ASSESSING THE RESISTANCE TO ROOT-LESION NEMATODE (*PRATYLENCHUS THORNEI*) IN A NEW COLLECTION OF WILD CHICKPEA (*CICER RETICULATUM* AND *C. ECHINOSPERMUM*) FROM TURKEY

A Thesis submitted by

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Abstract

Chickpea (*Cicer arietinum* L.) is an ancient grain legume first domesticated some 10,000 years ago and is now the second major pulse produced worldwide. The root-lesion nematode *Pratylenchus thornei* is a microscopic eelworm, and is the foremost nematode species affecting chickpea production worldwide with yield losses of intolerant Australian cultivars estimated to be up to 25%. Crop improvement has been challenged by the narrow genetic diversity within cultivated chickpea and a previously limited world collection of wild chickpea. Crop wild relatives are a rich source of genetic diversity for new allelic variation including disease resistance, and thereby play a major role in meeting challenges for 21st century agriculture. Recent collection missions in southeastern Turkey have boosted numbers of accessions of wild chickpea, namely *C. reticulatum* Ladizinsky and *C. echinospermum* P. H. Davis, the only wild species crossable with the cultigen *C. arietinum*. This study evaluated 174 accessions (133 *C. reticulatum* and 41 *C. echinospermum*) under controlled-environment conditions to identify levels of *P. thornei* resistance within the collection. Assessments were determined at chickpea species level, with geographic locations and genetic population groups also assessed to identify any clusters of resistance.

Accessions of both wild *Cicer* species were, on average, more resistant ($P < 0.001$) than the cultigen *C. arietinum*. Multi-environment analyses to determine genetic rankings of accessions showed 53 (30%) accessions were significantly more resistant than the least susceptible Australian cultivar PBA Seamer. Collection sites and genetic population groups differed significantly ($P < 0.001$) for mean *P. thornei* population densities. This is the first study to evaluate nematode resistance of this new collection and it has revealed novel sources of *P. thornei* resistance that can be exploited by breeding programs worldwide for chickpea improvement. Furthermore, the study provides valuable quantitative information for future genetic studies to identify candidate genes for *P. thornei* resistance in chickpea.

Certification of Thesis

This thesis is the work of Roslyn Ann Reen, except where otherwise acknowledged, with the majority of the authorship of the paper presented as a Thesis by publication undertaken by the student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Supervisor: Professor John Thompson

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Student and supervisors signatures of endorsement are held at the University.

Statement of Contribution

The following outlines the agreed share of contribution for the candidate and co-authors in the presented publication in this thesis:

This thesis incorporates a publication in the international journal *Phytopathology* published by the American Phytopathological Society which forms Chapter 3 of this thesis.

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I, Roslyn Reen declare that I am the first author of the paper published and incorporated in this thesis. Professor John Thompson and Michael Mumford are co-authors of this paper. Consequently, I acknowledge the contribution of the co-authors in planning, executing and reviewing the experimental work and in editing this paper. The contribution of authors is as follows

65% Roslyn Reen: Planning, design, experimentation, data collection, collation and interpretation of data, graphing, drafting, editing and revising of journal article for publication

15% Michael Mumford: Design, data analysis, graphing and editing of journal article

20% John Thompson: Concept design, reviewing of data collection, editing and revising of journal article for submission

I, Professor John Thompson as Principal Supervisor of Roslyn Reen at the University of Southern Queensland, confirm that the statement of Roslyn Reen is true and correct

Professor John Thompson

I, Mr. Michael Mumford as Biometrician and co-author at Leslie Research Facility Department of Agriculture and Fisheries confirm the statement of Roslyn Reen is true and correct.

Mr Michael Mumford

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Abbreviations

AMF	Arbuscular mycorrhizal fungi
BMS	Breeding Management System
cv.	Cultivar
CWR	Crop wild relative
GWAS	Genome-wide association study
ICARDA	International Centre for Agricultural Research in Dry Areas
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
Pi	Initial nematode population density
Pf	Final nematode population density
QTL	Quantitative trait loci
RLN	Root-lesion nematode

CHAPTER 1: INTRODUCTION

1.1 Thesis outline

Chapter 1 provides an overview of the economic importance of chickpea (*Cicer arietinum* L.), the constraint on production due to the root-lesion nematode (RLN) *Pratylenchus thornei* Sher & Allen and the challenge to improve crop productivity due to the limited genetic diversity in domesticated germplasm. Chapter 2 is a literature review documenting current knowledge of *P. thornei,* chickpea cultivation and management strategies for control of RLN within Australian agriculture. The emphasis is on research with the root-lesion nematode *P. thornei* and the role of wild relatives as sources of resistance. Chapter 3 presents the research undertaken to assess 174 accessions from the new collection of wild *Cicer* for resistance to *P. thornei.* The research is provided in the format of an article published in the international journal *Phytopathology.* vol. 109, no. 7, pp. 1270-79. A summary of the research and benefits for industry are outlined in Chapter 4 with a discussion on avenues for future research and advancement of the germplasm concludes the thesis.

1.2 Overview

Chickpea is the second major food legume worldwide after bean (*Phaseolus vulgaris* L.) in terms of production (FAOSTAT 2017). It is an integral rotational crop in many agricultural systems worldwide due to its capacity for biological nitrogen fixation and used as a disease break between crops. The agricultural value of chickpea, coupled with its nutrition and health benefits, and a consumer trend away from animal protein products, has led to an increase in global demand within the last decade (Daryanto, Wang & Jacinthe 2015; Perez-Hidalgo, Guerra-Hernández & García-Villanova 1997). Currently, the two major chickpea producing countries are India, producing ~9 million tonnes, and Australia ~5 million tonnes with the current world annual production being ~14.7 million tonnes (FAOSTAT 2017). The increase in global demand and its current value has led chickpea to become Australia's largest and most valuable pulse crop with over 1.1 million ha sown to chickpea, and annual gross production valued at ~ 2 billion AUD (FAOSTAT 2017; Pulse. Australia 2016).

Although chickpea is a major pulse crop, its susceptibility to biotic and abiotic stresses is a challenge for achieving greater productivity. Current average production worldwide is estimated to be less than 1 t/ha, well below its yield potential (Singh et al. 2015; Chauhan et al. 2017). Development of new cultivars to combat these stresses and increase yield potential has been hindered by the lack of genetic diversity within current modern day chickpea germplasm (Singh et al. 2015; Varshney et al. 2013).

1.3 The research problem

In Australia, chickpea is grown as a dryland winter crop with over 90% of production located within the sub-tropical grain region of eastern Australia. Chickpea within this region is often grown in rotation with wheat (*Triticum aestivum* L.). A major biotic stress for both crops is the root-lesion nematode (RLN) *Pratylenchus thornei*. This nematode, also known as the legume and cereal nematode is widespread in the Australian grain region and throughout the world (Carrasco-Ballesteros et al. 2007; Smiley et al. 2005; Thompson et al. 2010).

Susceptible chickpea cultivars increase *P. thornei* populations in the soil and consequently limit yields, thereby reducing the benefits of chickpea as a rotational crop in cereal cropping systems. Current management relies on rotation with resistant and tolerant crops, however, research indicates several resistant crops in sequence are required to effectively reduce *P. thornei* populations (Owen et al. 2014). To date, in Australia, there are no fully resistant chickpea or wheat cultivars. Incorporating genetic resistance through plant breeding is the only sustainable way to combat the high population densities of *P. thornei* remaining in the soil after growing susceptible crops (Trudgill 1991).

World gene bank collections of cultivated chickpea are limited in the diversity of traits needed by breeders for crop improvement (Collard, Pang & Taylor 2003; Rao et al. 2007). Due to this narrow genetic base, using crop wild relatives (CWR) provides a promising way to find genetic solutions for resistance to *P. thornei* in chickpea. Multiple studies indicate wild *Cicer* species have greater genetic variability for various traits and superior genetic disease resistance compared to the cultigen *C. arietinum* (Andeden et al. 2013; Gupta et al. 2017; Toker, Canci & Yildirim 2007). There are 43 wild species of chickpea, however, only two annual wild species *C. reticulatum* Ladizinsky and *C. echinospermum* P.H. Davis are cross compatible with the chickpea

cultigen *C. arietinum* (Ladizinsky & Adler 1976a). Both of these wild species occur naturally in a restricted geographic area of southeastern Turkey (Fig. 1.1a) (Berger, Abbo & Turner 2003; Tanno & Willcox 2006). Due to their crossability with chickpea these two wild *Cicer* are important for exploring the potential genetic diversity for disease resistance and germplasm improvement (Andeden et al. 2013; Kameswara Rao, Reddy & Bramel 2003; Sudupak, Akkaya & Kence 2002).

Pratylenchus thornei is the foremost nematode species attacking chickpea in Australia and a major constraint to chickpea production in the Mediterranean basin, the Indian subcontinent and North Africa (Castillo, Gomez-Barcina & Jiménez-Díaz 1996a; Di Vito et al. (1994a; 1994b). Previous research in Australia and overseas to identify resistance to *P. thornei* in the chickpea cultigen (*C. arietinum*) has been extensive but with limited success. In contrast, wild *Cicer* accessions from a previous limited collection offered improved resistance (Thompson et al. 2011) resulting in incremental gains of resistance to *P. thornei* in chickpea cultivars. The potential for further improvement was hindered by the low numbers of original wild *Cicer* accessions in world collections, with only a minor proportion of genetic diversity available in wild populations present in gene bank collections (Berger, Abbo & Turner 2003).

In an effort to address the gaps in available wild *Cicer* germplasm, an international consortium of scientists from Australia, United States of America and Turkey conducted a mission in 2013 to 2015 to collect accessions of wild *Cicer* from southeastern Turkey, where the progenitor of chickpea *C. reticulatum* originated (Tanno & Willcox 2006). Accessions were collected from 21 sites from within the five provinces of Sirnak, Urfa, Diyarbakir, Mardin and Adiyaman (Fig 1.1b). Genetic studies determined the wild *Cicer* collection to have 100 times more diversity than *C. arietinum* with accessions grouped into 12 populations comprising eight *C. reticulatum* and four *C. echinospermum* based on genetic structure (Von Wettberg et al. 2018). This new collection is the focus of an international research effort to identify new sources of genetic resistance to various abiotic and biotic stresses.

1.4 Aims and objectives

The aim of this thesis was to evaluate the new wider collection of annual wild *Cicer* accessions, namely *C. reticulatum* and *C. echinospermum*, to identify new sources of resistance to *P. thornei* for future introgression into cultivated chickpea. The main objectives of the research were to (i) assess 174 wild *Cicer* accessions collected from 21 locations within Turkey comprising 133 *C. reticulatum* and 41 *C. echinospermum* for resistance to *P. thornei*, (ii) to determine if any accessions had higher levels of resistance to *P. thornei* than currently present in a selection of Australian cultivars and (iii) compare the level of *P. thornei* resistance within the two wild species, (iv) to assess the effect of geographic location on level of resistance in the wild species, and (v) to assess the effect of population structure on the level of *P. thornei* resistance in the wild species.

This new collection provides a unique opportunity to access a wide diversity of potential sources of resistance to *P. thornei* that has never before been available for research and chickpea breeding purposes. Future introgression of *P. thornei* resistant wild *Cicer* into chickpea, will reduce nematode soil populations allowing more flexible rotational options and increased yields, resulting in more favourable economic outcomes for growers.

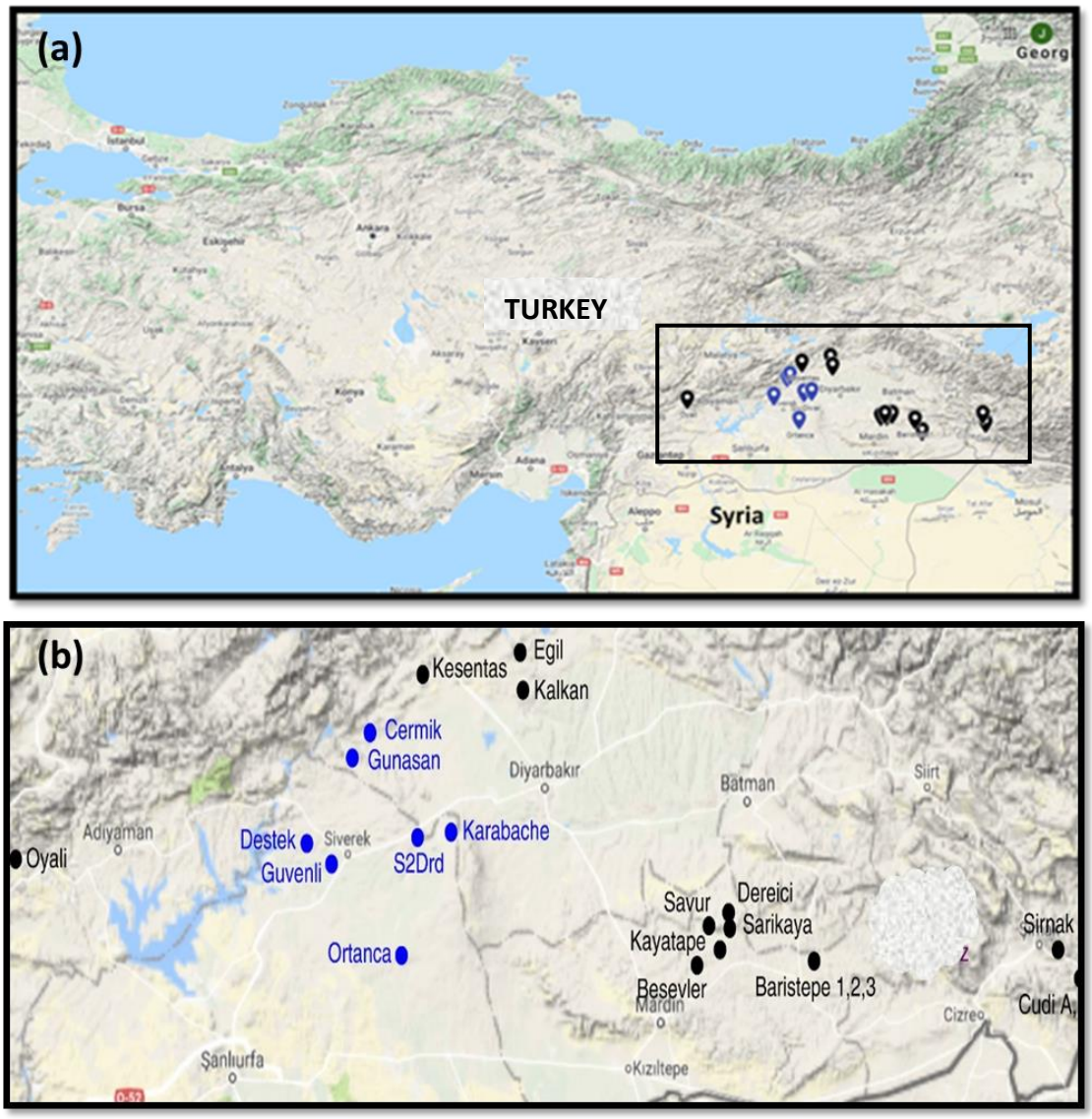

Figure 1.1: Maps of southeastern Turkey illustrating collection sites (a) Overview of collection sites with *Cicer echinospermum* sites (blue) and *C. reticulatum* sites (black) adapted from My Maps google and (b) an enlarged region with collection site names Von Wettberg et al. (2018, p. 5).

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Assessing crop resistance to nematodes requires an understanding of the host, nematode species, and knowledge of environmental and edaphic conditions that influence crop growth and nematode reproduction. The following literature review will provide current information on chickpea and the root-lesion nematode *Pratylenchus thornei* including the value of crop wild relatives and their role in sourcing resistance to this nematode.

2.2 Chickpea *Cicer arietinum*

Chickpea is a crop of global economic importance currently grown in over 50 countries in the five continents of Asia, Africa, Europe, America and Australia (Singh 1997; FAOSTAT 2017). Its ancestry aligns with the West Asian Neolithic founder crops assemblage, with cultivation dating back some 10,000 years ago at Nevali Cori in southeastern modern Turkey (Zohary & Hopf 2000). Chickpea is a self-pollinating diploid plant (2n=2x=16) (Singh et al. 2015), that belongs to the genus *Cicer*, the family *Leguminosae/Fabaceae* and the tribe *Cicereae* (Van der Maesen 1984).

Based on seed type, cultivated chickpea is categorised into (i) the kabuli type or "macrosperma" with seed white to cream in colour which was originally grown in the Western Mediterranean, and (ii) the desi type or "microsperma" with brown angular hard coated seed and originally grown in Central Asia and the Indian subcontinent (Andeden et al. 2013). Recent studies suggest that the desi type chickpea is more genetically linked to the wild species *C. reticulatum* than is the kabuli type (Gupta et al. 2017). Evidence suggests the kabuli type, originated from a larger seeded desi type within a small restrictive gene pool during domestication, resulting in kabuli having slightly less diversity than the desi type (Moreno & Cubero 1978).

2.2.1 Cultivation and agronomic value

Chickpea can grow in a vast array of climatic regions and on a wide range of soil types with major production areas being within the semi-arid regions of the world (Canci & Toker 2009). In agriculture, chickpea plays a vital role, contributing to soil nitrogen (N) in crop rotational systems, and in Australia it acts as a disease break for a major soil fungal pathogen in cereals such as crown rot *Fusarium graminearum* Schwab. (Felton et al. 1998). In Australia, chickpea are mainly grown as a dryland winter crop dependent on in-season rainfall. Over 90% of production occurs within the sub-tropical grain region of eastern Australia. The region covers approximately 4 million hectares extending from the Liverpool Plains region of New South Wales up to the semi-arid region of the Tropic of Capricorn in central northern Queensland (Dang et al. 2015). The area is characterised by summer dominant rainfall, with the annual median 600–800 mm (Thompson et al. 2008). Soil types within this region are characterised as being deep (0.9–1.5 m), with a high clay content and plant water-holding capacity that include Vertisols, Chromosols and Sodosols (Thompson, Mackenzie & Sheedy 2012). Chickpea are dependent on this stored moisture and in-crop rainfall during the growing season, as the climate can be highly variable with frequent dry periods and extremes of temperature (Chauhan et al. 2017). Average daily maximum air temperatures can range from 12–20ºC in winter and in summer 27–33ºC (Thompson et al. 2017).

Many Australian chickpea cultivars are long-day phenological types developed from cultivars originating from the Mediterranean and Indian subcontinent. For example, the first Australian cultivar Tyson, released in 1978 was a desi derived from the Indian cultivar C235 (Berger et al. 2004). Today, Australia is the world's largest exporter of chickpea with over 95% being exported to India and Pakistan with the majority being the desi type (Pulse Australia 2016).

Worldwide, chickpea production is constrained by a range of biotic and abiotic stresses which reduce annual world production by one third (Croser et al. 2003). Chickpea improvement has been hindered by limited genetic variation. The constrained gene pool is attributed to four main factors, (i) restricted geographic area of the progenitor compared to other founder crops such as wheat and pea (*Pisum sativum* L.), (ii) domestication, (iii) change from autumn to spring sowing and (iv), replacement of landraces with elite cultivars

through modern plant breeding (Abbo, Berger & Turner 2003). The modification of sowing time in the Bronze Age was possibly due to the need to escape the effects of Ascochyta caused by *Ascochyta rabiei* (Pass.) Lab. This, coupled with domestication is cited as having the greatest evolutionary effect in constraint of the genepool (Berger et al. 2005). Furthermore, lack of diversity within chickpea cultivars reduces their ability to cope with disease in agriculture environments and crop wild relatives are a key tool for addressing the limits of genetic variation and disease resistance for crop improvement (Dempewolf et al. 2017).

2.2.2 Major biotic and abiotic stresses affecting chickpea production

Plant parasitic nematodes (PPN) are one of the most important biotic stresses affecting yield potential in crops. Root-lesion nematodes (RLN) *Pratylenchus* spp. Filipjev, are the second most damaging nematode group in field and horticultural crops after the root-knot nematode (RKN) *Meloidogyne* spp. Goeldi, in relation to global economic loss (Castillo et al. 2008; Jones & Fosu-Nyarko 2014.) Root-lesion nematodes are microscopic worm-like plant parasites that feed and reproduce in the cortex of plant roots. In Australia, *P. thornei* is the foremost species affecting chickpea production (Thompson et al. 2000, 2011), and ranks second in importance of the five major diseases affecting chickpea yield in Australia (Murray & Brennan 2012). Similarly, this nematode species is a major constraint to production in the Mediterranean basin, the Indian subcontinent and North Africa (Castillo et al. 1998; Carrasco-Ballesteros et al. 2007; Di Vito et al. 1994a; 1994b).

Globally there are 50 diseases estimated to be affecting chickpea production, with the fungal diseases ascochyta blight *Ascochyta rabiei* (Syn. *Phoma rabiei*) and fusarium wilt (*Fusarium oxysporium* Schlecht. Emend. Synd. & Hans. f. sp *Ciceri* (Padwick) *S*ynd. & Hans.)) being the most damaging worldwide (Pande et al. 2005; Singh et al. 1993). Fusarium wilt symptoms are exacerbated by *Pratylenchus thornei* (Castillo et al. 1998), and future development of cultivars with *P. thornei* resistance has the potential to limit *Fusarium* infection.

Fusarium wilt of chickpea is not present in Australia, however, ascochyta blight is present and it currently rates as the major fungal pathogen affecting chickpea production (Collard, Pang & Taylor 2003).

Worldwide production of chickpea is mainly under dryland conditions and major abiotic stresses such as terminal heat and drought are key determinants of chickpea production (Berger 2007; Kumar & Abbo 2001). Australia has a variable climate, and within the four million hectares where chickpea production occurs, the crop may be exposed to drought, water-logging, high temperatures and chilling (Chauhan et al. 2017). Similarly, where chickpea is grown in the Mediterranean, West Asia and North Africa regions, soil moisture can be depleted during crop maturity and towards the end of the growing season when there are increasing temperatures (Toker et al. 2007; Whish, Castor & Carberry 2007). Water and nutrient stress can be exacerbated by root damage from *P. thornei.* Furthermore, when chickpea is subjected to water stress *Pratylenchus thornei* can significantly increase in the roots and soil (Castillo et al. 1995).

Finally, the impact of diseases and environmental stresses has led to a loss of local chickpea ecotypes. In view of this and coupled with the low genetic variability within domesticated germplasm, breeders are sourcing wild relatives as an alternative genetic resource for chickpea improvement (Croser et al. 2003).

2.3 Crop wild relatives

Current domesticated crops are closely associated with ancestral species including their progenitors. The Russian plant geneticist Nicolai Vavilov was the first to realise the potential of ancestral species as sources of diversity and genetic resistance to biotic and abiotic stresses (Maxted et al. 2010). The genus *Cicer* comprises 43 wild species and the one cultivated species *C. arietinum*. Among these there are nine annual and 35 perennial species. The nine annual species include *C. arietinum* and eight wild *Cicer,* comprising *C. reticulatum, C. echinospermum, C. pinnatifidum* Jaub. & Sp., *C. judacium* Boiss., *C. bijugum* K.H. Rech., *C. cuneatum* Hochst., *chorassanicum (*Bge.) M.Pop. and *C. yamashitae* Kitam. (Ladizinsky & Adler 1976a).

Wild species can be classified into primary, secondary and tertiary gene pools depending on compatibility and success of producing fertile hybrids with the cultivated chickpea, Ladizinsky & Adler (1976a, 1976b). Only the two wild species *C. reticulatum* and *C. echinospermum* belong to the primary gene pool of chickpea. Both are cross-compatible with cultivated chickpea and only occur in southeastern Turkey

where chickpea originated. The secondary gene pool consists of *C. bijugum, C. judacium* and *C. pinnatifidum,* while the tertiary gene pool comprises *C. cuneate, C. yamashitae* and *C. chorassanicum* (Crosser et al. 2003).

The two wild annual species *C. reticulatum* and *C. echinospermum* originating in southeastern Turkey are the focus of the research described in this thesis. *Cicer reticulatum* is the original progenitor, first discovered by Ladizinsky in 1975 (Ladizinsky and Adler 1976b) in Dereici, Mardin. *Cicer echinospermum* was first discovered in Siverek, Sanliurfa in 1957 by P.H. Davis (Talip et al. 2018). Both species have a distinct seed coat and morphology. *Cicer reticulatum* has a reticulate seed coat and larger leaflets and flowers similar to the cultivated chickpea. In contrast, *C. echinospermum* seed is smaller with a spiny or echinate seed coat and smaller leaflets and flowers than *C. reticulatum* (Talip et al. 2018). Differences in seed coat and flowers are illustrated in Fig. 2.1.

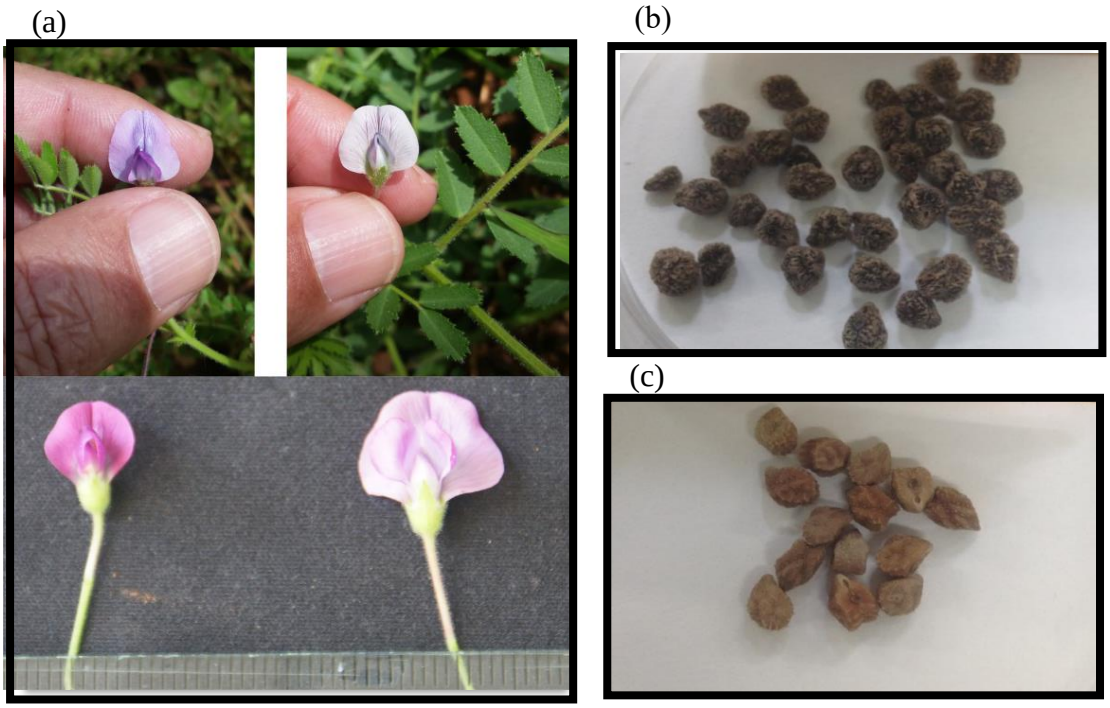

Figure 2.1: Flowers of wild *Cicer* (a) *Cicer echinospermum* on left, *C. reticulatum* on right. Photo sourced from Talip et al. (2018 p. 20). Seed of (b) *C. echinospermum* and (c) *C. reticulatum.*

Based on potential to cross-breed with chickpea and cytogenetic studies, *C. echinospermum* was originally nominated within the secondary genepool (Ladizinsky & Adler 1976a).

However, successful hybridisation for several biotic stresses including RLN (Collard, Pang & Taylor 2003; Knights et al. 2008; Singh & Ocampo 1997; Thompson et al. 2011) led Croser et al. (2003) to propose *C. echinospermum* be placed in the primary gene pool.

In terms of seed size *Cicer echinospermum* does have some similarities to cultivated chickpea and this eliminates the major difficulty in recovering backcross progeny (Knights et al. 2008). However, the variable fertility with *C. echinospermum* hybridisation has contributed to a reluctance of breeders to use *C. echinospermum* germplasm for chickpea improvement (Kahraman et al. 2017). Moreover, for this new wild *Cicer* collection from southeastern Turkey, Kahraman et al. (2017) conducted a study on F1 hybrids of *C. echinospermum* from six collection sites and revealed two distinct subgroups of *C. echinospermum* associated with hybrid sterility or breakdown. They proposed further molecular studies will facilitate future introgression breeding of unique desirable traits from *C. echinospermum* germplasm. Furthermore, a recent study with *C. echinospermum* derivative crosses possessing improved RLN and *Phytophthora* root rot resistance (Knights et al. 2008), were shown to maintain seed quality traits crucial for commercialisation and export (Woods et al. 2019).

Crop wild relatives are a vital resource of genetic diversity, critical for improving disease resistance and the adaptive capacity of agriculture crops worldwide (Dempewolf et al. 2017). They play a vital role in sustaining global food security through the provision of beneficial traits that lead to yield stability (Redden 2015). Introgression of genes from wild *Cicer* species into chickpea for *Pratylenchus thornei* resistance will facilitate widening the genetic base for *P. thornei* resistance, resulting in more robust and genetically diverse cultivars.

2.4 Root-lesion nematode

Root-lesion nematodes belong to the genus *Pratylenchus* and are classified in the order *Rhabditia*, Suborder *Tylenchina*, Superfamily *Hoplolaimidae* and Subfamily *Pratylenchinae* (Castillo & Volvas 2007). Based on feeding behaviours, plant-parasitic nematodes can be categorised into three major groups, (i) sedentary endoparasites which mainly remain within the roots,

(ii) migratory endoparasites, which move within roots and between roots and soil and (iii) ectoparasites which feed on the outside of roots (Peng & Moens 2003). *Pratylenchus* spp. are polyphagous, migratory, root endoparasites with current taxonomic studies indicating over 89 morphometrically described species (Carrasco-Ballesteros et al. 2007).

2.4.1 Morphology

Morphological identification of species within *Pratylenchus* are based on characteristics of adult females. The key diagnostic features include body length and body shape index determined by the de Man ratio of *a* = body length/width ratio (Thorne 1961), number of lip annuli (2–4), stylet length, vulva position in the body, and shape of tail and terminus. The presence or absence of males is also considered (Castillo & Vovlas 2007; Handoo & Golden 1989).

The morphometric characteristics of *P. thornei* are a body length range between 0.41–0.71 mm, *a* value of 0.25–0.36 and a tail terminus bluntly rounded to truncate in shape. The mouth region is characterised by three lip annules and a stylet or spear measuring approximately 17–19 µm in length. A key diagnostic feature is the position of the vulva which is situated at 74–79% of the total body length measured from the anterior end (Sher & Allen 1953). In the sub-tropical grain region of eastern Australia 52 *P. thornei* adults from a Vertosol (Australian classification, Isabell 1996) had an average body length of 0.569 mm, *a* value of 0.32 and vulva position situated at 77% of total body length (Thompson, Rostad & Whish 2017).

2.4.2 Pathogenic variability

Pathogenic variability of *Pratylenchus* or existence of different races has been reported for several species, however, little to none has been reported for *P. thornei* (Castillo & Vovlas 2007). In Spain, four populations of *P. thornei* from different locations were tested on chickpea and no differences in pathogenicity or reproductive fitness were found (Castillo et al. 1998). Moreover, molecular research with *P. thornei* isolates originating from Italy and Spain found no variation within the species (De Luca et al. 2011). In Australia, a study with *P. thornei* and *P. neglectus* (Rensch) Filipijev and P. Schuurmans Stekhoven across states within the country found wheat cultivars ranked similarly in resistance indicating no pathogenic diversity

with these species (Sheedy et al. 2015). Furthermore, recent molecular testing for the two major species *P. thornei* and *P. neglectus* affecting the Australian grain cropping regions concluded there was little diversity within these two species (Giblot-Ducray et al. 2017).

2.4.3 Life cycle

Depending on species, temperature and moisture, the life cycle of *Pratylenchus* can vary from 3–9 weeks. Under optimal conditions on a susceptible host this can be brief as 26 days, for example *P. vulnus* on carrots (Jones & Fosu-Nyarko 2014). The life cycle range for *P. thornei* species is between 4–6 weeks with a generation normally completed within 45 days (Larson 1959; Thompson, Rostad & Whish 2017) (Fig. 2.2).

Although *Pratylenchus* spp. are migratory, their life cycle imitates the general nematode life cycle, which involves four juvenile stages transitioned by moulting stages before reaching the adult stage. The first stage juvenile is within the egg (J1), and the second stage (J2) hatches from the egg approximately seven days later (Blake & Baxter 1968), followed by the third (J3) and fourth juvenile (J4) stages before reaching adult stage (Jones & Fosu-Nyarko 2014) (Fig. 2.3).

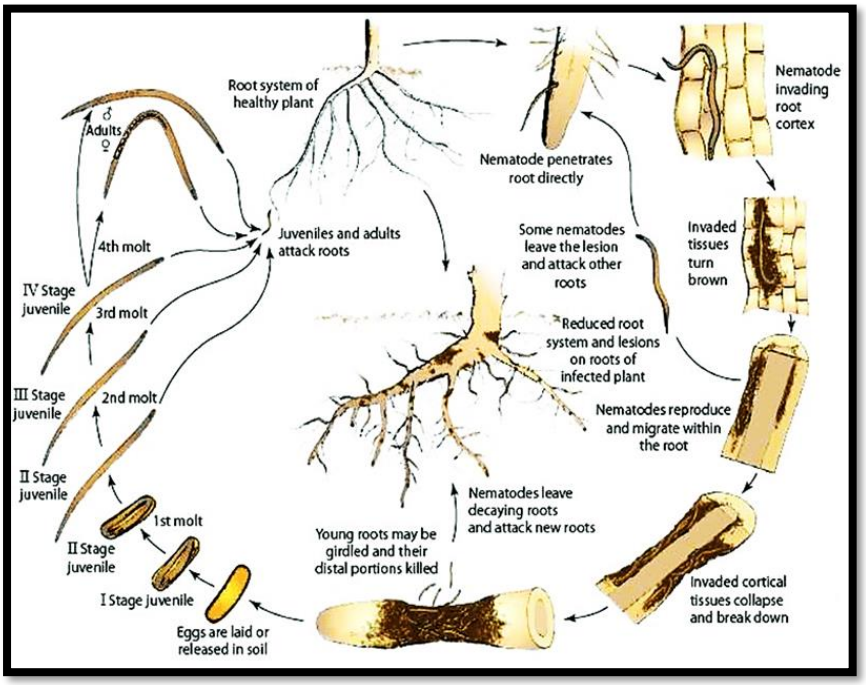

Figure 2.2: Life cycle of root-lesion nematode (*Pratylenchus* spp.). (AG Agrios 1988, p. 127.).

Plant parasitic nematodes possess a stylet or hollow spear situated in the head region with attached knobs that are quite prominent in *Pratylenchus* spp. (Castillo et al. 2008). The stylet situated in the head region acts like a protractible needle to pierce the parenchymal roots of plants to gain entry. Once inside the root, the nematode releases secretions into the plant tissues via the oesophageal glands connected to the stylet. These secretions contain cell-wall degrading enzymes which are also known as effectors that allow nematode migration and modification of the cells for ingesting of nutrients (Hussey 1989; Jones & Fosu-Nyarko 2014). For chickpea, there is no preferential penetration site with both juveniles and adults of *P. thornei* noted entering roots within one to 11 days after inoculation, with the numbers penetrating increasing over time (Castillo, Trapero-Casas & Jimenez-Diaz 1996b; Castillo, Vovlas & Jiménez 1998). Migration within chickpea roots was observed through the epidermal and cortical cells, with damage to the endodermal cells occassionally being noted in some susceptible lines (Castillo, Vovlas & Jiménez-Diaz 1998). Direct feeding inside the roots eventually causes cell death that results in small necrotic lesions and browning of the root tissue (Jones & Fosu-Nyarko 2014).

Figure 2.3: Photomicrographs illustrating adult and juvenile (J4, J3 and J2) life stages of *Pratylenchus thornei* (Thompson, Rostad & Whish 2017 p. 82.).

Pratylenchus mainly inhabit roots but being migratory can lay eggs either singularly or in groups in the root cortex or in nearby soil (Jones & Fosu-Nyarko 2014) (Fig. 2.4). Females reproduce by mitotic parthenogenesis, while *P. thornei* males are a rare occurrence (Castillo et al. 1995). Adult females of *Pratylenchus* multiply continously and can lay up to five eggs per day (Linsell et al. 2014). This growth of *Pratylenchus* and changes in population densities with time has been described as increasing in an exponential form (Seinhorst 1966).

In controlled environment studies, population densities of *P. thornei* peaked for chickpea between 18 and 19 weeks after inoculation (Reen & Thompson 2009; Reen, Zwart & Thompson 2016; Thompson et al. 2011). In field situations depending on the crop and temperature, several generations of *P. thornei* can be produced within a growing season (Thompson, Clewett & O'Reilly 2015).

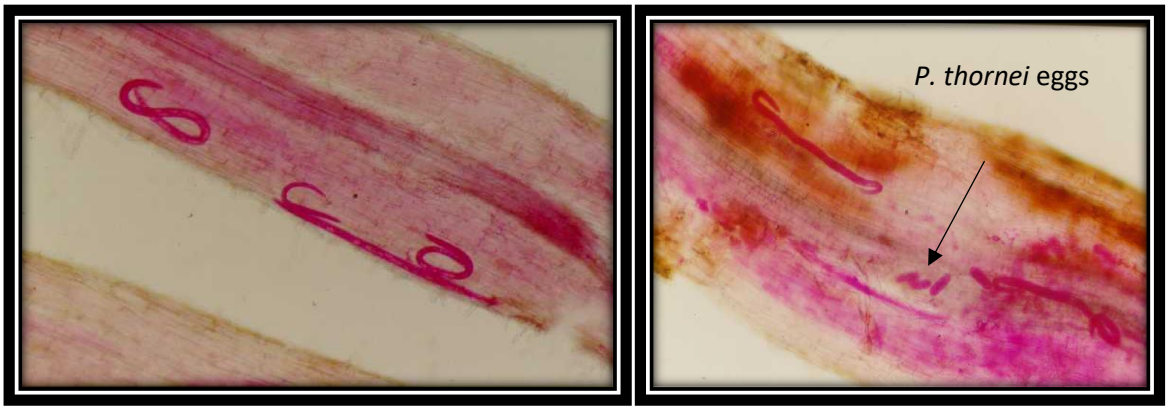

Figure 2.4: Photomicrographs illustrating sections of chickpea roots infested with vermiform *Pratylenchus thornei* and eggs stained with acid fuchsin. Samples collected at Formartin, Queensland, Australia.

2.4.3.1 Anhydrobiosis

Pratylenchus species are well adapted to abiotic stress due to their ability to undergo a process known as cryptobiosis. The procedure involves *Pratylenchus* eggs, juveniles and adults slowing their metabolic activity during adverse conditions which allows them to survive during periods of dryness, heat and cold making eradication difficult (Castillo & Vovlas 2007; Demeure and Freckman 1981). During dry periods *Pratylenchus* undergo a form of cryptobiosis, known as anhydrobiosis where they

slowly dehydrate and rehydrate when moisture is adequate. In this anhydrobiotic state they can withstand temperatures up to 40ºC, with 3% surviving three cycles of the wetting and drying process, and then multiplying twice as much in roots compared to fresh nematodes (Glazier & Orion 1983).

A laboratory study on soil desiccation by Thompson, Rostad & Whish (2017), found that *P. thornei* survival in a Vertisol soil with a starting moisture of 45% w/w (matric potential pF 3.3), was dependent on how fast the soil dried. Survival rate at 15% soil moisture was only 5% after fast drying (10.5 h), while slow drying (91.5 h) had no significant effect on *P. thornei* population densities. In the Australian grain region, low numbers of *P. thornei* have been known to survive extended fallows for up to eight years (Peck et al. 1993). In the sub-tropical grain regions of eastern Australia fallows are often used in rotations with chickpea to conserve moisture, and the survival of *P. thornei* within these soils emphasises the need for having resistant chickpea cultivars as the long term control strategy.

2.4.3.2 Edaphic factors affecting reproduction

Understanding factors that influence reproduction of *P. thornei* is critical when optimising conditions for resistance testing of crop cultivars. Reproduction rates of *P. thornei* are temperature dependent, and for chickpea, highest numbers of *P. thornei* invading roots occurs between 20–25ºC, with optimum reproduction temperature being 20ºC (Castillo, Trapero-Casa & Jimenez-Diaz 1996b). Similarly, optimum temperatures for *P. thornei* reproduction in wheat ranges between 20–25ºC with little reproduction at 15ºC, and no reproduction at 30ºC (Thompson, Clewett & O'Reilly 2015).

Identifying chickpea genotypes with resistance is normally conducted in controlled environment facilities where environmental influences that affect host and nematode reproduction such as temperature, inoculum density, soil moisture and texture, are better controlled (Nombela & Romero 1999) (Fig. 2.5). Assessment of resistance in the field is not recommended due to seasonal influences and the non-uniformity of nematode population densities (Boema & Hussey 1992). Furthermore, although screening processes are under controlled conditions, the same accession can vary in final nematode population densities in different experiments, therefore it is integral to

have good statistical design that encompasses sufficient replication and two independent screening tests (Kaplan 1990).

Owing to edaphic effects on the *Pratylenchus* life cycle, the soil type used for testing resistance should be typical of the local region where the crop is produced (De Waele & Elsen 2002). Furthermore, initial nematode density (Pi) should be calculated to a level where there is little damage to the plant but sufficient to establish a population (Peng & Moens, 2002a). A study by Castillo et al. (1995), found reproduction factors for *P. thornei* in chickpea growing in potting mix decreased when inoculum density exceeded 5,000 *P. thornei* per plant. Methods of inoculating plants vary, with nematode suspensions often pipetted into holes in the soil near the plant base, however, this may cause root damage (Peng & Moens 2003). Furthermore, studies with *P. penetrans* on alfalfa (*Medicago sativa* L. cv. Du Puits) roots, found penetration decreased with root age and the majority of females penetrated within two days after inoculation (Olthof 1982). Nematodes rely on soil moisture to move and feed, and moist soil at inoculation and during the growing period is imperative (Castillo & Volvas 2007). Soil moisture at soil field capacity provides optimum conditions for movement and reproduction (Wallace 1973).

Figure 2.5: Screening of wild *Cicer* for resistance to *Pratylenchus thornei* in a controlled environment glasshouse.

Fertiliser type can also influence nematode reproduction and plant growth, particularly in the case of mineral nitrogen, where ammonium nitrtae (NH_4NO_3) has been shown to decrease *P. neglectus* reproduction (Kimpinski, Wallace & Cunningham 1976). A study by Thompson, Clewett & O'Reilly (2015) determined that nitrate as Ca $(NO_3)^2$ was the most appropriate nitrogen source for assessing resistance to *P. thornei* in wheat. Experiments with chickpea for assessing *P. thornei* resistance, revealed a solution-based fertiliser of nitrogen (N) phosphorus (P) potassium (K) gave higher reproduction of *P. thornei* compared to Osmocote®native gardens plus micro nutrients (17-1.6-8.7 NPK) slow release fertiliser (Scott's Australia Pty. Ltd. Baulkham Hills Australia (Reen, Zwart and Thompson 2016).

2.5 Symptoms of *Pratylenchus thornei* damage on chickpea

Above-ground symptoms even under high populations of *Pratylenchu*s are frequently nondescript in chickpea (Sharma, Smith & McDonald 1992). Due to impaired root function resulting in moisture and nutrient stress particularly in dry seasons, symptoms in chickpea plants can include stunting, yellowing and reddish pigmentation on leaflets. Thus, symptoms appear as nutrient deficiencies and consequently infestations often go undiagnosed. Moreover, other biotic and abiotic stresses will also cause chickpea to exhibit similar physiological symptoms.

2.5.1. Root symptoms

Pratylenchus being migratory nematodes tend to feed from the root hairs, epidermal, and cortical cells. The J2 and J3 feed more frequently from the root hairs, while the later stages tend to feed within the roots (Jones & Fosu-Nyarko 2014). In chickpea, feeding by *P. thornei* in the cortical parenchyma, results in small necrotic brown lesions on the root and a reduction in root growth (Castillo, Vovlas & Jiménez-Diaz, 1998). Measurement of lesions of the infective roots (Ali & Ahmad 2000) is not recommended for determining levels of resistance due to the lesions being symptoms of infestation and root damage, and are not indicative of actual *P. thornei* numbers present in the roots and soil. Damage to chickpea roots by *Pratylenchus* spp. in glasshouse experiments is illustrated in Fig. 2.6.

Figure 2.6: Root browning and lesions caused by root-lesion nematode *Pratylenchus* spp. on chickpea *Cicer arietinum*

Damage to the roots by *P. thornei* allows secondary colonisation by other pathogenic organisms such as *Fusarium oxysporum* causing fusarium wilt (Castillo, Vovlas & Jiménez-Diaz, 1998*;* Castillo et al. 2008). In field situations, root discolouration cannot be conclusive of RLN damage and can be confused with infections from other soil borne pathogens such as *Phytophthora medicaginis* and dry root rot (*Rhizoctonia bataticola* Taub.) (Ali & Sharma 2003). *Pratylenchus thornei* can also reduce the number of Rhizobium nodules and root weight (Sharma, Smith & McDonald 1992; Castillo et al. 1995). Although *Pratylenchus* species cause root damage resulting in subsequent yield losses, even under high nematode pressure plant death does not occur due to efficient parasitism (Castillo & Volvas 2007).

2.5.2 Damage thresholds

Pathogenicity relates to the amount of physiological damage a pathogen can cause to a host plant (Shaner et al. 1992). Damage thresholds are minimum population densities that cause economic damage in a crop and are used as a management tool based on nematode population densities in the soil at planting, or roots and soil during the growing season (Ferris 1981). Growers can decide which crop to plant in a particular

field based on these damage thresholds. Damage thresholds can also be referred to as tolerance limits and were described by Seinhorst (1970) using a sigmoidal equation, that relates the relationship between initial population density and yield. Crop species can vary in the amount of damage they exhibit with some sustaining damage at low populations, while others can support large populations with little damage. The damage thresholds for *Pratylenchus* vary with species and geographic location (Castillo & Volvas 2007).

In Syria a field study showed the tolerance limit of chickpea was estimated at 0.031 *P. thornei*/cm^3 soil causing 40% yield loss (Di Vito, Greco & Saxena 1992). In eastern Australia, damage thresholds for wheat in the sub-tropical grain region are estimated at 2,000 *P. thornei* /kg soil (Thompson, Clewett & O'Reilly 2015). An Australian study comparing yields after growing chickpea on different *P. thornei* population densities ranging from as low as 187/kg soil after canaryseed (*Phalaris canariensis*) to high 11,654 /kg after wheat (*Triticum aestivum*) resulted in yield losses of 5–20% (Reen et al. 2014). Chickpea cultivars in this study also tended to be more tolerant to *P. thornei* than wheat. Currently, in Australia there are limited published studies related to assessing yield losses for current chickpea cultivars following different population densities of *P. thornei.*

2.6 Australian geographic distribution and host range of *P. thornei*

Pratylenchus thornei is widespread throughout the Australian grain region, occurring in 67% of fields surveyed (Hollaway et al. 2008; Thompson et al. 2010). It is the dominant species within the sub-tropical grain region of eastern Australia (Thompson et al. 1999) whilst also occurring with the species *P. neglectus* in 26% of fields surveyed (Thompson et al. 2010). Both chickpea and wheat are hosts to *P. neglectus* (Nene, Sheila & Sharma 1996; Taylor et al. 2000; Thompson et al. 2000).

The distribution of *Pratylenchus* species can be influenced by soil texture and pH, with some species preferring lighter sandy soils to heavier textured soils, and optimum pH varying with species of the host plant (Castillo and Volvas 2007). Surveys of the sub-tropical grain region by Thompson et al. (2010) found both species, *P. thornei* and *P. neglectus,* occurred in a wide range of textured soils and pH. The highest occurrence of *P. thornei* was in heavy textured soil (48% clay, 37% sand) with a pH of 8.2.

Pratylenchus neglectus occurred more frequently in the top 0–15 cm soil depth, as opposed to *P. thornei* generally found deeper in the soil profile at 30–40 cm.

Pratylenchus thornei tend to occur throughout the soil profile including depths of 1 m (Owen et al. 2014; Reen et al. 2014; Thompson et al. 1999). A study by Owen, Clewett & Thompson (2010) found that high *P. thornei* population densities after wheat harvested in November, declined substantially within the topsoil (0–15 cm) during the fallow period compared to deeper in the soil profile. Similarly, in the same study, numbers of *P. thornei* after chickpea cv. Amethyst also declined during the fallow from 5,470.to 1,050/kg soil. This rapid decline in *P. thornei* densities in the topsoil has been attributed to higher temperatures (Whish et al. 2017). A study with wheat and *P. thornei* revealed soil temperatures above 35ºC contributed to a rapid decline in soil populations within the top 15 cm (Thompson et al. 2018). Survival at depth within heavy textured soils with a high water holding capacity, and later population establishment in surface layers has been indicated as a means of survival (Whish et al. 2017).

Pratylenchus thornei has a wide host range, infesting both summer and winter crops including cereals and legumes that are grown in the Australian grain region (Table 2.1). The table illustrates that within the cropping region where chickpea are mainly grown, only three crops cotton (*Gossypium hirsutum*), millet (*Panicum miliaceum*) and sorghum (*sorghum bicolor*) have high levels of resistance making rotational options for control limited.

Table 2.1. Host range and ratings of resistance to *Pratylenchus thornei* for the main crops grown in the Australian grain region. Table adapted from Sheedy et al. (2008), Thompson et al. (2008); Vanstone, Hollaway & Stirling (2008). Cultivars within crops may vary in their resistance to *P. thornei*.

Common name	Botanical name	Resistance to *Pratylenchus thornei*
Canaryseed	*Phalaris canariensis*	High resistance
Cotton*	*Gossypium hirsutum*	High resistance
Field Pea	*Pisum sativum.*	High resistance
Lablab	*Lablab purpureus*	High resistance
Lentil	*Lens culinaris*	High resistance
Linseed	*Linum usitatissimum*	High resistance
Medic	*Medicago* spp.	High resistance
Millet*	*Panicum miliaceum*	High resistance
Pigeon Pea	*Cajanus cajan*	High resistance
Rye	*Secale cereale*	High resistance
Sorghum*	*Sorghum bicolor*	High resistance
Sunflower*	*Helianthus annuus*	Moderate resistance
Barley*	*Hordeum vulgare*	Moderate resistance
Canola	*Brassica napus*	Moderate resistance
Durum wheat*	*Triticum turgidum*	Moderate resistance
Maize*	*Zea mays*	Moderate resistance
Oats *	*Avena sativa*	Moderate resistance
Persian clover	*Trifolium resupinatum*	Moderate resistance
Triticale*	*Triticosecale*	Moderate resistance
Black gram*	*Vigna mungo*	Susceptible
Chickpea*	*Cicer arietinum*	Susceptible
Cowpea	*Vigna unguiculata*	Susceptible
Faba bean*	*Vicia faba*	Susceptible
Mung bean*	*Vigna radiata*	Susceptible
Navy bean	*Phaseolus vulgaris*	Susceptible
Soybean*	*Glycine max*	Susceptible
Subterranean clover	*Trifolium subterraneum*	Susceptible
Vetch	*Vicia sativa*	Susceptible
Wheat *	*Triticum aestivum*	Susceptible
Narbon bean	*Vicia narbonensis*	Very susceptible

*Crops commonly grown in the sub-tropical grain region of eastern Australia where chickpea are grown.

2.7 Crop management strategies for *Pratylenchus*

Management of *P. thornei* relies on an integrated system involving farm hygiene (control of soil erosion, water runoff and clean machinery) in combination with (i) correct species identification, (ii) rotations with non-host crops or weed free fallow periods, and (iii) use of tolerant and resistant cultivars (Owen, Clewett & Thompson 2010; Thompson 2008). Due to the wide host range of *P. thornei,* limited options are available for rotations and research indicates that several resistant crops in sequence are warranted to effectively reduce *P. thornei* levels in the soil (Owen et al. 2014; Thompson, Mackenzie & Sheedy 2012). Apart from cultural practices, the deployment of resistance genes through breeding cultivars with increased levels of resistance is the more achievable and sustainable way to control *Pratylenchus* (Castillo and Volvas 2007; Roberts 1992).

The use of other strategies to minimise damage, such as nematicides, is uneconomical in broad-acre dryland cropping systems. Nematicides are hazardous for the environment (Rich, Dunn & Noling 2004) moreover, 98% of cytotoxic compounds fail due to metabolism effects and the impermeable cuticle of nematodes (Burns et al. 2010). Furthermore, they are ineffective in dry seasons, and only effective in the upper layers of soil and not deep in the soil profile where *P. thornei* occur (Doyle et al. 1987; Reen et al. 2014; Thompson, Mackenzie & Sheedy 2012).

Suppression of nematodes via organic amendments based on nitrogen tend to fail due to the nematicidal compounds being relatively short lived (Li, Stirling & Seymour 2017; Oka 2010; Rahman, Whitelaw-Weckert & Orchard 2014; Stirling 2011). Suppression by microbial activity is insufficient for complete control of RLN found deep in the soil profile (Doyle et al. 1987; Stirling 2011). Furthermore, a review of organic amendments showed no decisive evidence linking organic soil amendments to suppressing nematodes (Termorshuizen, Korthals & Thoden 2011). Another biological approach is the use of antagonists such as the bacterial parasite *Pasteuria thornei* the only known host-specific parasite of *Pratylenchus* (Starr & Sayre 1988). However, while *Pasteuria thornei* can regulate nematode population densities, the levels of parasitism are insufficient to prevent nematode damage to the crop (Stirling 2014).

Recently the effect of gene silencing was investigated on *P. thornei* and *P. zeae* through the application with double stranded RNA (dsRNA) interference which reduces nematode multiplication (Tan, Jones & Fosu-Nyarko 2013). Although adding to our understanding of *Pratylenchus,* the research is still developing and while *in vitro* studies show promise, the technology has yet to be developed for successful application in broad-acre cropping situations.

2.7.1 Resistance

A major research focus of managing plant parasitic nematodes is to identify and breed cultivars with resistance or tolerance. Resistance and tolerance are separate genetic traits (France & Brodie 1996). Resistance is the ability of a plant to suppress nematode reproduction or multiplication (Trudgill 1991), as opposed to tolerance being the ability of the plant to endure the damaging effects of nematode invasion and yield well in nematode infested soil (Cook 1987).

Cultivars have varying degrees of resistance that can range through from fully resistant to partially resistant to fully susceptible. A cultivar that is termed highly resistant is one that supports little or no nematode reproduction, whereas partial resistance allows low levels of reproduction. In contrast, susceptible cultivars allow nematodes unhindered reproduction (Boerma & Hussey 1992). Chickpea cultivars can differ in their resistance levels to *P. thornei* (Castillo et al. 2008; Thompson 2008).

Natural resistance involves plant genes which can be dominant, recessive or additive in expression. The resistant genes, known as R-genes, can be identified as single major genes or a combination of two or more genes and are identified statistically as quantitative trait loci (QTLs) (Molinari 2011). The majority of research with natural genetic resistance involving R-genes has been with sedentary endoparasitic nematodes namely root-knot nematode *Meloidogyne* spp. (Jones & Fosu-Nyarko 2014). Resistant genes or QTLs described as polygenic and additive in nature, have been identified for *P. thornei* and *P. neglectus* on the A, B and D genomes in synthetic hexaploid wheats (Thompson et al. 2008; Zwart, Thompson & Godwin 2005), however, none have been identified in chickpea (Zwart et al. 2019). Genetic control of multiplication of *P. thornei* and *P. neglectus* in previous research with chickpea was shown to be governed by independent genes (Thompson et al. 2011). Introgression of the wild species *C.*

echinospermum and *C. reticulatum* into domesticated cultivars to improve RLN resistance revealed this resistance was highly heritable and quantitative (Rodda et al. 2016; Thompson et al. 2011). To date the mechanisms governing *Pratylenchus* resistance in chickpea are unknown. Plant resistance is complex involving both biochemical reactions by the plant and nematode response with little being known of the mechanisms involved for *Pratylenchus spp.* (Castilo & Volvas 2007). Identifying resistant wild *Cicer* in this current study will support future molecular studies on the mechanisms involved in chickpea resistance.

In instances where a pathogen has a high capacity to produce new recombinant types there is the potential of the pathogen to overcome resistance (McDonald & Linde 2002). However, often for nematodes there is durability of resistance, even when resistance is conferred by a single dominant gene (Roberts 1992) and to date no breakdown of resistance has been reported for *Pratylenchus.* Breakdown in resistance is more likely to occur when there are sexual reproduction systems, high mutation rate, large population sizes and high potential for gene flow, however, when these factors are low such as in *P. thornei*, and there is strict asexual reproduction there is less chance of this occurring (McDonald & Linde 2002).

2.7.2 Tolerance

The use of tolerant cultivars is integral to management of root-lesion nematodes however, tolerant cultivars can also be susceptible allowing reproduction and build-up of nematode population densities within the soil. Tolerance in crops such as pulses and cereals is measured by grain yield in the field. This can be assessed on either high populations of nematodes, or on plots of high and low nematode population densities that include reference cultivars with known tolerance responses for calibration (Thompson et al. 1999). Crop cultivars can differ in their levels of tolerance to *Pratylenchus* with some very tolerant cultivars supporting large nematode populations without any adverse effects on the plant and yield, as opposed to intolerant cultivars that sustain heavy yield losses. Tolerance ratings to *P. thornei* in current, Australian commercial cultivars range from moderately intolerant–intolerant (MII) to tolerant (T) (GRDC 2020).

In chickpea breeding programs, selection of tolerance to nematodes has been beneficial (Ansari et al. 2004; Sharma et al. 1995). Incorporating both resistance and tolerance traits is valuable for chickpea, due to the suppression

of *P. thornei* reproduction being combined with good yield ability (Reen et al. 2014; Starr, Bridge & Cook 2002)This strategy has been successfully used for wheat breeding where combined resistance and olerance to *P. thornei* has resulted in increased yields compared with cultivars being merely tolerant (Sheedy & Thompson 2009).

2.7.3 Crop rotation

Crop rotations are integral for managing levels of *P. thornei* in the soil. Crop cultivars vary in their resistance and tolerance status to each RLN species, therefore, effective management relies on correct species identification. A study on chickpea rotations by Reen et al. (2014) found lower chickpea yields resulted after wheat where the highest populations of *P. thornei* occurred (~11,600/kg soil), and highest yields were after canaryseed (*Phalaris canariensis* cv. Moroccan) with lower *P. thornei* residual populations ~1,000/kg soil. A similar study with several summer crop rotations by Owen et al. (2014) showed highest *P. thornei* populations and lowest wheat yields were after mungbean (*Vigna radiata*), black gram (*V. mungo*) or soybean (*Glycine max*), while highest wheat yields followed sorghum, sunflower, and millet/panicum cultivars which had lowest *P. thornei* populations.

Crop selection is important in crop sequences with chickpea due to its dependency on natural soil-borne arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhiza fungi are involved in the uptake of both phosphorous (P) and zinc (Zn) (Farzaneh et al. 2011). Australian soils are known for their low fertility in phosphorus and zinc with zinc deficiency being the most widespread of micronutrients (Alloway, Graham & Stacey 2008). In the sub-tropical eastern grain region, wheat is commonly grown in the winter season (May to July), followed by a weed-free summer fallow period of about 6 months (November to April), and with chickpea planted in the following winter season. In field situations, the use of weed-free fallows and non-mycorrhizal crops such as canola, while reducing *P. thornei* populations, also reduce AMF levels which was found detrimental to chickpea yields (Reen et al. 2014).

Controlled environment studies show the most common effect of AMF is the promotion of tolerance within the plant to nematodes (Anwar & Zaki 2005; Hussey & Roncadori 1982). Castillo and Volvas (2007) cite many studies worldwide reporting the diversity of interactions between AMF and nematodes, however, less is known about the effect of migratory nematodes particularly *P. thornei* and

interactions with AMF and chickpea in promoting tolerance.

2.8 Sources of resistance to *Pratylenchus thornei* in chickpea

The search to identify *P. thornei* resistant chickpea cultivars has been extensive, however, there has been little success with the cultigen *C. arietinum.* In Syria, Greco et al. (1988) screened 97 chickpea accessions and found all were susceptible. In India, over 850 *C. arietinum* lines were screened during the period 1992–2000 with 35 lines identified as resistant (Zwart et al. 2019). Research by Thompson et al. (2011) screened over 453 *C. arietinum* accessions for *P. thornei* and *P. neglectus* resistance. Entries comprised international cultivars sourced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the International Centre for Agricultural Research in Dry Areas (ICARDA) plus 29 Australian domestic cultivars, 171 breeding lines and 107 wild *Cicer* genotypes. No international cultivars showed any greater resistance to *P. thornei* than the current Australian cultivars, with the exception of the Indian cultivar ICC11323.

In contrast, the wild *Cicer* species *C. reticulatum*, *C. echinospermum* and *C. bijugum* showed improved resistance compared to domestic chickpea (Thompson et al. 2011). Progeny from crosses of accessions of *C. echinospermum* and *C. reticulatum* with chickpea cultivars Jimbour and Howzat resulted in some improved *P. thornei* resistant breeding lines (Reen, Thompson & Knights 2011; Rodda et al. 2016; Thompson et al. 2011). Moreover, the limited collection of original wild *Cicer* accessions available at that time hindered efforts to find improved resistance and diversity. Similarly, of 147 wild *Cicer* tested by Di Vito et al. (1995), six *C. bijugum*, three *C. cuneatum*, 11 *C. judacium* and one *C. yamashitae* were identified with good resistance, however, these wild *Cicer* species are not crossable with domesticated chickpea.

Worldwide, there are 44 gene banks with stored collections of 98,313 accessions of chickpea landraces, cultivars and wild relatives (Smýkal et al. 2015). Major collections of chickpea germplasm for resistance breeding are stored within six of these gene banks with the largest two being at ICRISAT in India and at ICARDA in Morocco. The three major genebanks housing the world collection of annual wild *Cicer* are ICARDA, The National Genetic Resources Program Pullman (USDA-ARS) in the United States, and the Australian Grains Genebank Australia (AGG) (Berger, Abbo & Turner. 2003). Collections of wild chickpea compared to landraces and cultivars are

underrepresented, constituting less than 1% of the total global chickpea accessions, even though they are genetically rich in comparison (Singh, Upadhyaya & Bisht 2013).

In the Australian chickpea breeding program, nine out of 36 breeding lines in advanced germplasm trials in the year 2000 had wild *C. echinospermum* in their pedigree (Berger, Abbo & Turner 2003). The importance of wild annual *Cicer*, led Berger, Abbo &. Turner (2003) to undertake a review of the world collection of annual wild *Cicer* species. The review identified (i) that original collections consisted of only 18 original accessions of *C. reticulatum* and ten of *C. echinospermum* (Table 2.2), (ii) there was multiple duplication of these original accessions, (iii) only a small amount of disease resistance was reported for wild *Cicer*, and (iv) genetic diversity for multiple traits present in wild germplasm was compromised due to the state of world collections, the limited access and low numbers of original annual wild accessions (Abbo, Berger & Turner 2003; Berger, Abbo & Turner 2003). The review resulted in an international collaborative effort by scientists to boost collections of wild *Cicer*, particularly, *C. reticulatum* and *C. echinospermum.* The resulting collection is the focus of this thesis. The collection spans geographic and climate variables of the wild *Cicer* species that influence evolutionary genetics, resulting in more genetically diverse germplasm previously not available in world genebanks (Von Wettberg et al. 2018). It is envisaged that any future introgression of resistance to *P. thornei* resulting from results of this thesis will deliver more robust and diverse *P. thornei* resistant chickpea cultivars for growers use.

Table 2.2. Comparison of total numbers of annual wild *Cicer* accessions in genebanks prior to the recent 2013 to 2015 collection to actual original accessions held in world collections, based on data from the genebanks of the Consultative Group of International Agriculture Research (CGIAR) system including those registered with the International Plant Genetic Resources Institute. Table adapted from Berger, Abbo & Turner (2003).

Cicer species	Gene pool	Total accessions	Original
C. echinospermum	Primary	43	10
C. reticulatum	Primary	139	18
C. bijugum	Secondary	104	20
C. judacium	Secondary	135	34
C. pinnatifidum	Secondary	98	28
C. chorassanicum	Tertiary	13	2
C. cuneatum	Tertiary	12	1
C. yamashitae	Tertiary	14	3
Total		558	116

2.8.1 Exploiting crop wild relatives

The use of crop wild relatives for crop improvement dates back some 70 years (Hajjar and Hodgkin 2007). A survey by Hajjar and Hodgkin (2007) of 13 major world crops, found the majority of beneficial traits conferred by CWR genes were related to pest and disease resistance. The survey revealed chickpea at the time had no cultivars with disease resistance derived from wild relative genes and only one cultivar from India (BG1103) derived from a *C. reticulatum* had been released with drought tolerance.

Exploiting wild species for crop improvement is not a new concept and in the case of wheat, substantial advances have been made for incorporation of resistance to *P. thornei* (Sheedy & Thompson 2009; Sheedy, Thompson & Kelly 2012; Thompson 2008). Interspecific hybridisation of wild annual *Cicer* species with cultivated chickpea has the potential to widen the genetic base of cultivated chickpea without penalty for yield or adaptation (Koseoglu et al. 2017; Singh & Ocampo 1997; Singh et al. 2005; Singh et al. 2015). Furthermore, breeding programs benefit the most when

wild germplasm encompasses the complete geographical and climatic range of the species (Von Wettberg et al. 2018).

Chickpea genotypes with multiple resistance are also valuable in breeding programs, and wild *Cicer* tend towards having genes for multiple resistances to major biotic and abiotic stresses compared to *C. arietinum*. Breeding efforts have been directed towards introgressing individual sources of resistance into one adaptive genotype (Singh et al. 1993). Earlier research involving wild relatives *C. bijugum*, *C. pinnatifidum* and *C. echinospermum* revealed at least one accession of each species had multiple resistance to ascochyta blight (*Ascochyta rabiei*), fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*), leaf miner (*Liriomyza cicerina* Rodani), bruchid (*Callosobruchus chinensis* L.) cyst nematode (*Heterodera ciceri* Vovlas, Greco & Divito), and cold tolerance (Singh 1998). Interestingly, for the eight different countries where accessions originated the multiple resistance principally originated from accessions of Turkish origin (Singh 1998). Later research found higher and more stable yields were possible with the introgression of *C. reticulatum* into chickpea cultivars, where progeny displayed improved yield, drought tolerance and *Fusarium wilt* resistance (Yadav et al. 2004). Similarly, for the pod borer (*Helicoverpa armigera),* a major pest in Australia and India, moderate resistance was identified in *C. reticulatum* (Sharmad et al. 2005). Furthermore, a small sub-set of *C. echinospermum* accessions had improved resistance to both *P. thornei* and *Phytophthora medicaginis* with several derivatives also having excellent seed quality for food processing (Knights et al. 2008; Reen, Thompson & Knights 2011; Woods et al. 2019). Where genetic variability and disease resistance in cultivated germplasm is low, introducing genetic diversity using wild relatives is crucial to sustain productivity and prevent virulent biotypes within diseases (Kameswara Reddy & Bramel 2003).

2.9 Summary of research literature and benefits of this research

In summary, it is evident that *P. thornei* has a broad host range and is prevalent in the grain cropping regions of Australia and overseas. Genetic solutions through plant breeding for resistance offers the most sustainable and long term solution for chickpea improvement. The development of resistant cultivars that reduce *P. thornei* populations are needed for successful crop rotations and will help address industry demands for a more profitable legume (Thompson et al. 2008). Furthermore,

collections of wild *Cicer* from the primary genepool such as the one assessed in this study, are integral to combating future disease challenges in the 21st century and imperative for assessing new allelic variation for crop improvement.

This thesis will provide the first data on resistance to *P. thornei* of chickpea in this new wild *Cicer* collection. Characterising the new collection for *P. thornei* resistance will (i) offer plant breeders and scientists, resistance rankings that can be utilised at national and international levels and (ii) provide the essential base for future identification of the genetics controlling *P. thornei* resistance. Furthermore, the results from this thesis will have international linkages, and the future deployment of wild *Cicer* affords the opportunity to develop more resilient chickpea with multiple resistance to stresses, that are better equipped to meet challenges in the future.

CHAPTER 3 PUBLICATION

3.1 Overview

This chapter is presented as published in the American *Phytopathology* Journal under the citation:

Reen RA, Mumford MH, Thompson JP, 2019, 'Novel sources of resistance to root-lesion nematode (*Pratylenchus thornei*) in a new collection of wild *Cicer* species (*C. reticulatum* and *C. echinospermum*) to improve resistance in cultivated chickpea (*C. arietinum*)*'. Phytopathology,* vol. 109, no. 7, pp. 1270-79. doi: 10.1094/PHYTO-02-19-0047-R

This paper outlines the research involved in characterising 174 new accessions of wild *Cicer* collected from 21 locations in southeastern Anatolia (Turkey) to identify levels of resistance to the root-lesion nematode *Pratylenchus thornei*. An overview of chickpea, the impact of *P. thornei* within the chickpea industry and previous research to identify resistance is outlined. Information on the new collection is reported plus details on the methodology used to screen the collection. The paper concludes with a summary of the findings in terms of levels of resistance to *P. thornei*, how these levels compare to what is currently available in Australian germplasm and how the findings can benefit the chickpea industry. Supplementary tables to this paper are in Appendix A following Chapter 4.

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Nematology

e-Xtra*

Novel Sources of Resistance to Root-Lesion Nematode (*Pratylenchus thornei*) in a New Collection of Wild *Cicer* Species (*C. reticulatum* and *C. echinospermum*) to Improve Resistance in Cultivated Chickpea (*C. arietinum*)

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ABSTRACT

Pratylenchus thornei, a nematode species that feeds and reproduces in chickpea (*Cicer arietinum*) roots, is widespread throughout the Mediterranean basin and Indian subcontinent. In Australia, it can cause yield losses up to approximately 25% of intolerant chickpea cultivars. Potential for improvement has been hindered by the narrow genetic diversity of cultivated chickpea and a limited world collection of original wild *Cicer* spp. in the primary gene pool, consisting of 18 *C. reticulatum* and 10 *C. echinospermum* accessions. Recently, collections of *C. reticulatum* and *C. echinospermum* from Turkey have substantially increased the number of accessions. This study evaluated 133 *C. reticulatum* and 41 *C. echinospermum* accessions from the new collection for resistance to *P. thornei* under controlled conditions in repeated glasshouse pot experiments. The aim of the study was to identify accessions with resistance superior to that currently available in Australian germplasm. Both wild *Cicer* spp. were found, on average, to be more resistant to *P. thornei* ($P < 0.001$) than *C. arietinum.* Combined analyses across experiments to determine genetic rankings showed that 13 (7%) wild accessions were significantly more resistant than the most resistant *C. echinospermum* reference ILWC 246, while another 40 (23%) accessions were significantly more resistant than the least susceptible Australian chickpea cultivar PBA Seamer. Mean *P. thornei* population densities differed significantly between collection sites in Turkey and within each of the genetic population groups. The sites Kayatepe and Baristepe1, and genetic population groups Ret_A and Ret_F associated with sites Oyali and Baristepe1, produced the lowest *P. thornei* population densities. This is the first report assessing the resistance to *P. thornei* of this new collection which offers novel sources of *P. thornei* resistance and untapped genetic diversity valuable for international chickpea breeding programs to exploit.

Keywords: disease control and pest management, genetics and resistance, nematology, root biomass, root-lesion nematode, wheat *Triticum aestivum*, wild chickpea

In the past decade, there has been a major focus in agriculture worldwide oriented toward increased pulse production. Pulses such as chickpea (*Cicer arietinum*) supply starch, protein, and cholesterol-lowering dietary fiber (Perez-Hidalgo et al. 1997). Chickpea belong to the small group of crop species (<20) that feed the world population today (Smýkal et al. 2018). Over 95% of chickpea production occurs in developing countries, where it has an important role in improving food security and reducing malnutrition (Kozgar 2014). Global demand has rapidly increased since 2004, resulting in valuable exports from developed countries such as Australia, where the export value of chickpea in 2017 was approximately 2 billion Australian dollars (ABARES 2017; Chauhan et al. 2017). Currently, India is the largest producer and Australia ranks as the world's largest exporter of chickpea (FAOSTAT 2017; Pulse Australia 2016).

Over 90% of chickpea production in Australia is within the subtropical, semiarid grain region in the northeast of the country (Chauhan et al. 2017). In this region, chickpea also plays a beneficial role as a rotational crop in cereal-dominated systems by fixing nitrogen (N) and reducing the incidence of *Fusarium pseudograminearum* (crown rot) (Dalal et al. 1998; Felton et al. 1998). Counteracting these rotational benefits is the presence of the root-lesion nematode (*Pratylenchus thornei*), a migratory endoparasite that feeds and reproduces in the roots of cereals and pulses, destroying cortical cells (Fortuner 1977). Globally, the negative impact on chickpea production by root-lesion nematodes (*Pratylenchus* spp.) ranks second to root-knot nematode (*Meloidogyne* spp.), particularly in the Mediterranean basin and Indian subcontinent, where *P. thornei* is a major constraint to legume and cereal production (Carrasco-Ballesteros et al. 2007; Castillo et al. 1998a, 2008). *P. thornei* is also the most common plant-parasitic nematode species within the subtropical grain region of Australia (Thompson et al. 1999, 2010). Where chickpea is often grown in rotation with wheat (*Triticum aestivum*), yield losses of up to 25 and 50%, respectively, in intolerant cultivars have been attributed to *P. thornei* (Reen et al. 2014; Thompson et al. 2000). To date, there is no published evidence of information regarding totally resistant chickpea or wheat cultivars and current management relies on growing tolerant cultivars and rotation with resistant crops. Growing several resistant crops in a cropping sequence or maintaining weed-free fallow over approximately 3 years is necessary to effectively reduce *P. thornei* populations in the soil to below the damage threshold of 2,000 nematodes/kg of soil (Owen et al. 2014; Reen et al. 2014; Thompson et al. 2010, 2017; Whish et al. 2017). Incorporating genetic resistance through plant breeding into cultivars of susceptible crop species is the most effective and economical way to manage the overall problem by reducing the high

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*The *e-Xtra* logo stands for "electronic extra" and indicates that two supplementary tables are published online.

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population densities of *P. thornei* remaining in the soil after growing susceptible crops.

Resistance and tolerance to nematodes of crop cultivars are considered independent genetic traits (France and Brodie 1996). Resistance is the ability of a plant to reduce nematode reproduction (Trudgill 1991) and tolerance is the capacity of the plant to yield well in nematode-infested soil (Cook and Evans 1987). Current breeding efforts struggle to produce disease-resistant chickpea cultivars that will extensively increase production (Singh et al. 2015) owing to the narrowing of genetic diversity of traits resulting from domestication and a series of selective constraints during the evolution of the crop (Abbo et al. 2003; Ladizinsky and Adler 1976; Rao et al. 2007; Varshney et al. 2013). In contrast, multiple studies have shown that wild *Cicer* spp. offer superior sources of genetic resistance and diversity, making them a valuable resource for chickpea improvement (Abbo et al. 2003; Andeden et al. 2013; Muñoz et al. 2017; Rao et al. 2007; Sudupak et al. 2002; Toker et al. 2007). Interspecific hybridization of wild annual *Cicer* spp. has the potential to widen the genetic base of cultivated chickpea without penalty to yield or adaptation (Koseoglu et al. 2017; Singh and Ocampo 1997; Singh et al. 2015; Singh et al. 2005).

The annual species *C. reticulatum* and *C. echinospermum* in the primary gene pool of chickpea are the only crop wild relatives (CWR) cross-compatible with domestic chickpea (Croser et al. 2003). Both species occur only in a restricted area of southeastern Anatolia (Turkey) within rocky slopes, open forests, orchards, vineyards, and fields (Ballard et al. 2006; Tanno and Willcox 2006). Both species are currently under threat from urbanization, climate change, grazing, and industrial development (Talip et al. 2018; Von Wettberg et al. 2018). In early research, resistance to *P. thornei* was found in *Cicer* spp. from the secondary gene pool (*C. bijugum*, and *C. judaicum*) and tertiary gene pool (*C. cuneatum* and *C. yamashitae*) but not in *C. reticulatum* or *C. echinopsermum* (Di Vito et al. 1995). In subsequent research, resistance to *P. thornei* was identified in some accessions of *C. reticulatum* and *C. echinospermum*, resulting in incremental gains for resistant chickpea (Rodda et al. 2016; Thompson et al. 2011). However, at that time, the genetic diversity in wild germplasm held in seed gene-bank collections was compromised due to a limited world collection that consisted of only 18 original *C. reticulatum* and 10 *C. echinospermum* accessions (Abbo et al. 2003).

Recently (2013 to 2015), new collection missions in southeastern Turkey that spanned both the genomic diversity and environmental range of the wild progenitor species *C. reticulatum* (Ladizinsky and Adler 1976) resulted in numbers of accessions of *C. reticulatum* and *C. echinospermum* increasing 21-fold and 9-fold, respectively (Von Wettberg et al. 2018). A study of the genetic variation in this collection through genotyping-by-sequencing and analysis in the program STRUCTURE identified four different genetic population groups of *C. echinospermum* and eight of *C. reticulatum* (Von Wettberg et al. 2018). These genetic populations formed a cline largely related to the location of collection sites in an east-west transect through southeast Anatolia. To understand the consequences of domestication, whole-genome sequencing was performed on 26 diverse wild accessions. From these studies, it was concluded that 94 to 98% of the progenitor genetic variation in this CWR collection is currently lacking in breeding programs of the cultigen *C. arietinum.* The 26 diverse wild accessions were chosen for construction of introgression populations, with seven international elite *C. arietinum* cultivars representing major global climatic chickpea production zones (Von Wettberg et al. 2018).

Characterizing this new, more extensive collection of chickpea wild relatives for *P. thornei* resistance will enable selection of germplasm that will potentially widen the genetic base for resistance and the adaptive diversity of chickpea. Growing chickpea cultivars with a high level of resistance will result in reduced nematode populations in the soil that will allow diversified rotational options and more economical yields for growers. The purpose of this research was to (i) characterize individual accessions in this new, wider collection of chickpea wild relatives for resistance to *P. thornei,* in comparison with a selection of Australian chickpea cultivars; (ii) compare the level of resistance within the two wild species *C. reticulatum* and *C. echinospermum* with the cultivated species *C. arietinum*; (iii) assess the effect of geographic location on level of resistance in the CWR; and (iv) assess the effect of genetic population groups on level of resistance in the CWR.

MATERIALS AND METHODS

Plant phenotyping. Two experiments were conducted during the winter to spring season in Australia (July to November) in 2016 (experiment 1) and 2017 (experiment 2) to assess the resistance to *P. thornei* of 174 annual wild *Cicer* accessions collected from Turkey in 2013. Accessions obtained from the Australian Grains Genebank consisted of 133 *C. reticulatum* and 41 *C. echinospermum* collected from 21 sites within five provinces of southeastern Turkey. The number of accessions collected within each site varied from 1 to 17. Each site and province had only *C. reticulatum* or *C. echinospermum* present, with the exception of the province Diyarbakir, where both species were collected (Table 1). In all, 22 reference cultivars that ranged in levels of *P. thornei* resistance and 1 inoculated unplanted control treatment were included in the experiments. The reference cultivars included 11 Australian desi chickpea cultivars that were moderately susceptible (PBA Boundary, PBA HatTrick, PBA Seamer, PBA Pistol, Flipper, Howzat, and Yorker), susceptible (Jimbour, Sona, and Sonali), and very susceptible (Kyabra) (GRDC 2019). One desi cultivar found to be resistant in India (ICC11323) and one moderately resistant *C. reticulatum* derivative (00283-1095-1002 from the cross ILWC 140 × Jimbour) were also included. Based on our earlier research (Thompson et al. 2011), four other reference wild *Cicer* accessions were included which consisted of one resistant and one susceptible *C. reticulatum* (ILWC 123 and ILWC 184, respectively) and one resistant and one moderately susceptible *C. echinospermum* (ILWC 246 and ILWC 39, respectively). Hexaploid wheats were also included as references for a range of resistance or susceptibility to *P. thornei*; namely, susceptible (Petrie and Batavia), moderately susceptible (Sunzell), moderately resistant (QT8343) (Sheedy and Thompson 2009), and resistant (CPI133872) (Zwart et al. 2005).

Assessing accessions for resistance to *P. thornei.* All accessions were tested twice, with the exception of 23 accessions comprising 14 *C. reticulatum* and 9 *C. echinospermum*, which were not available in time for experiment 1 and were tested once in experiment 2. Resistance levels for each accession were determined by final nematode populations (Pf) in the soil and roots after 18 weeks of growth. Accessions with higher Pf values were considered more susceptible than those with lower Pf values.

Both experiments were conducted in controlled-environment glasshouses with air temperatures maintained at approximately 20 to 25°C. Under-bench heating maintained the soil temperature in pots at approximately 22°C, the optimum temperature for *P. thornei* reproduction (Thompson et al. 2015b). Benches were fitted with a bottom-watering system set at 2 cm of water tension regulated by a float valve. Accessions were replicated three times in a row–column randomized block design. Single plants were grown in 70-mm square pots (150 mm high) containing 330 g (oven-dry equivalent) of black Vertosol (Isbell 1996) of the Waco series (Beckmann and Thompson 1960), representative of soils in which chickpea is grown in the region. Soil was pasteurized with aerated steam at 85°C for 45 min (modified from Thompson [1990]). A solution-based fertilizer of nitrogen, phosphorus, and potassium (NPK) supplying NO_3-N at 200 mg/kg of soil, P at 25 mg/kg of soil, K at 88 mg/kg of soil, S at 36 mg/kg of soil, and Zn at 5 mg/kg of soil was mixed with 80% of the total soil volume in each pot. In both experiments, the

seeds of wild *Cicer* spp. were cut at the endosperm end with a scalpel to enable imbibition for germination. Inoculum was produced by open-pot culture on susceptible wheat (O'Reilly and Thompson 1993) using a pure culture of *P. thornei* originating from 10 specimens collected from a field at Formartin (27.46401°S, 151.42616°E, 364 m in elevation, 70 km west of Toowoomba, Australia) (Thompson 2008). *P. thornei* nematodes were extracted from soil and roots of the cultures using the Whitehead tray method (Whitehead and Hemming 1965). The suspension of nematodes for inoculum consisted of adult females and juveniles and was adjusted to deliver 3,300 *P. thornei* per pot, equivalent to 10,000 nematodes/kg of soil (oven-dry equivalent). Final ratios of adults to juveniles for inoculum suspensions were 32% adults and 68% juveniles for experiment 1 and 35% adults and 65% juveniles for experiment 2. At planting, the seed was placed on top of moistened soil within each pot and inoculated with a 0.5-ml slurry of rhizobium group N *Mesorhizobium ciceri* strain CC1192 on peat carrier. This was followed by a 10-ml nematode suspension pipetted around the seed. Following nematode inoculation, the remaining 20% soil volume was placed to cover the seed, rhizobium, and nematode inoculum.

Plant harvest, nematode extraction, and enumeration. Plants were harvested at 18 weeks with the water supply to benches turned off approximately 2 to 3 days prior to harvest date to allow the soil to dry to approximately 45% moisture content. At harvest, the plant height and plant growth stage of chickpea (Lancashire et al. 1991) and of wheat (Zadoks et al. 1974) were recorded. Plant biomass was measured by collecting plant tops cut at soil level and dried in a forced-draft oven at 80°C for 48 h. The soil from each pot was teased apart, roots were cut into approximately 10-mm lengths, and soil and roots mixed together manually. Nematodes were extracted by the Whitehead tray method (Whitehead and Hemming 1965) by placing a 150 g of moist subsample of soil and roots from each pot on a single Kimwipe tissue (KIMTECH; Kimberly-Clark Worldwide, Inc.) for 48 h at 22°C. Soil gravimetric moisture content was determined on a 100-g subsample dried in a forced-draft oven at 105°C for 48 h. Nematodes were collected on a 20-µm aperture sieve in approximately 15 ml of tap water, transferred into 30-ml vials, and stored at 4°C until enumeration. *P. thornei* were counted in a 1-ml Peters slide (Peters 1952) (purchased from Chalex Corporation) under a compound microscope (×40). Pf densities were expressed as number of nematodes per kilogram of soil and roots (oven-dried equivalent) or nematodes per gram of dry weight root, after determination of root biomass as described below.

Root biomass and *P. thornei* per gram of root. To determine whether there was any relationship between root biomass and final *P. thornei* densities, 167 chickpea and wild *Cicer* accessions from experiment 1 were assessed for root biomass. To assess root biomass, the 150-g subsample of soil and roots was retained immediately following nematode extractions and stored at 4°C in polypropylene plastic food containers (90 mm in diameter by 83 mm in height) until extraction of roots within the following 1 to 4 days. The roots were recovered by transferring each soil sample to a 10-liter bucket of tap water. Roots and soil were then manually agitated and decanted onto a sieve (250-µm aperture) to collect roots, which were washed under running tap water for further cleaning. This process was repeated several times until all roots were recovered from the sample. For final cleaning, the roots were returned to the original containers and the containers filled with water to allow further settling of any remaining sediment; floating debris was removed manually. The suspension of roots was then manually agitated and decanted onto a sieve (250-µm aperture) for final collection of roots. The roots were blotted with paper toweling, then dried in a forced-draft oven at 65°C for 4 days and values expressed as dry weight root per kilogram of soil. Determining root biomass enabled *P. thornei* numbers to be expressed as *P. thornei* per gram of dry weight root.

Statistical design and analyses. Linear mixed models were used to analyze *P. thornei* population densities by the residual maximum-likelihood procedure (Patterson and Thompson 1971) within the ASReml-R package (Butler et al. 2009) in the R software environment (R Core Team 2018). A logarithmic transformation $\log_e(x)$ was required for the analysis of *P. thornei* per kilogram (soil + roots) and *P. thornei* per gram (roots) (Marks and Proctor 1974).

The primary analysis investigated genetic effects for each individual accession across the two experiments using the methods

TABLE 1. Passport information on the collection of wild chickpea accessions in terms of code number, genetic population, and species aligned with province and collection site of origin for accessions collected in 2013 from southeastern Turkey and assessed for *Pratylenchus thornei* resistance[y]

Province, collection site	Species[z]	Group	*N*	Prefix	Suffix and accession code number
Adiyaman					
Oyali	*C. ret*	Ret_A	8	Oyali	071, 073, 076, 084, 100, 104, 105, 107
Mardin					
Baristepe1	*C. ret*	Ret_F	8	Bari1	062, 063, 064, 068, 069, 091, 092, 093
Baristepe2	*C. ret*	Ret_E	5	Bari2	062, 064, 067, 072, 074
Baristepe3	*C. ret*	Ret_E	17	Bari3	064, 065, 067, 072C, 073, 074, 075, 079,091, 092, 100, 101, 102, 103, 106D, 110, 112
Beslever	*C. ret*	Ret_D	8	Besev	061, 062, 065, 066, 074, 075, 079, 083
Dereici	*C. ret*	Ret_D	10	Derei	062, 065, 066, 069, 070, 072, 073, 074, 075, 078
Kayatepe	*C. ret*	Ret_D	7	Kayat	061, 063, 064, 066, 070, 077, 080
Sarikaya	*C. ret*	Ret_D	10	Sarik	061, 064, 065, 066, 067, 073, 074, 077, 078, 080
Savur	*C. ret*	Ret_D	1	Savur	063
Sirnak					
CudiB	*C. ret*	Ret_G	12	CudiB	004, 005, 006, 008B, 009, 011, 016, 017, 018, 019, 022C, 023
CudiA	*C. ret*	Ret_G	14	CudiA	101A, 103C, 104, 105, 122, 124, 127, 128, 151,152, 153, 154, 155, 221
Sirnak	*C. ret*	Ret_H	10	Sirna	060, 064, 071C, 081B, 082, 083, 084, 085, 104, 105
Diyarbakir					
Kesentas	*C. ret*	Ret_B	10	Kensen	062, 065, 066, 067, 071, 073, 075, 077, 101, 104
Egil	*C. ret*	Ret_C	7	Egil	063, 065, 066, 072, 073,074, 075
Kalkan	*C. ret*	Ret_C	6	Kalka	061, 064, 066, 067, 070, 074
Gunasan	*C. ech*	Ech_A	2	Gunas	062, 100
Cermik	*C. ech*	Ech_A	5	Cermi	063, 071, 072, 073, 075
Urfa					
Destek	*C. ech*	Ech_A	9	Deste	061, 063, 064, 071, 072, 073, 075, 079, 080
Siv-Diyar	*C. ech*	Ech_B	11	S2Drd	061, 062, 065, 100, 101, 102, 104, 105, 106, 107B, 109
Karabahce	*C. ech*	Ech_B	12	Karab	062, 063, 081, 082, 084, 086, 091B, 092, 162, 171, 172, 174
Ortanca	*C. ech*	Ech_C	2	Ortan	061, 066

[y] All wild *Cicer* accessions are identified by an original collection code number supplied by the Australian Grains Genebank (AGG). Group = genetic population group, *N* = number of accessions, and Prefix = code name used before the suffix number.

[z] Abbreviations: *C. ret.* = *Cicer reticulatum* (wild *Cicer*) and *C. ech.* = *C. echinospermum* (wild *Cicer*).

proposed by Smith et al. (2001). Because the main objective was genetic selection of resistance, experiment was fitted as a fixed effect while accession was considered as random. The genetic correlation between the two experiments was also estimated (Smith et al. 2001), resulting in accession predictions that were empirical best linear unbiased predictors (BLUPs). Residual variances were estimated for each experiment separately and spatial trend was accounted for using the methods of Gilmour et al. (1997).

Subsidiary analyses were performed to investigate whether significant differences existed between accessions nested within (i) *Cicer* spp., (ii) provinces, (iii) collection sites, and (iv) genetic population groups. Each of the subsidiary analyses was performed for each experiment separately, giving a total of eight subsidiary analyses. Contrasts were set up to test commercial cultivars and wild relatives as two separate groups for the nested variables and their interactions (Bell and Mumford 2017). In order to set up the contrasts, the corresponding nested variables were fitted as fixed effects, resulting in empirical best linear unbiased estimators (BLUEs). Comparisons of significant differences within the corresponding nested variable were completed using Fisher's protected least significant difference testing.

Reproduction factors (RF) were calculated as the ratio of the final population density of nematodes divided by the initial inoculation rate, both expressed per kilogram of soil. From the combined analysis, genotypes with final population densities significantly ($P < 0.05$) less than *C. echinospermum* ILWC 246 (RF range 0.28 to 0.40) were rated resistant while those significantly less than chickpea cultivar PBA Seamer (RF range 0.41 to 0.65) were rated moderately resistant.

RESULTS

Final *P. thornei* population densities (Pf) for accessions. In each experiment, *P. thornei* population densities were continuously distributed with significant ($P < 0.001$) differences between accessions. Population densities of *P. thornei* in experiment 1 ranged from the most resistant wild accession Oyali_071 (990 nematodes/kg of soil + roots) to susceptible chickpea reference cultivar Kyabra (89,199 nematodes/kg) (data not shown). Overall, 29 (19%) wild *Cicer* accessions produced fewer *P. thornei* than the unplanted reference treatment (3,337 nematodes/kg). For the wheat references, population densities of *P. thornei* ranged from 3,380 nematodes/kg for the resistant synthetic hexaploid wheat CPI133872 to 87,566 nematodes/kg for susceptible Petrie.

In experiment 2, *P. thornei* population densities for wild *Cicer* accessions ranged from 3,041 nematodes/kg for ILWC 123 to 108,012 nematodes/kg for Savur_063. Twenty (11%) of the wild *Cicer* accessions had lower population densities than the unplanted reference (4,915 nematodes/kg). The reference wheat accessions ranged from 5,432 nematodes/kg for resistant synthetic wheat CPI133872 to 271,034 nematodes/kg for susceptible Petrie (data not shown).

A multienvironment trial analysis was performed on the entire data set of chickpea and wild *Cicer* accessions across both experiments to determine the overall ranking of accessions for resistance. In each experiment, predictions were calculated separately (Fig. 1) and with the correlation between the experiments being strong ($r = 0.844$). genetic predictions (BLUPs) for the common accessions were averaged across the two experiments. Values ranged from the most resistant *C. reticulatum* Bari1_091 (2,844 nematodes/kg of soil, RF 0.28) to the susceptible reference Kyabra (55,167 nematodes/kg, RF 5.52), while *C. reticulatum* Savur_063 (41,794 nematodes/kg, RF 4.18) was the most susceptible of the new accessions tested. The chickpea cultivar with the lowest population density was ICC11323 (10,834 nematodes/kg, RF 1.08), followed by Australian cultivars PBA Seamer (11,175 nematodes/kg, RF 1.12), PBA Pistol (11,215 nematodes/kg, RF 1.12), and PBA HatTrick (18,887 nematodes/kg, RF 1.89). In total, 53 (30%) wild *Cicer* accessions were significantly ($P \leq 0.05$) more resistant than the best Australian cultivar PBA Seamer, with 38 (21%) of these more resistant than ICC11323 and 13 (8%) more resistant than ILWC 246 (7,431 nematodes/kg). For the wild *Cicer* reference cultivars, ILWC 123 (2,931 nematodes/kg) was significantly ($P < 0.05$) more resistant than ILWC 246. The full data set is available in Supplementary Table S1 and the frequency distribution of the BLUPs of $\log_e$ (*P. thornei* nematodes/kg of soil + roots) for all accessions is given in Figure 2. A summary of final *P. thornei* population densities for chickpea cultivars and wild accessions that were significantly more resistant than ILWC 246 is given in Table 2.

P. thornei population densities for the 26 diverse wild accessions (20 *C. reticulatum* and 6 *C. echinospermum*) selected internationally for trait marker association studies, and in Australia crossed with PBA HatTrick, revealed that 11 of the 26 produced lower

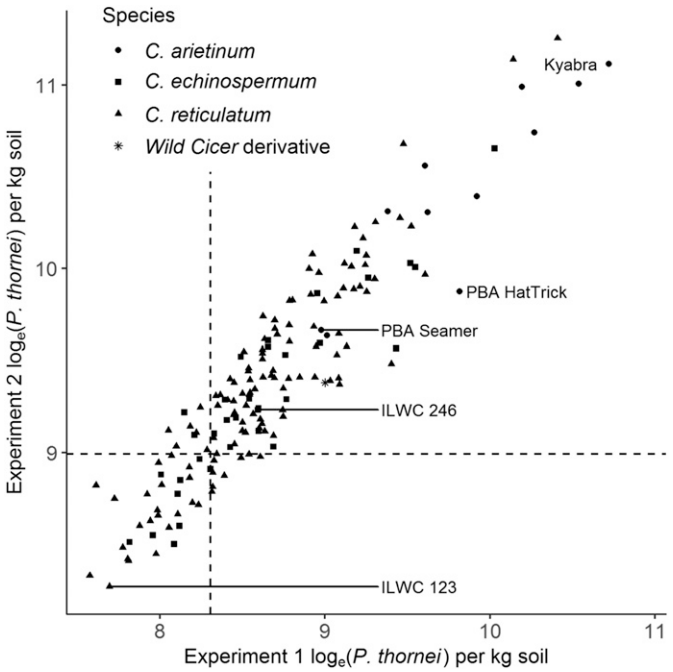

Fig. 1. Accession means (best linear unbiased predictors) of *Pratylenchus thornei* population densities for *Cicer* accessions included in both experiments showed a strong genetic correlation between experiments ($r = 0.844$, $n = 167$). Vertical and horizontal dashed lines denote the cut-off points for the top 20% genotypes for resistance to *P. thornei*.

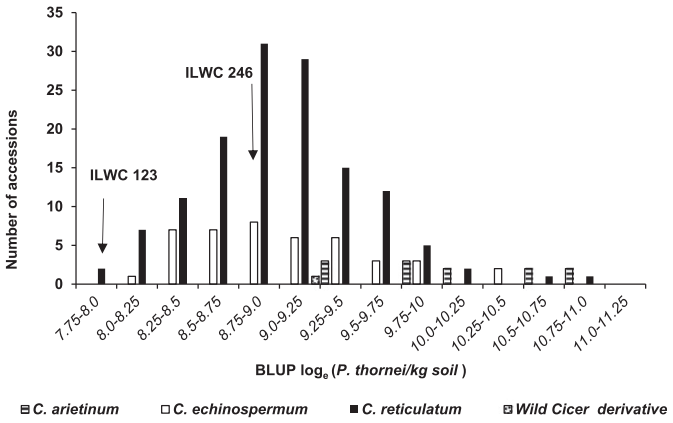

Fig. 2. Frequency distributions of chickpea and wild *Cicer* accessions from combined analysis of two experiments in classes of best linear unbiased predictions (BLUPs) of $\log_e$ (number of *Pratylenchus thornei* nematodes per kilogram of soil + roots) after 18 weeks of plant growth.

P. thornei population densities than ILWC 246. Of these 11 accessions, 2 of them—Gunas_062 (3,514 nematodes/kg, RF 0.35) and Kayat_077 (3,790 nematodes/kg, 0.38)—produced significantly ($P < 0.05$) lower population densities than ILWC 246 (Fig. 3).

***P. thornei* population densities according to species, province, collection sites, and genetic populations.** The number of accessions for each collection site varied from 1 to 17 and, due to the extra 23 accessions in experiment 2, numbers varied within the provinces, sites, and genetic populations for each experiment. Seed of accessions from one site (Guvenli) were not available for testing, resulting in assessment of 11 genetic population groups out of the 12 identified for this collection by Von Wettberg et al. (2018).

The results indicated significant ($P < 0.05$) interactions between genotype and (i) species, (ii) province, (iii) collection site, and (iv) genetic population group. Based on individual analysis for species, both experiments showed that, on average, accessions of the wild species *C. reticulatum* and *C. echinospermum* were equal in terms of *P. thornei* resistance, with accessions of both species significantly ($P < 0.001$) more resistant than *C. arietinum* (Table 3).

Mean population densities of *P. thornei* differed significantly ($P < 0.001$) among the five provinces in each of the experiments (Table 4). Accessions from the Adiyaman province had the lowest mean population densities for each of the two experiments, with 2,868 and 9,555 *P. thornei* nematodes/kg of soil, respectively, while the Sirnak province had the most susceptible accessions for both experiments, with 6,960 and 14,600 nematodes/kg of soil, respectively.

For each of the 21 collection sites, mean *P. thornei* population densities differed significantly ($P < 0.001$), as did individual accessions within the sites. The Oyali site had the lowest mean *P. thornei* population densities in experiment 1 (2,533 nematodes/kg) while Gunasan, with two accessions, had the lowest in experiment 2 (5,064 nematodes/kg). Overall mean *P. thornei* population densities were lowest for accessions from Kayatepe, with *P. thornei* population densities not exceeding 7,044 nematodes/kg. The Savur site, where only one accession originated, was the most susceptible in both experiments, with population densities of *P. thornei* of 22,902 and 108,102 nematodes/kg of soil in experiments 1 and 2, respectively (Fig. 4). The range of *P. thornei* population densities for each

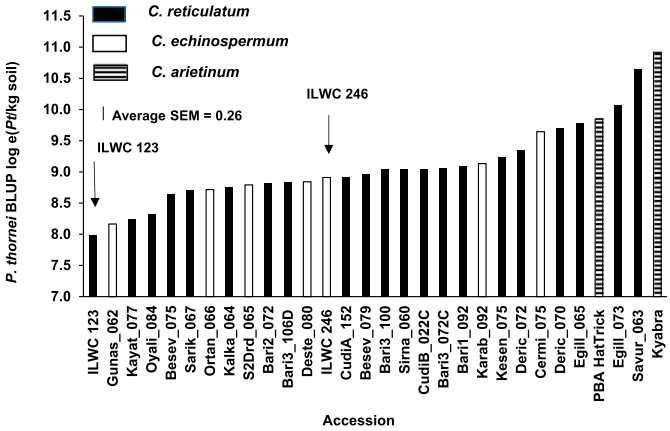

Fig. 3. *Pratylenchus thornei* densities as *P. thornei* best linear unbiased predictors (BLUPs; nematodes per kilogram of soil + roots) for 26 diverse wild *Cicer* selected internationally for nested association mapping studies compared with breeding parent PBA HatTrick, susceptible Kyabra, and resistant references *Cicer reticulatum* ILWC 123 and *C. echinospermum* ILWC 246. SEM = standard error of the mean.

TABLE 2. Best linear unbiased predictors of *Pratylenchus thornei* population densities (nematodes per kilogram of soil and roots) of 13 wild *Cicer* accessions significantly more resistant than reference *Cicer reticulatum* ILWC 246 and equivalent in resistance to reference *C. echinospermum* ILWC 123 in comparison with 13 other chickpea reference accessions from combined analysis of two experiments

Species[v]	AGG accession[w]	Code[x]	Group[y]	Nematodes[u]		Prob[z]
				Log_e	BTM	
C. ret	49797	Bari1_091	Ret_F	7.95	2,844	0.01
C. ret	ILWC 123			7.98	2,931	0.01
C. ret	49967	Kayat_063	Ret_D	8.11	3,331	0.02
C. ret	49795	Bari1_069	Ret_F	8.11	3,340	0.02
C. ret	49793	Bari1_068	Ret_F	8.13	3,392	0.02
C. ech	50135	Gunas_062	Ech_A	8.16	3,514	0.02
C. ret	49931	Deric_074	Ret_D	8.21	3,687	0.03
C. ret	50003	Oyali_071	Ret_A	8.22	3,705	0.03
C. ret	50005	Oyali_073	Ret_A	8.24	3,777	0.04
C. ret	49975	Kayat_077	Ret_D	8.24	3,790	0.04
C. ech	50150	Karab_082	Ech_B	8.25	3,845	0.04
C. ret	49803	Bari2_062	Ret_E	8.28	3,963	0.05
C. ech	50101	Cermi_072	Ech_A	8.29	4,001	0.05
C. ech	ILWC 246	…	…	8.91	7,431	N/A
C. ret der.	00283-1095-1002	…	…	9.19	9,809	0.76
C. ariet	ICC11323	…	…	9.29	10,834	0.81
C. ariet	PBA Seamer	…	…	9.32	11,175	0.86
C. ariet	PBA HatTrick	…	…	9.85	18,877	0.99
C. ariet	Yorker	…	…	9.85	18,897	0.99
C. ariet	Flipper	…	…	9.96	21,257	1.00
C. ariet	PBA Boundary	…	…	10.08	23,941	1.00
C. ariet	Jimbour	…	…	10.16	25,774	1.00
C. ariet	Sonali	…	…	10.51	36,499	1.00
C. ariet	Howzat	…	…	10.59	39,836	1.00
C. ariet	Sona	…	…	10.77	47,670	1.00
C. ret	ILWC 184	…	…	10.83	50,654	1.00
C. ariet	Kyabra	…	…	10.92	55,167	1.00

[u] *P. thornei* nematodes per kilogram of soil + roots. BTM = back-transformed mean.
[v] Abbreviations: *C. ret* = *C. reticulatum, C. ech* = *C. echinospermum, C. ret* der = *C. reticulatum* derivative, and *C. ariet* = *C. arietinum.*
[w] AGG = Australian Grains Genebank.
[x] Accession code.
[y] Genetic population group.
[z] Probability of accession having higher *P. thornei* density than ILWC 246.

collection site for the two experiments is available in Supplementary Table S2.

Analysis of the 11 genetic population groups revealed significant ($P < 0.001$) differences for mean *P. thornei* population densities (Fig. 5). Accessions in genetic population group Ret_A produced the lowest mean (2,533 nematodes/kg) for experiment 1 and Ret_F produced the lowest mean (6,843 nematodes/kg) for experiment 2. Both of these groups were aligned with the sites Oyali and Baristepe1. Across both experiments, accessions grouped in Ret_F, aligned with the site Baristepe1, produced the lowest mean *P. thornei* population densities. The most susceptible genetic population groups were Ret_H and Ret_C in experiments 1 and 2, respectively, with accessions in both of these genetic population groups aligned with collection sites Sirnak, Egil, and Kalkan.

Correlation of root biomass with *P. thornei* population densities. To investigate the effect of root biomass on final *P. thornei* population densities, the dry root biomass was determined for 167 chickpea and wild *Cicer* accessions in experiment 1. *C. echinospermum* accessions had significantly ($P < 0.001$) greater mean root biomass (4.94 g/kg of soil) than *C. reticulatum* (3.87 g/kg of soil). However, neither wild species differed significantly in root biomass from *C. arietinum* (4.16 g/kg of soil). Individual accessions differed significantly ($P < 0.001$) in root biomass and ranged from Karab_082 (1.02 g/kg of soil) to Deste_064 (11.63 g/kg of soil). Root biomass was greatest for *C. echinospermum* accessions Deste_064 (11.63 g/kg of soil) and S2Drd_065 (11.59 g/kg of soil) and *C. reticulatum* accessions Kalka_064 (9.01 g/kg of soil) and Kalka_074 (8.98 g/kg of soil).

TABLE 3. Population densities of *Pratylenchus thornei* in relation to species of *Cicer* best linear unbiased estimators (*P. thornei* nematodes per kilogram of soil + roots) after 18 weeks of plant growth in experiment 1 and experiment 2

Experiment, species	N^z	Nematodes/kg of soil + roots[y]	
		Log_e	BTM
1			
Cicer arietinum	11	10.13 a	25,804
C. echinospermum	34	8.61 b	5,503
C. reticulatum	121	8.59 b	5,359
2			
C. arietinum	12	10.42 a	33,390
C. reticulatum	135	9.38 b	11,897
C. echinospermum	43	9.35 b	11,487

[y] Values followed by the same letter within each experiment are not significantly different ($P < 0.05$). BTM = back-transformed mean.
[z] Number of accessions.

TABLE 4. Population densities of *Pratylenchus thornei* (number of nematodes per kilogram of soil + roots) after 18 weeks growth of wild *Cicer* accessions in relation to provinces of origin in Turkey

Experiment, province	Number of accessions		Nematodes/kg of soil + roots[z]	
	C. reticulatum	*C. echinospermum*	Log_e	BTM
1				
Sirnak	27	…	8.85 a	6,960
Urfa	…	25	8.68 ab	5,888
Diyarbakir	22	5	8.59 b	5,395
Mardin	64	…	8.52 b	5,000
Adiyaman	8	…	7.96 c	2,868
2				
Sirnak	36	…	9.59 a	14,600
Diyarbakir	23	7	9.48 ab	13,144
Urfa	…	34	9.37 bc	11,759
Mardin	66	…	9.23 d	10,162
Adiyaman	8	…	9.16 cd	9,555

[z] Values followed by the same letter are not significantly different ($P < 0.05$). BTM = back-transformed mean.

Correlation analysis for the accessions showed no significant relationship ($r = 0.11$) between final *P. thornei* population densities and root biomass. In contrast, there was a highly significant genetic correlation ($r = 0.88$, $P < 0.001$) between the population density of *P. thornei* expressed on a root weight basis or on a soil and root weight basis (Fig. 6). This indicated that differences among accessions in amount of root produced were not a major influence on nematode population densities and on interpretation of the levels of resistance or susceptibility of the accessions.

Plant maturity. Plant maturity of chickpea accessions at 18 weeks ranged from preflowering to full pod ripening (data not shown). For *C. echinospermum* accessions, 28% were at preflowering stage, 60% at flowering, and 12% at podding. Similarly, 22% of *C. reticulatum* accessions were at preflowering stage, 51% flowering, 26% podding, and 1% at pod ripening stage. *C. arietinum* accessions ranged from pod ripening stage to full maturity. Overall, *C. reticulatum* had a larger percentage of accessions at the podding stage compared with *C. echinospermum*. Although there were significant differences among accessions in numeric scores for plant maturity ($P < 0.001$), there was no significant correlation between *P. thornei* population density and the numeric score for plant growth stage, with $P = 0.31$ for experiment 1 and $P = 0.27$ for experiment 2.

DISCUSSION

This is the first study characterizing a new, more expansive collection of *C. reticulatum* and *C. echinospermum* accessions for resistance to a plant-parasitic nematode species; namely, *P. thornei.*

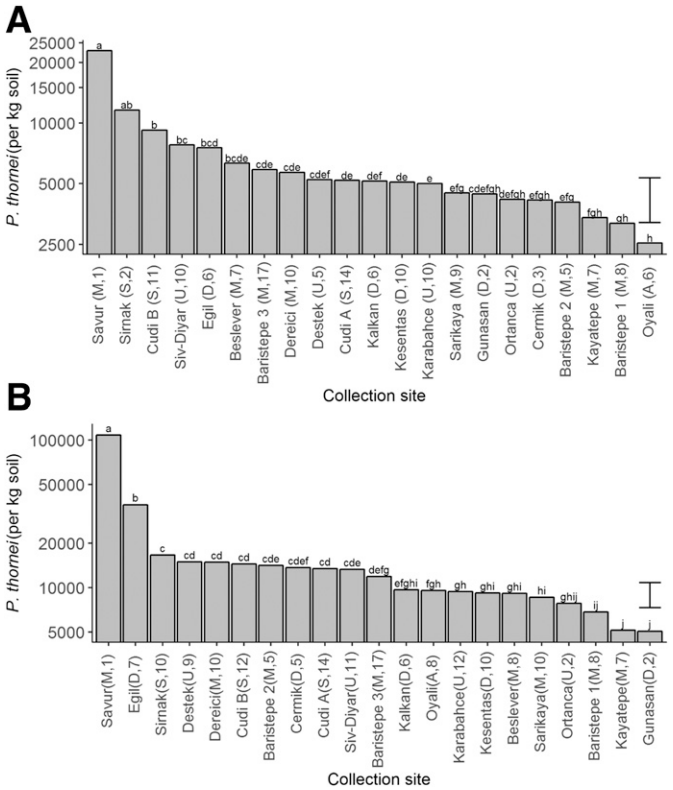

Fig. 4. Back-transformed means (best linear unbiased estimators) for population densities of *Pratylenchus thornei* nematodes per kilogram of soil for wild *Cicer* accessions from the 21 collection sites in southeast Turkey in **A,** experiment 1 and **B,** experiment 2. The letter in parenthesis after the site name indicates the Turkish province from east to west, where S = Sirnak, M = Mardin, D = Diyarbakir, U = Urfa, and A = Adiyaman. The number in parentheses indicates the number of accessions from the collection site in each experiment. Collection sites without a common letter are significantly different ($P \leq 0.05$). The vertical bar represents the average least significant difference value across the 21 collection sites.

On average, the wild *Cicer* spp. or accessions exhibited higher levels of resistance to *P. thornei* than the cultivated varieties (*C. arietinum*) assessed in this study. This agrees with earlier studies (Thompson et al. 2011), where improved levels of resistance were identified in a much smaller collection of accessions of 18 original *C. reticulatum* and 10 original *C. echinospermum*. The current study revealed a range of resistance within accessions for both wild species, with neither species appearing the more resistant. In contrast, in an earlier study with a limited number of accessions, *C. echinospermum* tended to have a distribution toward greater resistance than *C. reticulatum* (Thompson et al. 2011). A larger number of *C. echinospermum* and *C. reticulatum* accessions (41 and 133, respectively) from new collection sites were assessed in this current study compared with the earlier study and evaluation of a larger sample size has better characterized the resistance status of the two wild species.

Accessions in the new collection originated from a narrow geographical area that nonetheless spanned a range of physical and environmental scales based on climate and soil type, including a large elevational gradient (740 to 1,695 m) that was shown to affect genetic differentiation more than geographic distance (Von Wettberg et al. 2018). Furthermore, that study found that *C. reticulatum* specimens tended to occur at higher elevation than *C. echinospermum*. Also, *C. reticulatum* was found on soils developed from limestone and sandstone and *C. echinospermum* on soils developed from basalt, suggesting different edaphic requirements for the two species. In our study, there appeared to be no obvious trends for association of *P. thornei* resistance in terms of elevation and geographic distance because resistant accessions occurred at all elevations and at all sites, with the exception of the one and only accession from Savur. For example, this accession from Savur was the most susceptible and neighbored Kayatepe, where some of the most resistant accessions originated.

Von Wettberg et al. (2018) found evidence of ancestral gene flow from the wild relative *C. bijugum* into *C. reticulatum* at sites Oyali (17.1% *C. bijugum* DNA) and Kesentas (11.9% *C. bijugum* DNA). Interestingly, Thompson et al. (2011) found that a higher proportion of *C. bijugum* accessions had partial resistance to *P. thornei* than the *C. reticulatum* accessions tested. In the present study, these sites (Oyali and Kesantas) provided *C. reticulatum* accessions that tended to be more resistant to *P. thornei* than many of the other collection sites. Currently, *C. bijugum* cannot be successfully crossed with *C. arietinum* (Li et al. 2015) and these accessions of *C. reticulatum* from Oyali and Kesentas might provide a natural bridge to introgress novel resistance genes from *C. bijugum* into *C. arietinum*.

Our current study identified significant differences among the genetic population groups of wild *Cicer* spp. for mean *P. thornei* population densities. Predominantly, the genetic population groups identified by Von Wettberg et al. (2018) were linked to the site of origin for each accession and our analysis identified lower mean *P. thornei* population densities for accessions grouped under Ret_A and Ret_F. Interestingly, these two genetic groups aligned with the sites Oyali and Baristepe1, located at elevations below 1,000 m, while the most susceptible genetic population groups Ret _H and Ret_C aligned with the sites Sirnak, Egil, and Kalkhan, which were situated at elevations of 1,659, 986, and 840 m, respectively. However, similar to our findings, for accessions within the collection sites, there appeared to be no distinct trend for association of resistance with elevation or geographic distance.

The foremost trait to reduce soil populations of *P. thornei* is resistance, and our current study highlights a diverse, abundant, and stronger source of resistance, with 53 (30%) of wild accessions identified as more resistant than PBA Seamer, the most resistant Australian cultivar in this study. A recent study based on a single glasshouse experiment by Rodda et al. (2016) noted the recently developed commercial cultivar PBA Seamer (formerly CICA 0912) to be equal in resistance to ILWC 246, PBA HatTrick, and the specifically bred lines for *P. thornei* resistance CICA1432 and CICA1433, both derived from ILWC 246. Currently, PBA HatTrick is cited as offering the best level of resistance; however, to incorporate *P. thornei* resistance, past breeding efforts targeted disease resistance to *Ascochyta rabei* (Ascochyta blight) and *Phytophthora medicaginis* from wild *Cicer* spp. rather than *Pratylenchus thornei*, and this has

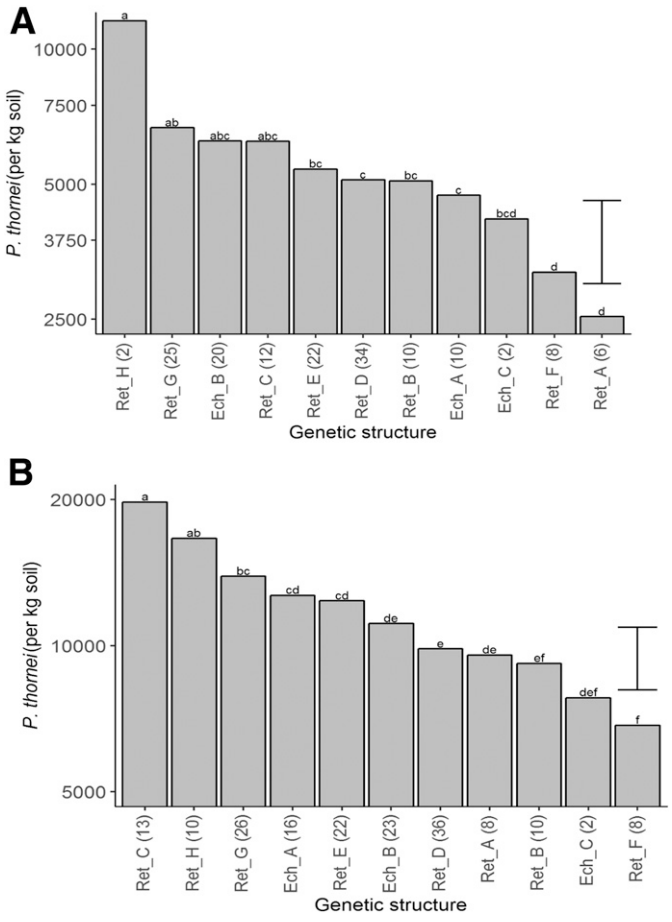

Fig. 5. Back-transformed means (best linear unbiased estimators) for population densities of wild *Cicer* accessions based on the 11 genetic population groups in **A,** experiment 1 and **B,** experiment 2. Numbers in parenthesis indicate the number of accessions in a genetic population group in each experiment. Genetic population groups without a common letter are significantly different ($P \leq 0.05$). The vertical bar represents the average least significant difference value across the 11 genetic population groups.

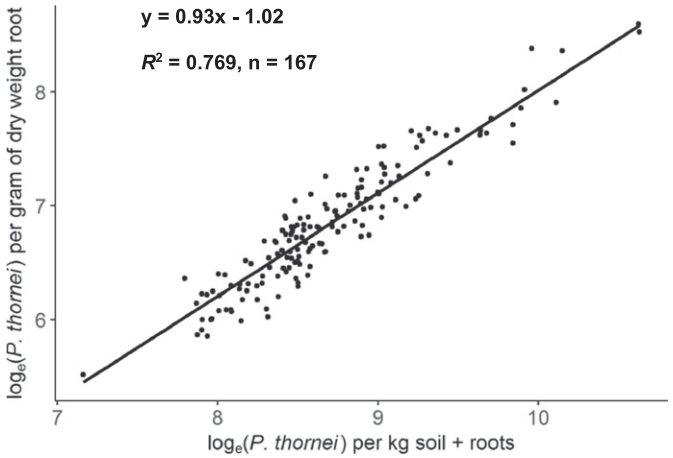

Fig. 6. Relationship between the predictions (best linear unbiased predictors) $\log_e$ (*Pratylenchus thornei*) expressed per gram of dry weight root or per kilogram of soil + roots in experiment 1.

hindered selection for *P. thornei* (Knights et al. 2008; Rodda et al. 2016). Our present results identified 13 wild accessions more resistant than ILWC 246, which offer the opportunity to exploit this resistance and broaden the base of current *P. thornei* resistance in chickpea cultivars.

Root biomass correlations with *P. thornei* population densities. In defining plant genotype resistance, nematode population densities can be assessed in terms of nematodes per unit mass of root, or on a whole-plant or pot basis as nematodes per unit mass of soil plus roots. Some wild *Cicer* spp. have been observed as having less root mass than cultivated chickpea (Kashiwagi et al. 2005). There have been concerns that genotypes that produce smaller root mass may result in lower Pf densities per plant or per unit weight of soil and roots and, therefore, accessions appear resistant. Limited research has been conducted in this area (Starr et al. 2002); therefore, in this study, we determined whether there were any correlations between root biomass and final *P. thornei* population densities.

Root biomass for both *C. reticulatum* and *C. echinospermum* accessions was similar to cultivated chickpea *C. arietinum*. This agrees with the study by Kashiwagi et al. (2005), who assessed roots after 35 days (earlier than in our study) and found that *C. reticulatum* and cultivated chickpea produced similar root biomass. They found that other wild species produced less root biomass but *C. echinospermum* was not part of their study. Although our study found that root biomass differed significantly among accessions, we found it had no significant influence on the characterization of *Cicer* genotypes for resistance or susceptibility to *P. thornei*, with there being no significant correlation between root biomass and final *P. thornei* population densities. Nonetheless, this is not to be confused with plants that grow poorly and have very weak root systems that will not support nematode reproduction. Root biomass and root architecture are important traits for drought tolerance and grain yield in crops (Kashiwagi et al. 2005, 2006; Robinson et al. 2018). Notably, the two accessions with maximum root biomass—*C. echinospermum* Deste_064 (11.63 g/kg of soil) and S2Drd_065 (11.59 g/kg of soil)—also produced low population densities of *P. thornei* across the two experiments, with means of 5,448 and 6,576 nematodes/kg of soil, respectively. Similarly, accessions of *C. reticulatum* Kayat_063 (7.37 g/kg of soil), Kayat_077 (7.96 g/kg of soil), and *C. echinospermum* S2Drd_107B (8.09 g/kg of soil) had final *P. thornei* mean populations below 5,000 nematodes/kg, with Kayat_063 being the third most resistant accession in this study. These accessions could be valuable for simultaneously introgressing desirable root production traits and *P. thornei* resistance into elite chickpea cultivars.

Designation of resistance. Resistance to *P. thornei* in chickpea appears to be a quantitative trait (Thompson et al. 2011), which was reinforced by the results of this study, where the range of Pf densities after growth of the accessions showed continuous variation. Thus, for comparison of genotypes, the quantitative values *P. thornei* nematodes per kilogram of soil as BLUPs provide accurate genetic rankings of the accessions. Another approach to assessing resistance of genotypes is to compare RF values and we have indicated these for some individual accessions, where appropriate, in the Results section. These RF values based on BLUPs are statistically conservative estimates. They are also conservative in that they have not been adjusted for the extraction factor which, for the Whitehead method used here, is 70% (Thompson et al. 2010). Furthermore, the inoculum density used in the denominator of RF is an overestimate because not all inoculated nematodes of *P. thornei* initiate reproduction, resulting in an underestimated RF value (O'Brien 1982; Thompson et al. 2015a). To categorize the best accessions as resistant or moderately resistant, we have preferred to compare them to reference genotypes included in both experiments where those called resistant had *P. thornei* population densities significantly less than *C. echinosperimum* ILWC 246 and moderately resistant had significantly less than the Australian chickpea cultivar PBA Seamer.

Multiple resistance. Cultivars with multiple disease resistance are desirable in most breeding programs, with wild *Cicer* spp. being a valuable source of multiple resistances compared with *C. arietinum* (Croser et al. 2003). Moreover, the value of wild chickpea species in resistance breeding is crucial to maintain crop productivity, particularly in crops where genetic diversity is low and disease pressure results in virulent biotypes (Kameswara Rao et al. 2003), such as in the fungal diseases Ascochyta bight (*A. rabei*) and Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris*), both major constraints in global chickpea production (Croser et al. 2003; Li et al. 2015). Little Ascochyta resistance has been recovered from world germplasm collections but improved resistance was identified in two *C. echinospermum* accessions (Collard et al. 2003; Reddy and Singh 1984, 1992). Furthermore, Fusarium wilt, while not a major disease in Australia, is devastating to chickpea in countries such as India and may be exacerbated by root-lesion nematode attack (Castillo et al. 1998b, 2008). Interestingly, among the 26 diverse wild chickpea accessions that are of international interest, we identified 12 with lower final population densities of *P. thornei* than ILWC 246. Two of these *P. thornei*-resistant accessions, Gunas_062 and Ortan_066, have recently been identified with excellent resistance to pod borer (*Helicoverpa armigera*), a major insect pest in chickpea production areas in Australia and other countries (Von Wettberg et al. 2018). Characterizing the wild *Cicer* accessions in this study provides an informative profile for *P. thornei* resistance status. Our data offers plant breeders the opportunity to select from the most resistant accessions and deploy germplasm that possibly includes combined disease resistance that will be more sustainable in the development of future disease-resistant cultivars.

Crop improvement is dependent on a rich and diverse germplasm collection (Saeed et al. 2011) and current chickpea germplasm has limited diversity. Von Wettberg et al. (2018) showed that this new collection of *C. reticulatum* and *C. echinospermum* is 100 times more genetically diverse than *C. arietinum*, offering promise for a range of useful traits. Already, studies of this new wild *Cicer* collection have identified accessions possessing desirable agronomic traits (Talip et al. 2018). It is this genetic diversity and the unparalleled opportunities to incorporate novel genes controlling important traits such as *P. thornei* resistance that is encouraging plant breeders to utilize wild species despite potential linkage drag caused by undesirable agronomic traits (Muñoz et al. 2017). Utilizing the resistant accessions identified in this study while broadening the base of *P. thornei* resistance will also harness the new genetic variability within the collection.

In conclusion, our study provides the first evaluation of *P. thornei* resistance for wild chickpea in this new collection of accessions. Our results showed that 30% of the wild accessions were more resistant than current Australian chickpea cultivars. Furthermore, our data provides an informative repository that will allow linkages with genetic diversity studies and provide novel insights into nematode resistance. Finally, the introgression of resistant wild *Cicer* spp. into cultivated chickpea will reduce *P. thornei* populations in soils and allow more flexible crop rotations and increased yields. The outcome will be the availability of more effective and durable genes for resistance to *P. thornei* that can be used in chickpea breeding programs worldwide.

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CHAPTER 4: SUMMARY AND FUTURE PERSPECTIVES

4.1 Summary

Australian research efforts have been extensive in routinely screening large volumes of chickpea accessions to identify resistance to *P. thornei*. The most resistant germplasm has been identified in wild *Cicer* accessions from studies in Australia (Thompson et al. 2011) and overseas (Di Vito 1995). This thesis evaluated *P. thornei* resistance in a new and extensive collection of wild *Cicer* that has never before been available. The outcome has been the identification of new and diverse sources of resistance currently not available in *C. arietinum* germplasm. The research highlights the abundant sources of resistance available within the collection, with 53 wild accessions having greater resistance than PBA Seamer which is also shown to be equal in resistance to the current breeding cultivar PBA HatTrick (Rodda et al 2016). Furthermore, the research is the first worldwide to assess resistance to a nematode species for the 2013 wild *Cicer* collection and offers valuable information for chickpea breeding worldwide. The study also contributes valuable information pertaining to the relationship between root biomass and final *P. thornei* numbers. There were no significant correlations, highlighting that while root biomass differed among accessions this trait did not significantly influence the resistance rankings of accessions.

Owing to the current limited genetic diversity within cultivated chickpea, that has hindered past breeding efforts, the identification of *P. thornei* resistant germplasm reinforces the global recognition of urgency and importance of CWR as crucial to meeting challenges posed by biotic stresses. Improvement in any crop breeding program is reliant on the amount of genetic variation available within the crop germplasm (Jha et al. 2014). In view of the diversity already identified in the new wild *Cicer* collection compared to *C. arietinum* (Von Wettberg et al. 2018), targeting and exploiting the resistant accessions identified in this research will provide an excellent opportunity to harness the genetic diversity and resistance for chickpea improvement.

Crop wild relatives are vital to meet future challenges such as climate change and their role in future food security (Redden 2015). The rankings of *P. thornei* resistance identified for the wild accessions in this research are the platform for pre-

breeding and are imperative in providing the first step to linking root-lesion resistance traits of CWR for future development of more resistant and diverse chickpea cultivars.

4.2 Future research priorities

4.2.1 Genomics assisted breeding in chickpea

In the last few decades, advances in the field of genomics has seen the development of molecular markers that include simple sequence repeat (SSR), diversity arrays technology (DArT) and single nucleotide polymorphisms (SNP) for advancing genetics and breeding within chickpea. Interspecific hybridisation of wild relatives into chickpea can be aided with the employment of genomic technologies such as DNA markers and sequencing to identify candidate genes for *P. thornei* resistance. The adaptation of molecular methods for breeding for resistance to nematodes such as marker-assisted selection derived from DNA polymorphism integration has been mainly restricted to root-knot (*Meloidogyne* spp.) and soybean cyst nematodes (*Heterodera* and *Globodera* spp.) (Molinari & Sergio 2011). For marker-assisted backcrossing in chickpea, the research has predominantly focused on ascochyta blight (*Ascochyta rabiei*) resistance and drought tolerance (Collard, Pang & Taylor 2003; Varshney 2016). Furthermore, while molecular studies to identify nematode resistant genes have been investigated in other crops, to date no information is available for nematode resistance in chickpea (Zwart et al. 2019).

4.2.1.1 Genome-wide association mapping

Due to lack of information regarding mechanisms of *P. thornei* resistance or chromosomal regions associated with resistance in chickpea, future breeding of more resistant cultivars necessitates an understanding of the inheritance of resistance genes. In view of this, a genome-wide association study (GWAS) to identify markers associated with resistance is the initial step towards identifying the resistance genes involved. A preliminary analysis of existing data has been undertaken, however, more wild accessions are needed to increase the statistical power of GWAS (Zwart, Reen & Thompson 2019). Another 53 wild accessions from the 2013 collection were recently made available, in addition to the release of 30 *C. echinospermum* and 128 *C.*

reticulatum accessions from the 2014 collection in Turkey, and phenotyping of these accessions is currently underway. The value of these forthcoming results will provide additional information on *P. thornei* resistance for wild *Cicer* accessions and expand the existing data enabling a GWAS to be performed for identifying genomic regions associated with resistance. Combining molecular marker-based resources with accurate phenotyping will facilitate more rapid introgression of *P. thornei* resistance through the indirect selection of resistance. Pyramiding of resistance genes and combining of multiple resistances to biotic stresses will result in cultivars with more durable *P. thornei* resistance and diversity (Zwart et al. 2019).

4.2.1.2 Nested association mapping

Breeding programs can choose several ways in which to harness genetic diversity from wild species. Accessions can be chosen for specific crossings based on traits characterised either by phenotypic or genotypic characterisation, collection locality or as combinations of these or alternatively, by crossing with wild taxa first and then screening progeny for beneficial traits (Dempewolf et al. 2017). Several strategies are being used for the new wild *Cicer* collection with Von Wettberg et al. (2018) assessing the collection initially and identifying 26 diverse accessions for further use. The selected wild accessions were used as parents and crossed with six elite chickpea cultivars from major growing regions of the world (Turkey, United States, Canada, Ethiopia, India and Australia) to create nested association mapping (NAM) populations. In Australia, the elite cultivar was PBA HatTrick and five bi-parental populations segregating for resistance to *P. thornei* have recently been selected for advancement using single seed decent. Accurate phenotyping of the segregating progeny from these dedicated mapping populations is needed in the future, along with genotyping to identify molecular markers linked to *P. thornei* resistance. Combining the phenotypic results with molecular resources will facilitate selection for *P. thornei* resistance with the possibility of pyramiding resistance genes and combining resistance to multiple diseases or traits of interest.

4.2.2 Resistance to *Pratylenchus neglectus*

Although *P. thornei* is the dominant species in the sub-tropical grain region of eastern Australia, *P. neglectus* is also present. It is found in ~32% of fields with both species

occurring together in 26% of fields surveyed (Thompson et al. 2010). *Pratylenchus neglectus* occurs throughout the southern and western grain growing regions of Australia and is the dominant *Pratylenchus* species within these regions (Riley & Kelly 2002; Taylor, Hollaway & Hunt 2000; Vanstone, Hollaway & Stirling 2008). Within these regions, chickpea, wheat and canola are susceptible hosts of *P. neglectus* (Vanstone et al. 1998; Taylor, Hollaway & Hunt 2000; Thompson et al. 2000; Thompson et al. 2010; GRDC 2017). The presence of *P. neglectus* in the Western Australian region in 83% of paddocks surveyed (GRDC 2017) is a significant factor for chickpea production and is further complicated by the presence of *Rhizoctonia* root rot (*Rhizoctonia solani*) and acidic soils (Seymour, M. 2019, pers. comm., 4th April).

Earlier research identified improved resistance to *P. neglectus* in *C. reticulatum* and *C. echinospermum* accessions (Thompson et al. 2011), however, the breeding focus at that time concentrated on *P. thornei* resistance, as this species is the dominant species in the major chickpea production area. To take full advantage of the diversity already present in the 2013 collection, phenotyping for resistance to *P. neglectus* is the next priority. Furthermore, phenotyping the collection for *P. neglectus* resistance offers the opportunity to identify accessions that carry dual resistance to both *Pratylenchus* species, which is a desirable goal for most breeding programs. It also avoids one species gaining an advantage when both are present (Roberts 2002).

Introgression of wild *Cicer* with dual RLN resistance into chickpea will offer more flexible rotations within those grain regions where *P. neglectus* is a threat and where both species exist in the soil. Additionally it will assist in strengthening research for identifying the mechanisms involved in genetic resistance to both species of root-lesion nematode.

4.2.3 Collaboration in the use of Crop Wild Relatives

Lack of adaptive diversity to a range of biotic stresses in cultivated chickpea highlights the necessity of systematic collection and conservation of wild chickpea (Andeden et al. 2013). Introgression of resistance genes into cultivars is paramount for the industry in the 21st century, particularly in view of the increased disease pressure and other challenges predicted for all food crops due to climate change (Garrett et al. 2006).

Furthermore, to utilise and deploy the diversity in CWR through biotechnology there is a need for international collaboration (Ford-Lloyd et al. 2011). Key requirements in CWR crop improvements include the co-ordinated evaluation and sharing of knowledge of genotypic and phenotypic data, which has been lacking in many CWR improvement programs (Dempewolf et al. 2017). In Australia, the database Breeding Management System (BMS) (https://www.integratedbreeding.net/) has been implemented to serve as a platform where evaluated results and data for each trait being studied on the wild *Cicer* collection can be deposited. Data presented in this thesis are available within this BMS and are accessible to all Australian researchers evaluating the wild *Cicer* collection. The current research project also has international linkages with over eight countries. Genotypic data generated by genotyping-by-sequencing of the 2013 wild *Cicer* collection by the University of California, Davis is publicly available through the National Centre for Biotechnology Information (NCBI) database (Von Wettberg et al. 2018). Collaboration in genomic resources and phenotypic evaluation will provide novel insights into nematode resistance in chickpea and unprecedented opportunities for Australian and international plant breeders and geneticists to sustain chickpea improvement.

4.3 Conclusion

The benefits of CWR collections such as the one studied in this thesis have been clearly demonstrated. New diverse sources of *P. thornei* resistant accessions have been identified in both *C. reticulatum* and *C. echinospermum*. Moreover, it is hoped the application of traditional breeding, coupled with innovative technologies will utilise the *P. thornei* resistance identified in this research. The outcome of harnessing this novel germplasm and genetic diversity, will be the provision of more robust and diverse *P. thornei* resistance genes in cultivars that allow more flexible rotations with a profitable legume in Australian and overseas chickpea cropping systems.

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APPENDIX A

Supplementary Table 1. Final population densities of *P. thornei*/kg soil and roots for 191 chickpea and wild *Cicer* accessions from combined analysis of two glasshouse experiments with probabilities of accessions having higher nematode population densities than PBA HatTrick, PBA Seamer and ILWC 246 calculated using best linear unbiased predictions (BLUPs).

Values of $P \leq 0.05$ indicate an accession is significantly more resistant than PBA HatTrick, PBA Seamer or ILWC 246. AGG Number = Australian Grains Genebank number; Genetic_Pop. = Genetic population group; *C. ret* = *C. reticulatum* species (wild species); *C. ech* = *C. echinospermum* species (wild *Cicer*); *C. ariet.* = *C. arietinum* (cultivated chickpea); *C. reticulatum* derivative = *C. ret*. der, BTM = back-transformed means; # exps. = Number of experiments in which accession was tested; RF = reproduction factor (final population per kg soil/initial inoculum rate per kg soil).

				P. thornei/kg soil + roots				Probability of accession having greater population density than:		
AGG Number	Accession	Species	Genetic_ Pop	Log_e	BTM	RF	#. Exps.	PBA HatTrick	PBA Seamer	ILWC 246
49797	Bari1_091	*C. ret*	Ret_F	7.95	2844	0.28	2	0.00	0.00	0.01
ILWC 123		*C. ret*		7.98	2931	0.29	2	0.00	0.00	0.01
49967	Kayat_063	*C. ret*	Ret_D	8.11	3331	0.33	2	0.00	0.00	0.02
49795	Bari1_069	*C. ret*	Ret_F	8.11	3340	0.33	2	0.00	0.00	0.02
49793	Bari1_068	*C. ret*	Ret_F	8.13	3392	0.34	2	0.00	0.00	0.02
50135	Gunas_062	*C. ech*	Ech_A	8.16	3514	0.35	2	0.00	0.00	0.02
49931	Deric_074	*C. ret*	Ret_D	8.21	3687	0.37	2	0.00	0.00	0.03
50003	Oyali_071	*C. ret*	Ret_A	8.22	3705	0.37	2	0.00	0.00	0.03
50005	Oyali_073	*C. ret*	Ret_A	8.24	3777	0.38	2	0.00	0.00	0.04
49975	Kayat_077	*C. ret*	Ret_D	8.24	3790	0.38	2	0.00	0.00	0.04
50150	Karab_082	*C. ech*	Ech_B	8.25	3845	0.38	2	0.00	0.00	0.04
49803	Bari2_062	*C. ret*	Ret_E	8.28	3963	0.40	2	0.00	0.00	0.05
50025	Sarik_061	*C. ret*	Ret_D	8.29	3980	0.40	1	0.00	0.01	0.08

AGG Number	Accession	Species	Genetic_ Pop	Log_e	BTM	RF	#. Exps.	PBA HatTrick	PBA Seamer	ILWC 246
50101	Cermi_072	*C. ech*	Ech_A	8.29	4001	0.40	2	0.00	0.00	0.05
50011	Oyali_084	*C. ret*	Ret_A	8.32	4116	0.41	2	0.00	0.00	0.06
50035	Sarik_073	*C. ret*	Ret_D	8.32	4121	0.41	2	0.00	0.00	0.06
49813	Bari3_064	*C. ret*	Ret_E	8.33	4167	0.42	2	0.00	0.00	0.06
49983	Kesen_066	*C. ret*	Ret_B	8.35	4221	0.42	2	0.00	0.00	0.07
50156	Karab_086	*C. ech*	Ech_B	8.36	4271	0.43	2	0.00	0.00	0.07
49855	Besev_066	*C. ret*	Ret_D	8.39	4386	0.44	2	0.00	0.00	0.08
50129	Deste_079	*C. ech*	Ech_A	8.40	4428	0.44	1	0.00	0.01	0.12
50037	Sarik_074	*C. ret*	Ret_D	8.42	4523	0.45	2	0.00	0.00	0.09
50148	Karab_081A	*C. ech*	Ech_B	8.44	4635	0.46	2	0.00	0.00	0.10
50199	S2Drd_107B	*C. ech*	Ech_B	8.44	4646	0.46	2	0.00	0.00	0.11
49977	Kayat_080	*C. ret*	Ret_D	8.46	4734	0.47	2	0.00	0.01	0.12
49979	Kesen_062	*C. ret*	Ret_B	8.47	4762	0.48	2	0.00	0.01	0.12
49839	Bari3_103	*C. ret*	Ret_E	8.47	4792	0.48	2	0.00	0.01	0.12
50193	S2Drd_104	*C. ech*	Ech_B	8.49	4850	0.49	2	0.00	0.01	0.12
49973	Kayat_070	*C. ret*	Ret_D	8.52	5021	0.50	2	0.00	0.01	0.15
49801	Bari1_093	*C. ret*	Ret_F	8.53	5046	0.50	2	0.00	0.01	0.15
49971	Kayat_066	*C. ret*	Ret_D	8.55	5161	0.52	2	0.00	0.01	0.17
49969	Kayat_064	*C. ret*	Ret_D	8.55	5176	0.52	2	0.00	0.01	0.17
50023	Oyali_107	*C. ret*	Ret_A	8.57	5253	0.53	2	0.00	0.01	0.18
49965	Kayat_061	*C. ret*	Ret_D	8.57	5253	0.53	2	0.00	0.01	0.18
49934	Deric_078	*C. ret*	Ret_D	8.59	5361	0.54	2	0.00	0.01	0.19
50111	Deste_064	*C. ech*	Ech_A	8.60	5448	0.54	2	0.00	0.02	0.20
49991	Kesen_073	*C. ret*	Ret_B	8.61	5464	0.55	2	0.00	0.02	0.21
50107	Deste_061	*C. ech*	Ech_A	8.61	5480	0.55	2	0.00	0.02	0.21
49857	Besev_074	*C. ret*	Ret_D	8.63	5610	0.56	2	0.00	0.02	0.23

AGG Number	Accession	Species	Genetic_ Pop	Log_e	BTM	RF	#. Exps.	PBA HatTrick	PBA Seamer	ILWC 246
49859	Besev_075	*C. ret*	Ret_D	8.64	5663	0.57	2	0.00	0.02	0.23
49955	Kalka_066	*C. ret*	Ret_C	8.65	5709	0.57	2	0.00	0.02	0.24
50191	S2Drd_102	*C. ech*	Ech_B	8.65	5729	0.57	2	0.00	0.02	0.24
50043	Sarik_080	*C. ret*	Ret_D	8.66	5786	0.58	2	0.00	0.02	0.25
49933	Deric_075	*C. ret*	Ret_D	8.67	5797	0.58	2	0.00	0.03	0.25
49845	Bari3_110	*C. ret*	Ret_E	8.67	5813	0.58	2	0.00	0.03	0.26
50103	Cermi_073	*C. ech*	Ech_A	8.68	5903	0.59	2	0.00	0.03	0.27
50021	Oyali_105	*C. ret*	Ret_A	8.70	5977	0.60	1	0.00	0.06	0.31
50033	Sarik_067	*C. ret*	Ret_D	8.70	6019	0.60	2	0.00	0.03	0.29
50178	Ortan_066	*C. ech*	Ech_C	8.72	6102	0.61	2	0.00	0.04	0.30
50019	Oyali_104	*C. ret*	Ret_A	8.72	6116	0.61	1	0.00	0.07	0.33
50176	Ortan_061	*C. ech*	Ech_C	8.73	6174	0.62	2	0.00	0.04	0.31
49949	Kalka_061	*C. ret*	Ret_C	8.73	6193	0.62	2	0.00	0.04	0.31
49879	CudiB_017	*C. ret*	Ret_G	8.74	6240	0.62	1	0.00	0.08	0.35
49817	Bari3_067	*C. ret*	Ret_E	8.75	6283	0.63	2	0.00	0.04	0.33
49951	Kalka_064	*C. ret*	Ret_C	8.75	6306	0.63	2	0.00	0.04	0.33
49789	Bari1_063	*C. ret*	Ret_F	8.76	6404	0.64	2	0.00	0.05	0.35
50185	S2Drd_065	*C. ech*	Ech_B	8.79	6576	0.66	2	0.00	0.06	0.37
49999	Kesen_101	*C. ret*	Ret_B	8.79	6587	0.66	2	0.00	0.06	0.37
49911	CudiA_154	*C. ret*	Ret_G	8.80	6656	0.67	2	0.00	0.06	0.38
49791	Bari1_064	*C. ret*	Ret_F	8.81	6690	0.67	2	0.00	0.06	0.39
49915	CudiA_221	*C. ret*	Ret_G	8.81	6724	0.67	2	0.00	0.06	0.40
49811	Bari2_072	*C. ret*	Ret_E	8.82	6800	0.68	2	0.00	0.07	0.41
50174	Karab_174	*C. ech*	Ech_B	8.83	6805	0.68	2	0.00	0.07	0.41
49919	Deric_065	*C. ret*	Ret_D	8.83	6808	0.68	2	0.00	0.07	0.41
49843	Bari3_106D	*C. ret*	Ret_E	8.83	6849	0.68	2	0.00	0.07	0.41
49987	Kesen_071	*C. ret*	Ret_B	8.83	6868	0.69	2	0.00	0.07	0.42

AGG Number	Accession	Species	Genetic_ Pop	Log_e	BTM	RF	#. Exps.	PBA HatTrick	PBA Seamer	ILWC 246
49901	CudiA_127	*C. ret*	Ret_G	8.84	6900	0.69	2	0.00	0.07	0.42
50131	Deste_080	*C. ech*	Ech_A	8.84	6919	0.69	2	0.00	0.08	0.42
49891	CudiA_103C	*C. ret*	Ret_G	8.85	6967	0.70	2	0.00	0.08	0.43
50172	Karab_172	*C. ech*	Ech_B	8.86	7027	0.70	2	0.00	0.08	0.44
50137	Gunas_100	*C. ech*	Ech_A	8.86	7043	0.70	2	0.00	0.08	0.44
49897	CudiA_122	*C. ret*	Ret_G	8.86	7070	0.71	2	0.00	0.09	0.45
49913	CudiA_155	*C. ret*	Ret_G	8.87	7097	0.71	2	0.00	0.09	0.45
49957	Kalka_067	*C. ret*	Ret_C	8.88	7153	0.72	2	0.00	0.09	0.46
49985	Kesen_067	*C. ret*	Ret_B	8.89	7238	0.72	2	0.00	0.10	0.47
50027	Sarik_064	*C. ret*	Ret_D	8.89	7243	0.72	2	0.00	0.10	0.47
49851	Besev_062	*C. ret*	Ret_D	8.89	7254	0.73	2	0.00	0.10	0.47
49853	Besev_065	*C. ret*	Ret_D	8.89	7254	0.73	2	0.00	0.10	0.47
49837	Bari3_102	*C. ret*	Ret_E	8.89	7291	0.73	2	0.00	0.10	0.48
50152	Karab_084	*C. ech*	Ech_B	8.91	7370	0.74	1	0.01	0.15	0.49
49905	CudiA_151	*C. ret*	Ret_G	8.91	7408	0.74	2	0.00	0.12	0.50
49907	CudiA_152	*C. ret*	Ret_G	8.91	7423	0.74	2	0.00	0.11	0.50
ILWC 246		*C. ech*		8.91	7431	0.74	2	0.01	0.14	
49909	CudiA_153	*C. ret*	Ret_G	8.92	7443	0.74	2	0.00	0.11	0.50
50195	S2Drd_105	*C. ech*	Ech_B	8.92	7458	0.74	2	0.00	0.11	0.50
50142	Karab_063	*C. ech*	Ech_B	8.92	7476	0.75	2	0.00	0.12	0.51
50001	Kesen_104	*C. ret*	Ret_B	8.93	7548	0.75	2	0.00	0.12	0.52
49787	Bari1_062	*C. ret*	Ret_F	8.94	7598	0.76	2	0.00	0.12	0.52
50029	Sarik_065	*C. ret*	Ret_D	8.96	7780	0.78	2	0.00	0.14	0.55
49861	Besev_079	*C. ret*	Ret_D	8.97	7867	0.79	2	0.00	0.15	0.56
49809	Bari2_074	*C. ret*	Ret_E	8.97	7871	0.79	2	0.00	0.15	0.56
49877	CudiB_016	*C. ret*	Ret_G	8.99	8004	0.80	2	0.01	0.16	0.58
49823	Bari3_074	*C. ret*	Ret_E	8.99	8022	0.80	2	0.01	0.16	0.58

AGG Number	Accession	Species	Genetic_ Pop	Log_e	BTM	RF	#. Exps.	PBA HatTrick	PBA Seamer	ILWC 246
49997	Kesen_077	*C. ret*	Ret_B	9.00	8091	0.81	2	0.01	0.17	0.59
50170	Karab_171	*C. ech*	Ech_B	9.01	8150	0.82	2	0.01	0.17	0.60
49899	CudiA_124	*C. ret*	Ret_G	9.02	8244	0.82	2	0.01	0.18	0.61
50166	Karab_162	*C. ech*	Ech_B	9.03	8337	0.83	2	0.01	0.19	0.62
50031	Sarik_066	*C. ret*	Ret_D	9.03	8337	0.83	2	0.01	0.19	0.62
49833	Bari3_100	*C. ret*	Ret_E	9.05	8499	0.85	2	0.01	0.21	0.64
50047	Sirna_060	*C. ret*	Ret_H	9.05	8511	0.85	2	0.01	0.21	0.64
49885	CudiB_022C	*C. ret*	Ret_G	9.05	8521	0.85	2	0.01	0.21	0.64
49883	CudiB_019	*C. ret*	Ret_G	9.07	8650	0.86	2	0.01	0.22	0.66
49819	Bari3_072C	*C. ret*	Ret_E	9.07	8656	0.87	2	0.01	0.22	0.66
49961	Kalka_074	*C. ret*	Ret_C	9.08	8778	0.88	2	0.01	0.24	0.67
49799	Bari1_092	*C. ret*	Ret_F	9.09	8871	0.89	2	0.01	0.25	0.68
49881	CudiB_018	*C. ret*	Ret_G	9.09	8880	0.89	2	0.03	0.29	0.66
50197	S2Drd_106	*C. ech*	Ech_B	9.09	8884	0.89	1	0.01	0.25	0.68
ILWC 39		*C. ech*		9.12	9092	0.91	2	0.02	0.28	0.70
50089	Sirna_105	*C. ret*	Ret_H	9.12	9110	0.91	1	0.04	0.31	0.68
49821	Bari3_073	*C. ret*	Ret_E	9.13	9189	0.94	2	0.02	0.28	0.71
50041	Sarik_078	*C. ret*	Ret_D	9.13	9210	0.92	2	0.02	0.28	0.72
50162	Karab_092	*C. ech*	Ech_B	9.13	9259	0.93	2	0.02	0.29	0.72
49849	Besev_061	*C. ret*	Ret_D	9.13	9266	0.93	1	0.04	0.32	0.69
50119	Deste_072A	*C. ech*	Ech_A	9.15	9379	0.94	2	0.02	0.3	0.73
50073	Sirna_085	*C. ret*	Ret_H	9.16	9545	0.95	1	0.05	0.35	0.72
49835	Bari3_101	*C. ret*	Ret_E	9.17	9648	0.96	2	0.02	0.33	0.76
50087	Sirna_104	*C. ret*	Ret_H	9.18	9659	0.97	1	0.05	0.36	0.73
49929	Deric_073	*C. ret*	Ret_D	9.18	9670	0.97	2	0.02	0.33	0.76
50007	Oyali_076	*C. ret*	Ret_A	9.18	9731	0.97	2	0.02	0.34	0.76
49959	Kalka_070	*C. ret*	Ret_C	9.19	9750	0.98	2	0.02	0.34	0.77

AGG Number	Accession	Species	Genetic_ Pop	Log_e	BTM	RF	#. Exps.	PBA HatTrick	PBA Seamer	ILWC 246
0283-1095-1002		*C. ret* der		9.19	9809	0.98	2	0.03	0.36	0.76
49815	Bari3_065	*C. ret*	Ret_E	9.19	9839	0.98	2	0.03	0.35	0.77
50063	Sirna_071C	*C. ret*	Ret_H	9.20	9901	0.99	1	0.06	0.38	0.74
50067	Sirna_082	*C. ret*	Ret_H	9.21	9966	1.00	1	0.06	0.39	0.75
49936	Egill_063	*C. ret*	Ret_C	9.21	9966	1.00	2	0.03	0.37	0.78
50039	Sarik_077	*C. ret*	Ret_D	9.21	10013	1.00	2	0.03	0.37	0.79
49831	Bari3_092	*C. ret*	Ret_E	9.23	10198	1.02	2	0.03	0.39	0.80
49995	Kesen_075	*C. ret*	Ret_B	9.24	10278	1.03	2	0.03	0.4	0.81
49981	Kesen_065	*C. ret*	Ret_B	9.24	10343	1.03	2	0.03	0.41	0.81
49893	CudiA_104	*C. ret*	Ret_G	9.26	10522	1.05	2	0.04	0.43	0.82
50140	Karab_062A	*C. ech*	Ech_B	9.28	10754	1.08	2	0.05	0.46	0.83
50071	Sirna_084	*C. ret*	Ret_H	9.29	10812	1.08	1	0.08	0.47	0.80
ICC11323		*C. ariet*		9.29	10834	1.08	1	0.09	0.47	0.81
50069	Sirna_083	*C. ret*	Ret_H	9.29	10836	1.09	1	0.09	0.47	0.81
49865	CudiB_004	*C. ret*	Ret_G	9.30	10949	1.09	2	0.05	0.48	0.85
49829	Bari3_091	*C. ret*	Ret_E	9.31	10998	1.10	2	0.05	0.48	0.85
50015	Oyali_100	*C. ret*	Ret_A	9.31	11032	1.10	2	0.05	0.49	0.85
50099	Cermi_071	*C. ech*	Ech_A	9.31	11057	1.11	1	0.09	0.49	0.82
49871	CudiB_008B	*C. ret*	Ret_G	9.32	11114	1.11	2	0.06	0.49	0.86
PBA Seamer		*C. ariet*		9.32	11175	1.12	2	0.06	NaN	0.86
PBA Pistol		*C. ariet*		9.32	11215	1.12	2	0.06	0.5	0.86
49927	Deric_072	*C. ret*	Ret_D	9.35	11546	1.15	2	0.07	0.54	0.88
49873	CudiB_009	*C. ret*	Ret_G	9.37	11674	1.17	2	0.08	0.55	0.89
49825	Bari3_075	*C. ret*	Ret_E	9.39	11921	1.19	2	0.08	0.58	0.90
50160	Karab_091B	*C. ech*	Ech_B	9.39	11955	1.20	1	0.13	0.57	0.86
50097	Cermi_063	*C. ech*	Ech_A	9.40	12132	1.21	1	0.14	0.58	0.87
49903	CudiA_128	*C. ret*	Ret_G	9.41	12199	1.22	2	0.1	0.6	0.91

AGG Number	Accession	Species	Genetic_ Pop	Log_e	BTM	RF	#. Exps.	PBA HatTrick	PBA Seamer	ILWC 246
50183	S2Drd_062	*C. ech*	Ech_B	9.41	12202	1.22	2	0.1	0.6	0.91
50117	Deste_071	*C. ech*	Ech_A	9.42	12286	1.23	1	0.14	0.59	0.87
49827	Bari3_079	*C. ret*	Ret_E	9.44	12606	1.26	2	0.12	0.64	0.91
49807	Bari2_067	*C. ret*	Ret_E	9.45	12726	1.27	2	0.12	0.65	0.92
49805	Bari2_064	*C. ret*	Ret_E	9.46	12878	1.29	2	0.13	0.66	0.93
49917	Deric_062	*C. ret*	Ret_D	9.47	12973	1.30	2	0.13	0.67	0.93
50201	S2Drd_109	*C. ech*	Ech_B	9.50	13350	1.34	2	0.15	0.7	0.94
49847	Bari3_112	*C. ret*	Ret_E	9.50	13367	1.34	2	0.15	0.7	0.94
49945	Egill_074	*C. ret*	Ret_C	9.50	13393	1.34	2	0.15	0.71	0.94
49895	CudiA_105	*C. ret*	Ret_G	9.53	13775	1.38	2	0.18	0.73	0.95
49887	CudiB_023	*C. ret*	Ret_G	9.56	14141	1.41	2	0.19	0.76	0.96
49875	CudiB_011	*C. ret*	Ret_G	9.56	14229	1.42	2	0.2	0.76	0.96
49947	Egill_075	*C. ret*	Ret_C	9.57	14379	1.44	2	0.21	0.78	0.96
49889	CudiA_101A	*C. ret*	Ret_G	9.59	14552	1.46	2	0.22	0.79	0.96
50109	Deste_063	*C. ech*	Ech_A	9.61	14859	1.49	2	0.24	0.8	0.97
49867	CudiB_005	*C. ret*	Ret_G	9.62	15092	1.51	2	0.25	0.82	0.97
49921	Deric_066	*C. ret*	Ret_D	9.63	15236	1.52	2	0.26	0.82	0.97
50105	Cermi_075	*C. ech*	Ech_A	9.64	15436	1.54	2	0.27	0.83	0.98
49923	Deric_069	*C. ret*	Ret_D	9.66	15691	1.57	2	0.29	0.85	0.98
49925	Deric_070	*C. ret*	Ret_D	9.70	16296	1.63	2	0.33	0.87	0.98
49940	Egill_066	*C. ret*	Ret_C	9.70	16376	1.64	2	0.34	0.87	0.98
50189	S2Drd_101	*C. ech*	Ech_B	9.77	17569	1.76	2	0.42	0.91	0.99
50181	S2Drd_061	*C. ech*	Ech_B	9.78	17636	1.77	2	0.42	0.91	0.99
49938	Egill_065	*C. ret*	Ret_C	9.78	17673	1.77	2	0.42	0.92	0.99
50065	Sirna_081B	*C. ret*	Ret_H	9.78	17707	1.77	1	0.44	0.87	0.98
49869	CudiB_006	*C. ret*	Ret_G	9.79	17800	1.78	2	0.43	0.92	0.99
50123	Deste_075	*C. ech*	Ech_A	9.81	18232	1.82	1	0.47	0.89	0.98

AGG Number	Accession	Species	Genetic_ Pop	Log_e	BTM	RF	#. Exps.	PBA HatTrick	PBA Seamer	ILWC 246
PBA HatTrick		*C. ariet*		9.85	18877	1.89	2	NaN	0.94	0.99
Yorker		*C. ariet*		9.85	18897	1.89	2	0.5	0.94	0.99
49863	Besev_083	*C. ret*	Ret_D	9.87	19264	1.93	2	0.52	0.95	1.00
50053	Sirna_064	*C. ret*	Ret_H	9.88	19476	1.95	2	0.54	0.94	0.99
Flipper		*C. ariet*		9.96	21257	2.13	2	0.64	0.97	1.00
49943	Egill_073	*C. ret*	Ret_C	10.08	23782	2.38	2	0.76	0.99	1.00
PBA Boundary		*C. ariet*		10.08	23941	2.39	2	0.76	0.99	1.00
49941	Egill_072	*C. ret*	Ret_C	10.11	24672	2.47	1	0.75	0.98	1.00
Jimbour		*C. ariet*		10.16	25774	2.58	2	0.82	0.99	1.00
50187	S2Drd_100	*C. ech*	Ech_B	10.34	30975	3.10	2	0.93	1.00	1.00
50121	Deste_073	*C. ech*	Ech_A	10.40	32899	3.29	1	0.92	1.00	1.00
Sonali		*C. ariet*		10.51	36499	3.65	2	0.98	1.00	1.00
Howzat		*C. ariet*		10.59	39836	3.98	2	0.99	1.00	1.00
50045	Savur_063	*C. ret*	Ret_D	10.64	41794	4.18	2	0.99	1.00	1.00
Sona		*C. ariet*		10.77	47670	4.77	2	1.00	1.00	1.00
ILWC 184		*C. ret*		10.83	50654	5.07	2	1.00	1.00	1.00
Kyabra		*C. ariet*		10.92	55167	5.52	2	1.00	1.00	1.00

Supplementary Table 2. The range of best linear unbiased estimates (BLUEs) for $\log_e$ (*P. thornei*/kg soil + roots) of accessions from each collection site in the two experiments. Other information on collection sites is given for elevation range, species, genetic population groups, and number of accessions *C. ret* = *C. reticulatum* species (wild *Cicer)*; *C. ech* = *C. echinospermum species* (wild *Cicer*) Genetic_Pop. = Genetic population group; # ACC = number of accessions; BTM = back-transformed mean.

		Elevation				Experiment 1		Experiment 2	
						Range		Range	
Province	Collection Site	Range (m)	Species	Genetic. Pop.	# Acc.	$\log_e$(*Pt*/kg)	BTM	$\log_e$ (*Pt*/kg)	BTM
Adiyaman	Oyali	918–940	*C. ret*	Ret_A	8	6.9–9.02	990–8266	8.50–10.11	4915–24588
Mardin	Baristepe 1	975–977	*C. ret*	Ret_F	8	7.35–8.86	1557–7055	8.19–9.78	3605–17677
	Baristepe 2	959–961	*C. ret*	Ret_E	5	7.72–8.95	2247–7686	8.47–10.38	4770–32209
	Baristepe 3	951–963	*C. ret*	Ret_E	17	7.88–10.64	2639–41894	8.41–10.51	4492–36680
	Beslever	878–922	*C. ret*	Ret_D	8	8.15–9.55	3451–14078	8.46–10.52	4722–37049
	Dereici	992–1000	*C. ret*	Ret_D	10	7.52–9.44	1853–12644	8.10–10.42	3294–33523
	Kayatepe	1083–1086	*C. ret*	Ret_D	7	7.71–8.48	2224–4821	8.18–8.86	3569–7044
	Sarikaya	915–1005	*C. ret*	Ret_D	10	7.76–9.52	2335–13622	8.34–9.80	4188–18034
	Savur	915	*C. ret*	Ret_D	1	10.04	22855	11.59	108012
Sirnak	CudiA	1285–1288	*C. ret*	Ret_G	14	8.94–9.77	3296–11445	8.96–10.16	7785–25848
	CudiB	1363–1369	*C. ret*	Ret_G	12	8.54–10.15	5098–25683	8.99–10.13	8022–25084
	Sirnak	1659–1661	*C. ret*	Ret_H	10	8.94–9.77	7606–17510	9.27–10.35	10615–31257
Diyarbakir	Kesentas	867–891	*C. ret*	Ret_B	10	7.65–9.66	2107–15600	8.59–9.87	5378–19341
	Egil	986–989	*C. ret*	Ret_C	7	8.38–9.26	4369–10562	9.97–11.24	21375–76115
	Kalkan	840–861	*C. ret*	Ret_C	6	8.15–9.01	3457–8198	8.71–9.90	6063–19930
	Gunasan	836–842	*C. ech*	Ech_A	2	7.78–9.02	2395–8248	8.33–8.73	4146–6186
	Cermik	770–778	*C. ech*	Ech_A	5	7.71–9.01	2223–8206	8.17–10.34	3533–30946
Sanliurfa	Destek	739–770	*C. ech*	Ech_A	9	8.08–9.38	3241–11821	8.47–11.24	4770–76115
	Siv-Diyar (S2 Drd)	1107–1126	*C. ech*	Ech_B	11	7.71–10.32	2223–30286	8.74–10.73	6248–45707
	Karabahce	1262–1268	*C. ech*	Ech_B	12	7.98–9.20	2909–9881	8.27–9.82	3905–18398
	Ortanca	857–861	*C. ech*	Ech_C	2	8.18–8.50	3564–4892	8.82–9.11	6768–9045

APPENDEX B

List of Publications

Poster Presentations

Reen, RA 2019 Wild Chickpea from Turkey can improve Australian chickpea cultivars'. *Centre for Crop Health, University of Southern Queensland Open Day*, Toowoomba, 2019, 5th February.

Reen, RA, Rostad, HE, Thompson, JP 2017 'Resistance to root-lesion nematode (*Pratylenchus thornei*) in a new collection of wild species of chickpea'. *Feed the Future Innovation Lab for Climate Resilient Chickpea 2017 Annual Meeting,* Hyderabad, India, 2017, 11–14th December. http://chickpealab.ucdavis.edu/index.php/events/2017-annual-meeting/abstracts/

Zwart, RS, **Reen, RA**, & Thompson JP 2019 'Association mapping of root-lesion nematode *Pratylenchus thornei* resistance in *Cicer reticulatum*, the wild progenitor of chickpea. *Australian Pulse Conference*, Horsham, 2019, 15-17th October.

Oral Presentations

Reen, RA, Mumford, MH, Zwart, RS, Owen, KA and Thompson JP 2019. Wild chickpeas from Turkey can provide nematode resistance for Australian chickpeas *Australasian Plant Pathology Society (APPS) Qld Plant Pathology Seminar Series* University of Southern Queensland; Toowoomba, 2019, October 21st.

Reen, RA, Zwart, RS & Thompson JP 2019 'Resistance of wild *Cicer* and chickpea to root-lesion nematode *Pratylenchus thornei'*. *Wild Cicer Annual National Project Meeting*, Perth, 2019, 4-5th April.

Reen, RA, Mumford, M & Thompson JP 2018 'Wild Chickpeas from Turkey can provide resistance for the Australian chickpea industry' *Higher Degree Research Student Week,* University of Southern Queensland, Toowoomba, 2018, 22nd June.