NA	NA
T14551B	2017	Clifton	QLD	4361	2	NA	NA	NA	NA	NA
T14551C	2017	Clifton	QLD	4361	2	NA	NA	NA	NA	NA
T14563A	2017	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14563B	2017	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14621	2018	Emerald	QLD	4720	2	NA	NA	NA	NA	NA
T14626	2018	Warra	QLD	4411	2	NA	NA	NA	NA	NA
T14632B	2018	Pampas	QLD	4352	2	NA	NA	NA	NA	NA
T14632C	2018	Pampas	QLD	4352	2	NA	NA	NA	NA	NA
T14632D	2018	Pampas	QLD	4352	2	NA	NA	NA	NA	NA
T14632E	2018	Pampas	QLD	4352	2	NA	NA	NA	NA	NA
T14633A	2018	Pampas	QLD	4352	2	NA	NA	NA	NA	NA

T14633B	2018	Pampas	QLD	4352	2	NA	NA	NA	NA	NA
T13922B	2015	Ranger's Bridge	NSW	2370	3	1	1	A	1	1
T14008	2015	Clifton	QLD	4361	3	1	1	A	1	1
T14177	2016	Brigalow	QLD	4412	3	1	1	B	1	1
T13636B	2013	Dalby	QLD	4405	3	NA	NA	NA	NA	NA
T14243	2016	Jondaryan	QLD	4357	3	NA	NA	NA	NA	NA
T14015B	2015	Hermitage	QLD	4370	4	1	1	A	1	1
T14005	2015	Kingaroy	QLD	4610	4	1	1	A	1	1
T14027	2015	Warra	QLD	4411	4	1	1	B	1	1
T13963	2015	Bellata	NSW	2397	4	NA	NA	NA	NA	NA
T14175	2016	Dulacca	QLD	4425	4	NA	NA	NA	NA	NA
T13946	2015	Baralaba	QLD	4702	5	1	1	A	1	1
T13750E	2014	Nobby	QLD	4360	5	1	1	A	1	1
T13981	2015	Yandilla	QLD	4364	5	1	1	A	1	1
T13636C	2013	Dalby	QLD	4405	5	1	1	A	1	1
T13911E	2015	Dalby	QLD	4405	5	NA	NA	NA	NA	NA
T13915D	2015	Dalby	QLD	4405	5	NA	NA	NA	NA	NA
T13922A	2015	Ranger's Bridge	NSW	2370	5	NA	NA	NA	NA	NA
T13971B	2015	Theodore	QLD	4719	5	NA	NA	NA	NA	NA
T14030	2015	Warra	QLD	4411	5	NA	NA	NA	NA	NA
T14154	2016	Brookstead	QLD	4364	5	NA	NA	NA	NA	NA
T14263	2016	Trangie	NSW	2823	5	NA	NA	NA	NA	NA
T14489A	2017	Kumbia	QLD	4610	5	NA	NA	NA	NA	NA
T14489B	2017	Kumbia	QLD	4610	5	NA	NA	NA	NA	NA
T14632A	2018	Pampas	QLD	4352	5	NA	NA	NA	NA	NA
PSP158	2018	Clifton	QLD	4361	NA	1	1	A	NA	NA

PSP157	2018	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP156	2018	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP155	2018	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP154	2018	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP152	2018	Oakey	QLD	4401	NA	1	1	A	NA	NA
PSP151	2018	Oakey	QLD	4401	NA	1	1	A	NA	NA
PSP150	2018	Oakey	QLD	4401	NA	1	1	A	NA	NA
PSP149	2018	Oakey	QLD	4401	NA	1	1	A	NA	NA
PSP136	2018	Warra	QLD	4411	NA	1	1	A	NA	NA
PSP135	2018	Warra	QLD	4411	NA	1	1	A	NA	NA
PSP133	2018	Pampas	QLD	4352	NA	1	1	A	NA	NA
PSP132	2018	Pampas	QLD	4352	NA	1	1	A	NA	NA
PSP131	2018	Pampas	QLD	4352	NA	1	1	A	NA	NA
PSP130	2018	Pampas	QLD	4352	NA	1	1	A	NA	NA
PSP129	2018	Pampas	QLD	4352	NA	1	1	A	NA	NA
PSP128	2018	Pampas	QLD	4352	NA	1	1	A	NA	NA
PSP127	2018	Pampas	QLD	4352	NA	1	1	A	NA	NA
PSP126	2018	Pittsworth	QLD	4356	NA	1	1	A	NA	NA
PSP124	2018	Pittsworth	QLD	4356	NA	1	1	A	NA	NA
PSP122	2018	Pittsworth	QLD	4356	NA	1	1	A	NA	NA
PSP121	2018	Pittsworth	QLD	4356	NA	1	1	A	NA	NA
PSP110	2017	Avondale	QLD	4670	NA	1	1	A	NA	NA
PSP109	2017	Avondale	QLD	4670	NA	1	1	A	NA	NA
PSP108	2017	Avondale	QLD	4670	NA	1	1	A	NA	NA
PSP107	2017	Avondale	QLD	4670	NA	1	1	A	NA	NA
PSP106	2017	Avondale	QLD	4670	NA	1	1	A	NA	NA

PSP105	2017	Springsure	QLD	4722	NA	1	1	A	NA	NA
PSP104	2017	Springsure	QLD	4722	NA	1	1	A	NA	NA
PSP103	2017	Springsure	QLD	4722	NA	1	1	A	NA	NA
PSP102	2017	Springsure	QLD	4722	NA	1	1	A	NA	NA
PSP101	2017	Springsure	QLD	4722	NA	1	1	A	NA	NA
PSP100	2017	Springsure	QLD	4722	NA	1	1	A	NA	NA
PSP099	2017	Springsure	QLD	4722	NA	1	1	A	NA	NA
PSP098	2017	Springsure	QLD	4722	NA	1	1	A	NA	NA
PSP095	2017	Emerald	QLD	4720	NA	1	1	A	NA	NA
PSP094	2017	Emerald	QLD	4720	NA	1	1	A	NA	NA
PSP093	2017	Emerald	QLD	4720	NA	1	1	A	NA	NA
PSP092	2017	Emerald	QLD	4720	NA	1	1	A	NA	NA
PSP091	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP090	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP089	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP088	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP087	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP086	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP084	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP083	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP082	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP081	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP080	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP079	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP074	2017	Pilton	QLD	4361	NA	1	1	A	NA	NA
PSP073	2017	Pilton	QLD	4361	NA	1	1	A	NA	NA

PSP072	2017	Pilton	QLD	4361	NA	1	1	A	NA	NA
PSP071	2017	Pilton	QLD	4361	NA	1	1	A	NA	NA
PSP070	2017	PIlton	QLD	4361	NA	1	1	A	NA	NA
PSP069	2017	Pilton	QLD	4361	NA	1	1	A	NA	NA
PSP068	2017	Nobby	QLD	4360	NA	1	1	A	NA	NA
PSP067	2017	Nobby	QLD	4360	NA	1	1	A	NA	NA
PSP066	2017	Nobby	QLD	4360	NA	1	1	A	NA	NA
PSP065	2017	Nobby	QLD	4360	NA	1	1	A	NA	NA
PSP064	2017	Nobby	QLD	4360	NA	1	1	A	NA	NA
PSP063	2017	Nobby	QLD	4360	NA	1	1	A	NA	NA
PSP062	2017	Nobby	QLD	4360	NA	1	1	A	NA	NA
PSP061	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP060	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP059	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP058	2017	Clifton	QLD	4361	NA	1	1	A	NA	NA
PSP048	2017	Warwick	QLD	4370	NA	1	1	A	NA	NA
PSP047	2017	Warwick	QLD	4370	NA	1	1	A	NA	NA
PSP046	2017	Warwick	QLD	4370	NA	1	1	A	NA	NA
PSP045	2017	Warwick	QLD	4370	NA	1	1	A	NA	NA
PSP044	2017	Warwick	QLD	4370	NA	1	1	A	NA	NA
PSP042	2017	Warwick	QLD	4370	NA	1	1	A	NA	NA
PSP012	2017	Dalby	QLD	4405	NA	1	1	A	NA	NA
PSP011	2017	Dalby	QLD	4405	NA	1	1	A	NA	NA
PSP010	2017	Dalby	QLD	4405	NA	1	1	A	NA	NA
PSP009	2017	Dalby	QLD	4405	NA	1	1	A	NA	NA
PSP008	2017	Dalby	QLD	4405	NA	1	1	A	NA	NA

PSP002	2017	Dalby	QLD	4405	NA	1	1	A	NA	NA
PSP001	2017	Dalby	QLD	4405	NA	1	1	A	NA	NA
K4260	2013	Kingaroy	QLD	4610	NA	1	1	A	NA	NA
K4257	2013	Kingaroy	QLD	4610	NA	1	1	A	NA	NA
K4258	2013	Kingaroy	QLD	4610	NA	1	1	A	NA	NA
27A	2016	Dalby	QLD	4405	NA	1	1	A	NA	NA
26A	2016	Chinchilla	QLD	4413	NA	1	1	A	NA	NA
3A	2016	King	QLD	4610	NA	1	1	A	1	1
2A	2016	Kingaroy	QLD	4610	NA	1	1	A	1	1
PSP153	2018	Oakey	QLD	4401	NA	1	1	B	NA	NA
PSP148	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP147	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP146	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP145	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP144	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP143	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP142	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP141	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP140	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP139	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP138	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP137	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP134	2018	Warra	QLD	4411	NA	1	1	B	NA	NA
PSP123	2018	Pittsworth	QLD	4356	NA	1	1	B	NA	NA
PSP120	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP119	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA

PSP118	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP117	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP116	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP115	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP114	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP113	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP112	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP111	2018	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP097	2017	Emerald	QLD	4720	NA	1	1	B	NA	NA
PSP096	2017	Emerald	QLD	4720	NA	1	1	B	NA	NA
PSP085	2017	Clifton	QLD	4361	NA	1	1	B	NA	NA
PSP078	2017	Clifton	QLD	4361	NA	1	1	B	NA	NA
PSP077	2017	Clifton	QLD	4361	NA	1	1	B	NA	NA
PSP076	2017	Clifton	QLD	4361	NA	1	1	B	NA	NA
PSP075	2017	Clifton	QLD	4361	NA	1	1	B	NA	NA
PSP043	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP041	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP040	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP039	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP038	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP037	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP036	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP035	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP034	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP033	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP032	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA

PSP031	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP030	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP029	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP028	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP027	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP026	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP025	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP024	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP023	2017	Pilton	QLD	4361	NA	1	0	B	NA	NA
PSP022	2017	Pilton	QLD	4361	NA	1	1	B	NA	NA
PSP021	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP020	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP019	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP018	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP017	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP016	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP015	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP014	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP013	2017	Warwick	QLD	4370	NA	1	1	B	NA	NA
PSP007	2017	Dalby	QLD	4405	NA	1	1	B	NA	NA
PSP006	2017	Dalby	QLD	4405	NA	1	1	B	NA	NA
PSP005	2017	Dalby	QLD	4405	NA	1	1	B	NA	NA
PSP004	2017	Dalby	QLD	4405	NA	1	1	B	NA	NA
PSP003	2017	Dalby	QLD	4405	NA	1	1	B	NA	NA
K4308	2013	Kingaroy	QLD	4610	NA	1	1	B	NA	NA
K4310	2013	Kingaroy	QLD	4610	NA	1	1	B	NA	NA

K4309	2013	Kingaroy	QLD	4610	NA	1	1	B	NA	NA
1A	2016	Hermitage	QLD	4370	NA	1	1	B	1	1

Table S2. List of PCR primers used in this study

Primer	Sequnence (5'-3')	Gene Target	Product size (bp)	Reference
avrPhE-F	GCGTTCGATCATAACGTTGA	hopX1 (avrPphE)	1400	(Stevens et al., 1998)
avrPhE-R	TCATTGGCAGAGCGATGAGT			
avrPhF-F	ATGAAGAATTCGTTCGACCG	hopF1 (avrPphF)	1400	(Tsiamis et al., 2000)
avrPhF-R	TCAGACCGAACTCTCAGACA			
ERIC1	ATGTAAGCTCCTGGGGATTCAC	Random	250-5000	(Versalovic et al., 1991)
ERIC2	AAGTAAGTGACTGGGGTGAGCG			
BOXA1R	CTACGGCAAGGCGACGCTGACG	Random	500-2000	(Versalovic et al., 1994)
IS50	GGTTCCGTTCAGGACGCTAC	Random	400-3500	(Sundin et al., 1994)

Table S3. Average nucleotide identity (ANI) between the K4287 and T11544 genome assemblies[1].

Metric	Value
OrthoANIu value (%)	99.98
Genome A (K4287) length (bp)	5,921,100
Genome B (T11544) length (bp)	5,965,980
Average aligned length (bp)	5,015,120
Genome A coverage (%)	84.7
Genome B coverage (%)	84.06

[1] https://www.ezbiocloud.net/tools/ani

Table S4. General features of the T11544 and K4287, *P. savastanoi* pv. phaseolicola genomes separated by chromosome and plasmid

	T11544 Chromosome	T11544 Plasmid A	T11544 Plasmid B	K4287 Chromosome	K4287 Plasmid A	K4287 Plasmid B
Size (bp)	5,562,967	50,165	16,100	5,525,885	57,207	21,883
G+C%	58.3	53.0	53.7	58.27	52.85	53.95
length of Coding regions	4,923,382	36,317	12,886	4,892,835	42,614	16,794
% Coding regions	88.50	72.40	80.04	88.54	74.49	76.74
No. Contigs	159	49	3	153	50	7
Contigs N50 (length >=200 bp)	84,045	2,209	12,377	84,046	3,238	12,377
No. of ORFs	5,255	79	23	5,208	88	30

Table S5. Type III secretion proteins in K4287 and T11544 genome assemblies. Genomic scaffolds were annotated using RAST and secreted Type III proteins were identified in each genome. Annotation as well as nucleotide and protein sequences are provided.

Scaffold_ID	RAST_ID	Gene_ID	Start	End	Strand	Description	Pathway	Nucleotide_sequences	Protein_sequences
K4287 genome									

K4287_ 000104| NODE_ 104_len gth_992 9_cov_ 69.5642 28	fig|319. 128.peg. 5403	K4287_000104|N ODE_104_length _9929_cov_69.56 4228_5708_7051	5708	7051	+	candidate type III effector HolPtoQ

atgcatcgtcttatcaccgcagcctatacc	MHRLITAAYTTSRLIL
acctcacgtctcatcctagatcagtcaaa	DQSKQISRTLSESSVQ
acaaatatcacgcaccctatcggaaagta	SALPQQTSMSSPVLER
gcgtgcaatcagcacttcctcagcaaac	SKSAPTLLTAAQRTM
aagtatgagcagcccagttctggagcgg	LAQVGACNAHLTSDE
tcgaaaagtgcgccaacgttattgactgc	NMAINELRLHKPRLPK
ggcacagcgcacgatgcttgcacaagtg	DTWFFTDPNKDPDDV
ggcgcatgcaacgctcatctgacctcag	VTYTLGKQLQAEGFV
acgaaaacatggccatcaatgaactgag	HITDVVATLGDAEVR
attacacaagccccggttacctaaggata	SQRAEMAKGVFNKLE
cgtggtttttcactgatcctaacaaggacc	LHDVHVSRGRDYAM
cggatgatgtcgtgacgtacaccttgggc	NSLQSKEHDKFLLEG
aagcaattgcaggctgagggctttgtaca	HALRAGPGEIHRDSLQ
catcacggacgtagtggcaacactgggt	DMSRRLARAPHGVGI

gatgctgaagttcgctctcaacgtgccga VVIAGMSDINALITTC

gatggccaaaggcgtgttcaacaagctt PDIVRERVDDITIMGG

gagttgcatgacgtgcatgtgtcgcgtgg VEPLKDADGFVQPDA

tcgggattacgcaatgaattcgcttcagtc RAYNNATDMDAARS

gaaggaacatgacaaatttttactggaag LYRKAQELGIPLRIVT

gtcatgctttaagggctggacctggtgaa KEAAYKTAVSPSFYE

atacaccgcgacagcttgcaggacatga GIAGSGHPVGHYLRD

gcaggcgcctggcccgtgcgccacatg VQKSALKGLWEGIQA

gagtcggtattgtcgtaattgcaggcatg GLLPGLDESWFFRTF

agtgatatcaatgcgctcatcactacctgc MPNAQIEAVQLDKNK

ccggatattgtgcgcgaacgggttgatg ESSFEDIWPKVTKLNL

acatcaccatcatgggcggcgtcgaacc YDPLTLLASVPGAAK

tttaaaggacgcagatggttttgtacagcc LLFKPKAIHTEGFGVV

tgatgcacgcgcttacaacaatgcgacc EQVGPDDVTHPEKAK

gacatggacgctgcgcgcagtctttatcg LLMSALAKSALVQST

aaaagcacaggagcttggcattccacttc VAPD

gtatagtgacaaaggaggcggcctataa

aacggcggtttcaccttcattttacgaagg

aatagcagggagcggacatccagtagg

ccactacctgagagacgttcagaagagt

gcgttgaaaggcctctgggaaggtattca

agctggattgcttcccgggttggatgaat

catggttctttcggacgttcatgccgaatg

cacagattgaagcagtacaactggataa

aaataaagagagttcgtttgaagatatctg

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gatcctctgacattactggcctcagtgcc

aggggcggcaaaactgctatttaaacca

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							atgctgtgcacaccgctgctattttcagcg catgccggagcagccggcacggcttct gaacaagccaatgtggaagtgatgattc	
							gccagctcaacgcgctcgaggccgtcg cgcagcgcagtgtcgatcttccacaaga cccggctcaacgctatcacctggactatc cccggttggtcagcgacatcgtgcgcat ccgccaaggtttgcaggactacctgtcg ccgtcccgcgcacagcctcgtgacccc	GTASEQANVEVMIRQ LNALEAVAQRSVDLP QDPAQRYHLDYPRLV SDIVRIRQGLQDYLSP SRAQPRDPVDISGHYN VSGDHTP

K4287_000054|NODE_54_length_40629_cov_38.2619	fig|319.128.peg.4291	K4287_000054|NODE_54_length_40629_cov_38.261914_39493_3874	3949	3874	-	Pantothenate kinase type III, CoaX-like (EC 2.7.1.33)	isu;Coenzyme_A_Biosynthesis_cluster isu;Coenzyme_A_Biosynthesis	gtagacatttccggccattacaacgtcag cggtgatcacacgccatga	atgattcttgagctcgactgtggcaacag cttcatcaagtggcggataacggcgaag aacgatgcggcagtgattagcggtggcg ttgtgggctccgatgctgccttgctgatgc acttgagtaatctgcctgacacatcattca ctgactgccgtctggtgagtgtgcgtagt gccgaagaaacggcgaggcttgtctcttt gctggctgacacctttttccgttgcgcctgt ctgcgccgaacctgctcaagagcttgct ggcgtgataaatggctatgacgacttcgc gcgtctggggctggaccgctggctggct ttcgtaggtgcttaccatctcgccagaag	MILELDCGNSFIKWRI TAKNDAAVISGGVVG SDAALLMHLSNLPDT SFTDCRLVSVRSAEET ARLVSLLADTFSVAPV CAEPAQELAGVINGY DDFARLGLDRWLAFV GAYHLARSACLVIDL GTAVTSDFVDAGGAH LGGFICPGMPLMRNQ LRTHTRRIRYDDTEAE KALARLVPGRATAEA

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gagaccactgggaagtgcccgtaaacc	VRAVSSHQTASASDR
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tcgtcacgaagaccttatgccaaaacaa

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acgccgaaacgcctcttgcctatccctct KTQRQALGMLREDLL

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cgaagatacgctgctgaaagcgttcttcg

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cccggtgttcagtttctcggacatgcaac

ccgagcaggcgaaaaaggcgcgattgc

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