
# RESISTANCE OF WILD RELATIVES (*CICER RETICULATUM* AND *C. ECHINOSPERMUM*) OF CHICKPEA (*C. ARIETINUM*) TO THE ROOT-LESION NEMATODE *PRATYLENCHUS NEGLECTUS*

A Thesis submitted by

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For the award of

Master of Science (Advanced Research Specialisation)

2022

# ABSTRACT

Chickpea (*Cicer arietinum* L.) is a major legume crop consumed worldwide. Its nutritional value as a pulse and its ability to fix atmospheric nitrogen denote its global importance in the cereal-pulse cropping systems. Australia is the largest exporter and second largest producer of chickpea after India. *Pratylenchus neglectus* (Rensch) Filipjev & Schuurmans-Stekhoven is a root-lesion nematode that invades, feeds, and reproduces in the roots of grain crops including chickpea and wheat (*Triticum aestivum* L.). In Australia, chickpea and wheat are commonly grown in rotation and damage by *P. neglectus* accounts for a large annual economic loss in production of both crops. Cultivated chickpea has little genetic diversity limiting the potential for improvement to abiotic and biotic resistance through plant breeding. However, the chickpea genepool may be expanded through introgression of favourable genes present in wild related species. New germplasm collections from southeast Turkey of two wild species, *C. reticulatum* Ladizinsky and *C. echinospermum* P.H. Davis, have substantially increased the previously limited world collection of these species. This research assessed 243 C. *reticulatum* and 86 *C. echinospermum* accessions from the 2013 and 2014 collection missions that spanned 32 collection sites within Turkey. The accessions were assessed in replicated pot experiments under controlled glasshouse conditions. Multi-experiment analyses to determine genetic rankings of accessions showed improved resistance in wild *Cicer* accessions compared to Australia's elite moderately resistant breeding cultivar PBA HatTrick. This study is unique, evaluating *P. neglectus* resistance of this collection and providing important information on *P. neglectus*-chickpea interactions which is lacking worldwide. This study has revealed new sources of *P. neglectus* resistance that can be introgressed into commercial chickpea cultivars to improve the diversity and level of resistance that chickpea has to this nematode species. Results from this study will contribute to a genome wide association study to identify markers and candidate genes for *P. neglectus* resistance. Chickpea cultivars with improved resistance provide growers with more flexible crop rotations, a reduction of *P. neglectus* population densities in infested fields and more profitable yields.

# CERTIFICATION OF THESIS

This thesis is the work of Hannah Elizabeth Rostad, except where otherwise acknowledged, with much 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: Dr Rebecca Zwart

Associate Supervisor: Professor John Thompson

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

# STATEMENT OF CONTRIBUTION

The following statement summarises the agreed share of contribution for the candidate and co-authors in the submitted publication in this thesis:

**Rostad HR**, Reen, RA, Mumford, MH, Zwart RS & Thompson JP (2022) Resistance to root-lesion nematode *Pratylenchus neglectus* identified in a new collection of two wild chickpea species (*Cicer reticulatum* and *C. echinospermum*) from Turkey. (Accepted for publication to Plant Pathology; Q1; Impact Factor: 2.59) **(Chapter 3)**.

I, Hannah Rostad declare that I am the first author of the paper submitted for publication and incorporated in this thesis. Mrs Roslyn Reen, Mr Michael Mumford, Dr Rebecca Zwart and Professor John Thompson 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:

60% Hannah Rostad: Planning, design, experimentation, data collection, collation and interpretation of data, graphing, drafting, writing, editing and revising of the journal article for publication.

10% Mrs Roslyn Reen: Assistance with planning, design, data collection, editing, and revising of the journal article for publication.

5% Mr Michael Mumford: Experimental design, data analysis and editing of the journal article for publication.

15% Dr Rebecca Zwart: Reviewing of data collection, editing, and revising of journal article for publication and submission.

10% Professor John Thompson: Concept, planning, editing and revising of the journal article for publication and submission.

# ACKNOWLEDGMENTS

This research has been supported by an Australian Government Research Training Program Scholarship. I would like to acknowledge the support of my supervisors Dr Rebecca Zwart and Professor John Thompson of the University of Southern Queensland (USQ) for their advice, encouragement and proof reading throughout the course of this thesis. Research experiments were undertaken at the Leslie Research Facility of the Queensland Department of Agriculture and Fisheries (QDAF), Toowoomba and USQ Agricultural Sciences Precinct, Toowoomba. I thank QDAF and USQ for the use of their laboratories and glasshouses that were essential for the undertaking of this research.

I would like to thank the funding bodies, the Grains Research and Development Corporation (GRDC), QDAF for support under the Broadacre Cropping Initiative with USQ for their financial support of this research. I also thank Dr Sally Norton and the staff at Australian Grains Genebank (AGG) for supply of seed, and Dr Jens Berger CSIRO Western Australia, and Prof. Douglas Cook of University of California Davis and teams, for the task of collecting the wild *Cicer* accessions in Turkey.

Furthermore, I would also like to extend my thanks and gratitude to the whole Crop Nematology Team of the Centre for Crop Health who gave me valuable input, encouragement, and feedback throughout my research. In particular I am grateful to Roslyn Reen for her technical assistance, proof reading and advice.

Finally, I thank my family and husband Jonathan for their ongoing support and always believing in me and pushing me to succeed.

# TABLE OF CONTENTS

# LIST OF FIGURES

# ABBREVIATIONS

| | |
|---|---|
| AMF | Arbuscular mycorrhizal fungi |
| cv. | Cultivar |
| GRDC | Grains Research and Development Corporation |
| GWAS | Genome-wide association study |
| NAM | Nested association mapping |
| PPN | Plant parasitic nematode |
| QTL | Quantitative trait locus |
| RLN | Root-lesion nematode |

# CHAPTER 1: INTRODUCTION

## 1.1 Thesis outline

This thesis encompasses four chapters. Chapter 1 outlines the importance of chickpea (*Cicer arietinum* L.) from both economic and nutritional standpoints. Limitations to chickpea production caused by the root-lesion nematode *Pratylenchus neglectus* (Rensch) Filipjev & Schuurmans-Stekhovenis and why finding new sources of resistance to this pathogen is important are discussed. Chapter 2 is a literature review encompassing relevant research on chickpea, *P. neglectus* and the new collection of wild *Cicer* germplasm which is the focus of this thesis. Chapter 3 presents the research undertaken in this project in the form of the manuscript accepted to the Q1 international journal Plant Pathology. Chapter 4 summarises the thesis, focusing on the research benefits and future directions for this research.

## 1.2 Overview

Chickpea is an important pulse crop, which plays a vital role in food security by providing the daily nutritional needs to populations of developing countries and is a strong economic resource for exporting countries. Demand for pulses has increased worldwide and current price rises has made chickpea more attractive for growers to produce. India is the largest producer of chickpea, generating 65% of total global chickpea production (Merga & Haji 2019, p. 4). Australia is the second largest producer of chickpea, accounting for 14% of chickpea production worldwide (Merga & Haji 2019, p. 4). Australia meets almost half of the global export demand providing an annual average of 570,000 tonnes of chickpea into the global market (Muehlbauer & Sarker 2018, p. 10). At its peak production in 2017, Australia exported 1,785,581 tonnes of chickpea into the global market (FAOSTAT, 2022). The latest statistics from 2019 show a downturn in export of chickpea at 423,755 tonnes (FAOSTAT, 2022). The cause of this downturn is related to growing conditions in Australia, going through a long period of drought, wavering demand from overseas and an increase in import taxes on chickpea imposed by India at the time. Although dependent on season, the value of chickpea export from Australia varied between 280M AUD to ~2B AUD at its highest peak value from 2009 to 2017 (Muehlbauer & Sarker 2018, p. 10; ABARES 2020). Chickpeas are also used as an

important rotational crop in Australia, replacing nutrients such as nitrogen into the soil which makes chickpea a valuable crop in farming systems as well as their value from an economic standpoint. In the growing period of 2017-18 chickpeas were grown across an area of 1,075,000 hectares in Australia (Bard 2019), highlighting their popularity and status as an important commodity in the Australian grains industry.

Chickpea is highly nutritious comprising ~60% carbohydrates, 30% protein, 6% fat and the remaining 4% consisting of folate, zinc, iron, magnesium, and B vitamins (Hulse 1994, p. 79; Wallace et al 2016, p. 2; Merga & Haji 2019, p. 10). The nutritional profile of chickpea fits into both the vegetable and meat food groups enabling it to fill the requirement of both (Wallace et al 2016, p. 2). Chickpeas have a low glycaemic index (Jenkins et al. 1983, p. 261; Venn et al. 2010, p. 367), have been shown to lower the risk of cardiovascular disease (Jukanti et al. 2012, p. 18; Gupta et al. 2017, p. 7), reduce cholesterol (Jukanti et al. 2012, p. 18; Wallace et al 2016, p. 6) and decrease systolic blood pressure (Mollard et al. 2012, p. 115; Gupta et al. 2017, p. 7).

Factors that impact the production of chickpea have a negative influence on growers' profits and supply to trade markets that can result in consumers having limited access to their food source and paying inflated prices for the product.

### 1.3 The nematode problem

The genus *Pratylenchus* is recognised globally as one of the major root-lesion nematode (RLN) species constraining many economically important crops (Castillo & Volvas 2007, p. 3). *Pratylenchus* spp. are soil inhabiting, obligate parasites that migrate into the roots of host plants causing major root damage, which results in nutrient deficiency, reduced water uptake by the plant, and overall yield loss in the crop (Castillo & Volvas 2007, p. 3). *Pratylenchus neglectus* is one of the most common and widely distributed species of the genus *Pratylenchus* worldwide (Bucki et al. 2020, p. 14). There is no global economic figure reported for crop loss from *P. neglectus*, however, it is understood to be a globally damaging nematode species to many crops including chickpea (Williams et al. 2002, p. 874; Smiley et al. 2005, p. 958; Ballard et al. 2006, p. 303; Oldach et al. 2014, p. 6; Smiley et al. 2014, p. 1344).

In Australia, crop losses, control measures, breeding efforts and cultural practices account for 9.6M AUD in costs due to *P. neglectus* every year (Murray & Brennan 2012, p. 8). Chickpea is susceptible to *P. neglectus*; however, it is understood to be more tolerant than other crops to *P. neglectus* damage (GRDC Grownotes 2016, p. 9). Tolerance can vary with pathogen load and rate of pathogen multiplication with some crops having higher tolerance levels than others (Pagán & García-Arenal 2020, p. 81). Therefore, when talking about chickpea tolerance, the definition most suited is the ability of a plant to endure the effects of nematode damage while nematode reproduction still occurs (Cook & Evans 1987, p. 186; Dalmasso et al. 1992, p. 467). Wheat (*Triticum aestivum* L.) is also susceptible to *P. neglectus* with crop losses ranging from 10–32% (Vanstone et al. 1998, p. 187; Taylor et al. 1999, p. 622; Vanstone et al. 2008, p. 226). Considering a common crop rotation in Australia is chickpea with wheat, the chickpea crop may still yield well because of its tolerance, but the subsequent wheat crop could have larger crop losses due to the increase in nematode populations in the soil, reducing the benefits chickpea has as a rotational crop (Taylor et al. 2000b, p. 596; Hawthorne & Bedggood 2007, p. 2). The most effective management strategy for *P. neglectus* is rotation of resistant crops in sequence as shown for *P. thornei* by Owen et al. 2014 (p. 228). Therefore, breeding cultivars with increased levels of resistance is a sustainable, economical, and effective long-term solution for managing nematodes (Dalmasso et al. 1992, p. 466; Castillo & Volvas 2007, p. 381; Zwart et al. 2019a, p. 2).

Identifying new sources of *P. neglectus* resistance in chickpea is vital to the improvement of the cultigen. Diversity within cultivated chickpea is small due to its limited genetic base due to four genetic bottlenecks during its domestication (Abbo et al. 2003, p. 1082) and a previously poor collection of original wild *Cicer* accessions located in genebanks, that could be accessed for breeding programs (Berger et al. 2003, p. 1077). To increase the number of wild *Cicer* accessions available in world genebanks, a new collection mission was organised. During 2013–2018 an international group of scientists from Australia, the United States of America and Turkey collected seed of wild *Cicer* from southeastern Turkey, where the progenitor of chickpea *C. reticulatum* Ladizinsky originated. This new collection successfully addressed diversity gaps in previously available wild *Cicer* germplasm, with genetic studies determining the new wild *Cicer* collection of the species

crossable with chickpea, *C. reticulatum* and *C. echinospermum* P.H. Davis, to have 100 times more diversity than cultivated chickpea *C. arietinum* (von Wettberg et al. 2018, p. 6). Utilising crop wild relatives in breeding programs provides an encouraging solution to incorporate new genetic diversity for abiotic and biotic resistance, and improved germplasm of cultivated chickpea. This is because wild *Cicer* is a rich source of genetic diversity that has not been subject to the bottlenecks of domestication and has already proved useful in finding new sources of resistance to abiotic and biotic constraints (Singh & Ocampo 1997, p. 420; Pande et al. 1998, p. 13; Collard et al. 2001, p. 272; Abbo et al. 2007, p. 338; Knights et al. 2008, p. 384; Thompson et al. 2011 p. 606; Talip et al. 2018, p. 961; von Wettberg et al. 2018, p. 8; Reen et al. 2019, p. 1277; Newman et al. 2021, p. 373).

In an international collaborative effort to effectively exploit the genetics of the new wild *Cicer* seed collected for improvement of cultivated chickpea, 26 genotypes, 20 *C. reticulatum* and six *C. echinospermum* were selected to represent the genetic and environmental diversity of the new wild *Cicer* collection (von Wettberg et al. 2018, p. 8). These 26 wild *Cicer* accessions were then crossed into seven chickpea cultivars that represented major chickpea growing regions worldwide to create nested association mapping (NAM) populations (von Wettberg et al. 2018, p. 8). The elite chickpea cv. chosen to represent Australia was PBA HatTrick. Early results from phenotypic evaluation of the NAM parents have indicated heat stress tolerance, pod borer (*Helicoverpa armigera* Hübner) resistance (von Wettberg et al. 2018, p. 8), *P. thornei* Sher & Allen resistance (Reen et al. 2019, p. 1273), aschochyta blight (*Aschochyta rabiei* (Passerini) Labrousse)) resistance (Newman et al. 2021, p. 374) and cottony soft rot (*Sclerotinia sclerotiorum* (Lib.) de Bary)) resistance present in the selected genotypes. These 26 accessions are included in the total number of accessions tested in this thesis.

This thesis is unique, evaluating the new wild *Cicer* collection for *P. neglectus* resistance, which provides information on *P. neglectus* and chickpea interactions, a subject with little information available worldwide.

### 1.4 Aims and objectives

The aim of this thesis was to evaluate the wider collection of annual wild *Cicer, C. reticulatum* and *C. echinospermum*, collected in Turkey during 2013 and 2014. This

was to identify new sources of resistance to *P. neglectus* for future introgression into cultivated chickpea. The main objectives of this study was to conduct glasshouse trials and combine experimental results from four experiments conducted over four years of experiments by multi-experiment analyses to (i) assess 329 wild *Cicer* accessions comprising 243 C. *reticulatum* and 86 *C. echinospermum* for resistance to *P. neglectus*, (ii) compare levels of resistance in the two wild species *C. reticulatum* and *C. echinospermum* to that in cultivated chickpea (*C. arietinum*), (iii) assess the geographical clustering of resistance to *P. neglectus*, if any and (iv) identify if any of 26 genetically diverse wild *Cicer* accessions used as NAM parents have resistance to *P. neglectus*.

This new collection provides an important opportunity to access new sources of resistance to *P. neglectus*, potentially contributing previously unavailable resistance genes for research and chickpea breeding purposes. Resistant accessions identified in this collection can be introgressed into commercial chickpea to improve current levels of resistance to *P. neglectus*. Cultivars with increased *P. neglectus* resistance and genetic diversity will have a positive impact worldwide by allowing more flexibility in grower rotations and reduction in *P. neglectus* population densities resulting in more profitable and resilient chickpea crops.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Introduction

The root-lesion nematode *Pratylenchus neglectus* is a major pathogen in the Australian grain regions causing financial losses to the Australian chickpea (*Cicer arietinum*) industry through crop damage and the cost of control measures to prevent further yield losses. Determining resistance of chickpea to nematodes necessitates a knowledge of the crop, the pathogen, and the ecological and environmental conditions. This chapter aims to provide a comprehensive review of current literature on chickpea and *P. neglectus*, focusing on the importance of chickpea wild relatives and how they can advance resistance breeding in Australia and globally.

## 2.2 Chickpea (*Cicer arietinum*)

One of the earliest cultivated legumes first grown in the Middle Eastern areas of the world is chickpea (Singh 1997, p. 161), a self-pollinating crop with a strong global demand. Chickpea cultivation is documented as early as 8,000 BC to 10,000 BC in northwest Syria and is one of the world's oldest cultivated plants (Tanno & Willcox 2006, p. 198). Subsequently, chickpea cultivation spread to Africa, Europe and particularly India (Merga & Haji 2019, p. 4). Its popularity has increased dramatically in western countries over the last decade because of its importance in human nutrition, supplying starch, protein, and cholesterol-lowering dietary fibre (Perez-Hidalgo et al. 1997, p. 66) and important amino acids (Jukanti et al. 2012, p. 11). Two main types of chickpea are grown, Kabuli and Desi (van der Maesen 1987, p. 12). They are differentiated by seed size, shape, and colour, with Kabuli being larger, round and a pale cream colour compared to Desi that is smaller and brown in colour (Figure 1).

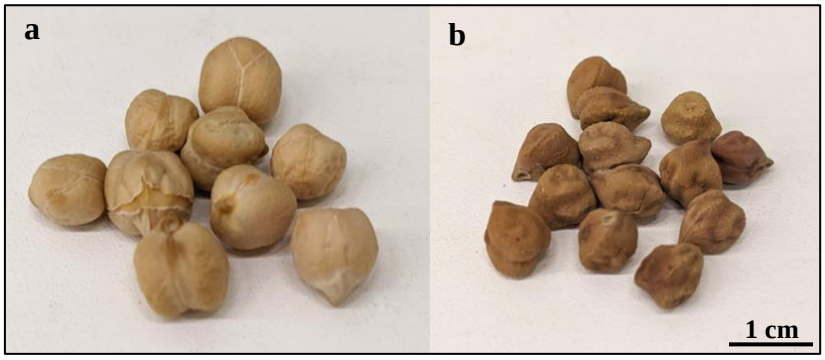


**Figure 1.** Differences between *Cicer arietinum* seed types, (a) Kabuli and (b) Desi (H Rostad 2021, personal photograph).

Chickpea is grown in a broad range of climates and soils, adapting to ecologically different parts of the world from where it was first grown in crumbly, stony soil rather than tilled regions (van der Maesen 1984, p. 95). The majority of chickpea grown in northern Australia is the Desi type, which prefers semi-arid tropical regions (Muehlbauer & Singh 1987, p. 100). Kabuli is favoured for growing in southern Australia, with this type preferring temperate regions (Muehlbauer & Singh 1987, p. 100).

Chickpea has a beneficial role as a rotational crop with cereals, supplying nitrogen to the soil (Herridge et al. 1995, p. 546) and as a non-host of the fungus *Fusarium pseudograminearum* O'Donnell & T. Aoki, the cause of crown rot in wheat and barley (*Hordeum vulgare* L.) (Dalal et al. 1998, p. 490). In the 2016–17 production year when all growing conditions were favourable, Australia produced 2004 kt of chickpea with the export value being ~2B AUD (ABARES 2020). Chickpea production and export dropped considerably to 281 kt with the export value being 306M AUD in the 2019–20 production year (ABARES 2020). This lower production value was due to Australia suffering a long period of drought.

Chickpea is grown in Australia's winter cropping period with sowing between mid-May to the start of July and harvest between September and November. Seventy-five percent of Australia's chickpea is produced in the subtropical grain belt of eastern Australia (central and southern Queensland and northern New South Wales) (ABARES 2020). The favoured soils in this area are Vertosols (Isbell 2021, p. 116), which have a high clay content and plant water holding capacity swelling when wet and shrinking when dry (Dinka & Lascano, 2012, p.

82). This region receives predominantly summer rainfall, and as chickpea is a winter crop, it relies heavily on stored moisture during the growing period (Chauhan et al. 2017, p. 132). Commercial chickpea crops were first grown in this region of Australia during the 1970s near the Queensland town of Goondiwindi (28.5387° S, 150.2983° E) (GRDC Grownotes 2016, p. xxiii). In this region chickpea are grown in rotation with winter crops wheat and barley and the summer crop sorghum (*Sorghum bicolor* (L.) Mönch)) (Thomas et al. 2010, p.4), all of which are susceptible to *P. neglectus* (Williams et al. 2002, p. 874; Owen et al. 2009, p. 1).

## 2.3 Constraints to chickpea production

### 2.3.1 Abiotic constraints to chickpea production

Abiotic stressors such as drought, heat, cold and salt are important factors that limit chickpea production. Globally, drought and heat stress account for 50% of production loss in chickpea (Ahmad et al. 2005, p. 245). Cold stress in chickpea is also a common abiotic constraint, leading to flower and pod abortion in temperatures below 15 °C (Clarke et al. 2004, p. 66) and killing of the plant in extreme cold of temperatures at and below –1.5 °C (Croser et al. 2003a, p. 193). However, the disadvantages of winter sowing in Australia are outweighed by the advantages. Winter sowing in Australia is advantageous over traditional spring sowing as it increases yield, improves water use efficiency of the crop, and utilises stored moisture from summer rainfall (Millan et al. 2006, p.85; Heidarvand et al. 2011, p. 157).

Rhizobia are bacteria that colonise the roots of chickpea forming nodules that fix atmospheric nitrogen (Singh et al. 2019, p. 2), allowing chickpea to grow well in soils over a range of soil nitrogen conditions. However, sodic and saline soils increase stress on chickpea by retarding the production of nodules after rhizobia infection, decreasing the ability of nodules to fix nitrogen, and reducing photosynthesis by the plant and water uptake (Rao et al. 2002, p. 569; Singh et al. 2005, p. 491).

### 2.3.2 Biotic constraints to chickpea production

Biotic constraints that affect chickpea yields include diseases, insects, and plant-parasitic nematodes (PPNs). Major fungal diseases that impact chickpea production are ascochyta blight, fusarium wilt (*Fusarium oxysporum f. sp. ciceri* (Padwick) Matuo & K. Sato)), phytophthora root rot (*Phytophthora medicaginis* Hansen & Maxwell), and botrytis grey mould (*Botrytis cinerea* Persoon) (Ahmad et al. 2005, p. 234; Knights et al. 2008, p. 383). Ascochyta blight causes lesions on the leaf tissues, pods, and branches (Akem 1999, p. 132) resulting in up to 100% loss of chickpea yield in extreme cases (Nene and Reddy 1987). The effects of the fungus on all above-ground plant parts cause seed abortion and can essentially ring-bark the stem of the chickpea plant, eventually causing it to break (Akem 1999, p. 132). Chickpea cultivars such as PBA Seamer are grown in the subtropical grain belt of eastern Australia because they are resistant to aschochyta blight (GRDC 2020). However, PBA Seamer is also moderately susceptible to *P. neglectus* and susceptible to botrytis grey mould (GRDC 2020), which creates problems for growers who can have multiple diseases to manage.

Fusarium wilt as the name suggests causes the plant to wilt, followed by discoloration of the leaves, desiccation and eventually breakdown of the whole plant (Jiménez-Díaz et al. 2015, p. 20). This disease can be exacerbated by RLN (Castillo et al. 1998b, p. 371) with Taheri et al. 1994 (p. 184) stating that a combination of *P. neglectus* and *F. oxysporum* increased root lesions and fungal infection within the roots of wheat. It has been suggested that when *Pratylenchus* spp. and *F. oxysporum* are present together in chickpea fields, greater damage by fusarium wilt is seen than if *F. oxysporum* is present alone (Castillo et al. 1998a, p. 828). Therefore, identifying chickpea accessions with *P. neglectus* resistance has the potential not only to lower RLN reproduction in chickpea roots but also to limit fusarium wilt infection.

Phytophthora root rot is a fungal disease that thrives under wet soil conditions and can be detrimental to chickpea, causing crop losses of 50–100% depending on rainfall events and soil moisture (Knights et al. 2008, p. 383). Symptoms of phytophthora root rot include wilting, defoliation, decay, and brown lesions visible on the roots (Vock et al. 1980, p. 117).

Botrytis grey mould is a destructive disease that persists in cool, overcast conditions with high humidity (Pande et al. 2006, p. 1137). Symptoms start as grey, brown lesions on the flowers, pods and stems that turn into rot which eventually destroys the entire plant (Williamson et al. 2007, p. 562), with loss of the entire crop in severe disease events (Pande et al. 2006, p. 1138).

Major insect pests that affect chickpea production are helicoverpa pod borer (*H. armigera* and *H. punctigera* Wallengren) and leaf miner (*Liriomyza cicerina* Rondani) (Ahmad et al. 2005, p. 248; Materne et al. 2011, p. 222). *Helicoverpa armigera* is distributed throughout Asia, Africa, the Mediterranean region, and the Oceania region, while *H. punctigera* is native to and widely distributed throughout Australia (Patil et al. 2017, p. 2). Both species can cause 80 to 90% chickpea crop loss under favourable conditions for the pod borer (Sehgal & Ujagir 1990, p. 30). The nature of damage includes larvae feeding on the chickpea foliage, then as they move to the caterpillar stage the insects feed on the seeds by boring through the pod, hence their name (Patil et al. 2017, p. 3).

Leaf miner invade both winter- and spring- planted chickpea. This insect causes major crop losses through damage in both the early germination and seedling growing period and by later hindering crop development (Soltani et al. 2018, p. 1216). Damage by leaf miner is primarily caused by the larvae, which feed on the inner leaf tissue resulting in cavities between the upper and lower epidermis and early leaf fall (Weigand 1990, p. 54).

Plant-parasitic nematodes are reported from all major chickpea growing regions worldwide. Nematode species causing crop losses to chickpea include reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira), root-knot nematodes (*Meloidogyne* spp. Goeldi), cyst-forming nematodes (*Heterodera* Schmidt and *Globodera* spp. (Skarbilovich) Behrens) and RLN (*Pratylenchus spp*.) (Rasool et al. 2015, p. 73). It is estimated that these species of PPNs cause an estimated annual yield loss of 14% to chickpea production worldwide (Castillo et al. 2008, p. 840; Materne et al. 2011, p. 53).

Reniform nematodes are semi-endoparasites that partially penetrate the plant roots on which they feed causing reduced root growth, necrosis and stunting in chickpea (Jones et al. 2013, p. 954). Root-knot nematodes cause yield loss in

chickpea through the formation of permanent feeding sites in the root cortex and vascular tissue (Castillo et al. 2008, p. 840). Feeding sites deform and block the vascular tissues, limit water and nutrient uptake, reduce nodulation and cause root rot, suppressing plant growth and reducing yield of chickpea (Thompson et al. 2000, p. 492; Castillo et al. 2008, p. 840). Cyst-forming nematodes (*Heterodera* and *Globodera* spp.) are geographically more widespread than other PPNs as the eggs within the cysts can tolerate long periods of drought, persist in the soil without a host, and are easily dispersed through rain events and improper farm biosecurity of infested sites (Perry & Moens 2006, p. 110). Above-ground symptoms of cyst nematodes appear at flowering with leaves showing reduced colour, number of flowers, pods with little or no seed, and overall stunted growth of the plant (Greco et al. 1992, p. 3; Castillo et al. 2008, p. 843). Below ground, roots show little or no nodulation from nitrogen-fixing rhizobia; the overall infected root system looks unhealthy and poorly developed and adult female cyst nematodes are visible to the naked eye (Greco et al. 1988, p. 98; Greco et al. 1992, p. 3).

Root-lesion nematodes have a global economic impact on chickpea that ranks third to root-knot and cyst nematodes due to their wide host range and ability to thrive in diverse climates (Castillo & Vovlas 2007, p. 1). *Pratylenchus neglectus* and *P. thornei* are the major RLN species causing yield losses in chickpea worldwide. Root-lesion nematodes cause extensive damage to the root system of infected plants, attacking the epidermis and feeding on the parenchymatous cells of the root cortex. This feeding results in lesions on the infected tissue and eventual necrosis of the tissue. (Castillo & Vovlas 2007, p. 1; Thompson et al. 2008, p. 236). In the field, damage from RLN in chickpea can be seen in patches with plants exhibiting reduced above-ground growth and paler colour. Below-ground, there is a reduction of root growth and under extensive infestation root death (Castillo et al. 2008, p. 842). *Pratylenchus* spp. have a strong ability to survive in the absence of a host crop, possibly surviving inside roots remaining in the soil, and/or feeding on the roots of weeds in fallow between crops (Whish et al. 2017, p. 50; Vanstone & Russ 2001, p. 248). *Pratylenchus* spp. can also utilise survival mechanisms where they can suspend their metabolic activity in response to environmental conditions that are unfavourable to survival (Jackson-Ziems

2016, p. 72; Ribeiro et al. 2020, p. 1). Anhydrobiosis is one such survival mechanism that is induced by extreme dry conditions. (Jackson-Ziems 2016, p. 72). Anhydrobiosis is the principal reason eradication is challenging with 'reactivation' of the nematodes during rainfall and availability of subsequent food sources from post fallow crops (Glazer & Orion 1983, p. 333; Talavera & Valor 2000, p.79).

### 2.4 *Pratylenchus neglectus*

*Pratylenchus neglectus* is an endoparasitic, vermiform nematode that feeds, reproduces, and migrates within the root cortex of host crops causing necrosis and reduced root branching (Oldach et al. 2014, p. 2) (Figure 2).


**Figure 2.** Root browning and lesions caused by *Pratylenchus neglectus* on roots of susceptible chickpea cv. Sona (H Rostad 2020, personal photograph).

*Pratylenchus neglectus* is 0.4–0.5 mm in length when fully developed (Townshend & Anderson 1976, p. 2; Thompson et al. 2017, p. 361). *Pratylenchus neglectus* has a wide host range including many cereals and pulses. The nematodes enter plant roots using their stylet through a sharp thrusting action while secreting lytic enzymes to break down the plant cell wall (Castillo & Volvas 2007, pp. 355–356). As *Pratylenchus* spp. feed, the root cortex is damaged limiting the capacity of the plant to take up vital nutrients and water, restricting growth and grain yield (Agrios 1988, p. 728). *Pratylenchus neglectus* reproduces via mitotic parthenogenesis with five life stages, egg, juvenile stage

J1 inside the egg, and juvenile stages J2, J3, J4 and adult, all motile in the soil and plant roots (Taylor 2000a, p. 5) (Figure 3). The full life cycle is completed in 4–6 weeks depending on food source availability and optimum environmental conditions (Thompson et al. 2017, p. 357). Although *P. neglectus* are obligate parasites they can survive in dry soil for long periods, entering a state of anhydrobiosis (Taylor 2000a, p. 5).


**Figure 3.** Photomicrographs illustrating adult and juvenile (J4, J3 and J2) life stages of *Pratylenchus neglectus* (Thompson et al. 2017, p. 361).

### 2.4.1 *Pratylenchus neglectus* distribution

*Pratylenchus neglectus* is found in soils globally and in Australia it is present in all grain growing regions (Sharma et al. 2011, p. 1321; Sheedy et al. 2015, p. 175). Globally, the geographic distribution of *P. neglectus* in chickpea has been documented in Algeria, the United States of America, Kenya, (Nene et al. 1996, p. 9; Smiley et al. 2014, p. 1345) Turkey, (Behmand et al. 2019, p. 357) and Italy (Di Vito et al. 2002, p. 104). Thompson et al. 2010 (p. 256) surveyed 795 wheat fields in the subtropical grain belt of eastern Australia and found that 32% had *P. neglectus* present. Chickpea is grown in rotation with wheat in this region therefore chickpea grown at these sites would also be affected by the *P. neglectus* present. Chickpea production in the western region of Australia is a small industry (Paterson & Wilkinson 2019), however, *P. neglectus* population densities at yield limiting levels were found in at least 40% of grain cropping

fields (Riley & Kelly 2002, p. 54). Chickpea is grown in the southern region of Australia in rotation with cereals and the oilseed crop canola (*Brassica napus* L.) (Hawthorne & Bedggood 2007, p. 2). It has been documented that wheat yields have been poor after chickpea crops grown on *P. neglectus* infested sites in the southern region (Hawthorne & Bedggood 2007, p. 2). Hawthorne & Bedggood 2007 (p. 2) also stated that *P. neglectus* adversely affects the growth and yield of chickpeas but has a greater negative effect on the following cereal crop.

### 2.4.2 Management of *Pratylenchus neglectus*

Measures that can be taken to manage *Pratylenchus* spp. include employing rigorous hygiene measures, such as maintaining clean farm machinery, vehicles, and footwear to reduce contamination between sites (Zhan et al. 2015, p. 30). Biological suppression of nematodes can occur using integrated management techniques such as zero tillage and stubble retention, however, these are only temporary measures as the nematicidal components of organic materials degrade quickly (Stirling 2014, p. 312). Crop residues can decrease nematode population densities through alterations in temperature, moisture and stimulating antagonistic soil microorganisms (Minton 1986, p. 139).

Antagonists of *Pratylenchus* spp. such as predatory nematodes (Khan & Kim 2007, p. 370), nematode trapping fungi (Thompson et al. 1980, p. 194; Khan et al. 2006, p. 671), arbuscular mycorrhizal fungi (AMF) (de La Peña et al. 2005, p. 169; Gough et al. 2020, p. 7) and parasitic bacteria such as *Pasteuria thornei* (Thorne) Sayre & Starr also contribute to suppression of *Pratylenchus* spp. (Starr & Sayre 1988, p. 22; Tian et al. 2007, p. 198). There are no specific studies on predation of *P. neglectus* by predatory nematodes, however, Bilgrami et al. 1986 (p. 193) showed from inspection of the intestinal contents of 33 species of monochid nematodes that *Pratylenchus* spp. was commonly found in their gut (Figure 4). An important point stated by Castillo & Volvas 2007 (p. 396), is that the use of dorylaimid, diplogastrid and mononchid predatory nematodes as a biological control method for *Pratylenchus* spp. has not been studied thoroughly and its possible importance as a control measure is unknown.

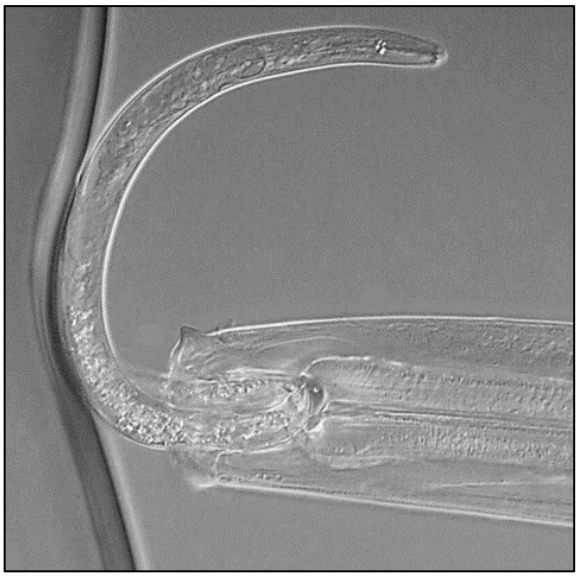

**Figure 4.** Mononchid predatory nematode feeding on a plant-parasitic nematode (Barbosa 2003).

In a study by Khan et al. 2006 (p. 671), *P. neglectus* was susceptible to the knob producing nematode-trapping fungus *Monacrosporium lysipagum* Drechsler with 81% of *P. neglectus* infected and killed within 20 h of exposure to the fungus. As *P. neglectus* colonise inside the roots of host plants, nematode trapping fungi as a biological control method would only be effective on nematodes in the rhizosphere (Castillo & Volvas 2007, p. 395).

The parasitic bacterium *Pasteuria* species that specifically infects *Pratylenchus* spp. is known as *Pasteuria thornei* (Starr & Sayre 1988, p. 22; Castillo & Volvas 2007, p. 397) (Figure 5). A greenhouse study relating specifically to *P. neglectus* showed there was population decline when *Pasteuria thornei* spores were inoculated into soil already containing *P. neglectus*, with spores visible on the cuticle and in the body cavity of the nematode after one week (Ornat et al. 1999, p. 14). However, it was unclear if the *Pasteuria thornei* lowered population densities of *P. neglectus* to below damage thresholds, with tillage and fallowing found to be more successful control methods for this nematode (Ornat et al. 1999, p. 14). Therefore, even though studies have shown *Pasteuria thornei* can reduce *Pratylenchus* spp. populations, the number of spores and parasitism levels are inadequate to lower population densities of *Pratylenchus* spp. to below damage thresholds (Stirling 2014, p. 216).

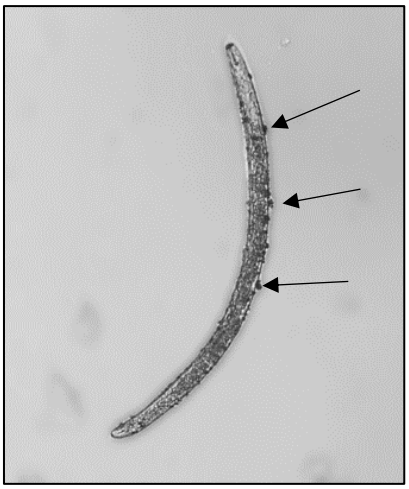

**Figure 5.** A dead *Pratylenchus neglectus* infected with the bacterium *Pasteuria thornei*; arrows indicating the bacterium (Image by J Lin 2016, unpublished).

Many studies have suggested that AMF improve host tolerance of plants infected by *Pratylenchus* spp. by boosting nutrient uptake, growth rate, reducing lesions on infected roots and reducing *Pratylenchus* spp. population densities (Forge et al. 2001, pp. 186–192; Smith & Reed 2008, p. 602; Gough et al. 2020, p. 8). A review on effects of AMF on *Pratylenchus* spp. by Gough et al. 2020 (p. 7) showed that inoculation with the AMF genera *Glomus* Tul. & C. Tul. and *Funneliformis* C. Walker & Shüßler, reduced or had no effect on *Pratylenchus* spp. densities in host roots in various studies. Therefore, there is evidence AMF can increase plant tolerance levels and offer a level of suppression against *Pratylenchus* spp. populations. However, there is little evidence to suggest AMF can be classed as a successful biological control of *Pratylenchus* spp. without further study (Castillo & Volvas 2007, p. 398–400).

Break crops can be utilised on infested sites, whereby growing a non-host will lower nematode population densities. Common break crops that help control *P. neglectus* are narrow leaf lupin (*Lupinus angustifolius* L.) (Collins et al. 2017, p. 72), field pea (*Pisum sativum* L.) (Taylor et al. 2000b, p. 596; Smiley et al. 2005, p. 966), faba bean (*Vicia faba* L.) (Taylor et al. 2000b, p. 596), triticale (*Triticale hexaploide* Lart.) (Taylor et al. 2000b, p. 596) and annual medic (*Medicago* spp. L.) (Ballard et al. 2006, p. 307). *Pratylenchus neglectus* can also be controlled with a rotation of resistant wheat and barley crops (Smiley et al. 2013, p. 538).

*Pratylenchus neglectus* and *P. thornei* occur together in 26% of fields in the subtropical grain belt of eastern Australia (Thompson et al. 2010, p. 257) and both have a wide host range. However, host resistance to one species does not always mean resistance to the other, highlighting the importance of testing fields for individual RLN species before applying management measures (Holloway et al. 2000, p. 600). Volunteer crops and susceptible weeds must also be controlled during fallow periods to successfully reduce *P. neglectus* populations in the soil (Vanstone & Russ 2001, p. 248). The most reliable control method is rotation of resistant and tolerant crops with recent research showing that growing resistant crops in sequence effectively reduces *Pratylenchus* spp. populations (Owen et al. 2014, p. 228). Through breeding crop cultivars with increased levels of resistance an economical and effective long-term solution for managing nematodes can be established (Castillo & Volvas 2007, p. 381; Zwart et al. 2019a, p. 2).

### 2.4.3 Breeding for resistance to *Pratylenchus* spp.

Resistance is defined as the ability of a plant to reduce nematode reproduction (Trudgill 1991, p. 169), whereas nematode numbers increase when a plant is susceptible (Figure 6).

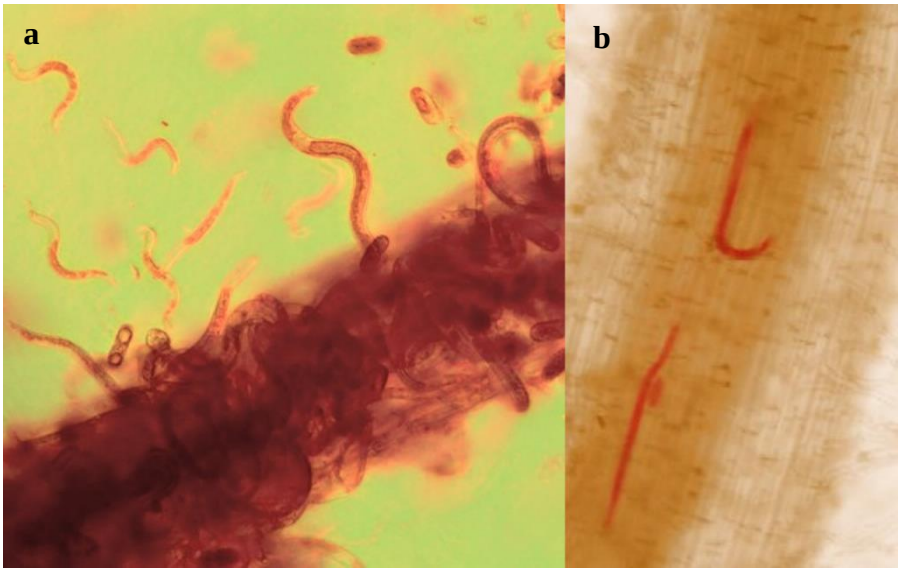


**Figure 6.** *Pratylenchus neglectus* colonising (a) a susceptible plant root and (b) a resistant plant root (*Agriculture - Root Lesion Nematodes (*Pratylenchus neglectus*) swimming through culture media and attacking barley root in petri dish. Eggs are capsule shaped. (20X);* Oregon State University 2008).

Resistance screening is best undertaken in controlled environmental conditions where temperature, soil moisture, fertiliser, inoculum, and other influences can be regulated (Nombela & Romero 1999, p. 386). Controlled environments also allow maintenance of optimal temperature for plant growth and maximum nematode reproduction. Phenotyping of wild *Cicer* conducted from 1996 to 2002 showed that accessions of *C. reticulatum* and *C. echinospermum* were largely more resistant to *P. neglectus* or *P. thornei* or to both nematode species than the commercial chickpea cultivars Howzat, Sonali, Jimbour and Sona (Thompson et al. 2011, pp. 606–607). However, only a small number of unique wild *Cicer* accessions were screened in that study (nine *C. reticulatum* and five *C. echinospermum*), because of the small number of wild *Cicer* accessions available in global genebanks at that time (Berger et al. 2003, p. 1081). The *C. reticulatum* accession ILWC 140 was shown to have good resistance to both *P. neglectus* and *P. thornei* (Thompson et al. 2011, p. 606). ILWC 140 was then crossed to the commercial cultivars Jimbour and Howzat, with several of the $F_4$ progeny producing lower *P. neglectus* population densities than the resistant parent ILWC 140 (Thompson et al. 2011, p. 609).

Recently, a larger collection of wild *Cicer* was screened for resistance to *P. thornei* by Reen et al. 2019 (p. 1270) comprising 133 *C. reticulatum* and 41 *C. echinospermum*. With the increase in wild *Cicer* germplasm available for testing, superior resistance to that of current Australian chickpea cultivars was found, with 23% of wild *Cicer* accessions tested being significantly more resistant than Australia's most *P. thornei* resistant chickpea cv. PBA Seamer (Reen et al. 2019, p. 1270). Therefore, using wild *Cicer* in resistance screening programs is an effective strategy to identify *Pratylenchus* spp. resistant accessions that can be used in subsequent breeding programs. Current commercial chickpea cv. ratings for *P. neglectus* are listed as moderately resistant to moderately susceptible (Matthews et al. 2021, p. 96). Low variation in the resistance ratings of existing chickpea cultivars is evidence that sources of better resistance are needed.

### 2.5 Background of *Cicer* genetics

Chickpea is a diploid crop with $2n=2x=16$ chromosomes (Ahmad et al. 2005, p. 229). A pan-genome was recently published on chickpea which encompassed all

genes and genetic variation within the species, comprising over 3000 individuals (Varshney et al. 2021, p. 623). Utilising previously published work on the Kabuli chickpea cv. CDC Frontier (Varshney et al. 2013, p. 241), the *C. reticulatum* genome (Gupta et al. 2017, p. 4), the ICRISAT Desi breeding line ICC 4958 and de-novo assembled sequences of wild and cultivated *Cicer* accessions, the chickpea pan-genome was shown to have 592.58 Mb and a total of 29,870 genes.

### 2.6 Wild *Cicer* relatives

Archaeological evidence suggests two centres of origin for wild *Cicer* (Vavilow 1951, p. 44). The first centre of origin is the geographic area of south western Asia and the Mediterranean region, mainly the area known today as southeast Turkey, neighbouring Syria (Vavilow 1951, p. 44; van der Maesen 1987, p. 11). The second point of origin is Ethiopia in east Africa (Vavilow 1951, p. 44). The genus *Cicer* contains 46 wild species in total, comprising ten annual and 36 perennial species (Toker et al. 2021, p. 2). Of the 10 annual species, *Cicer reticulatum*, the wild progenitor of domestic chickpea (Ladizinsky & Adler 1976, p. 198) along with *C. echinospermum* (Croser et al. 2003b, p. 435) form the primary chickpea genepool with *C. arietinum*. These two wild *Cicer* species can be directly crossed to *C. arietinum* (cultivated chickpea) to produce strong, fertile progeny (Maxted et al. 2006, p. 2677) and has been utilised widely for *C. reticulatum* and *C. echinospermum* introgressions into *C. arietinum* as shown by the following studies: Ladizinsky & Adler 1976, p. 200; Pundir & Mengesha 1995, p. 242; Singh & Ocampo 1997, p. 419; Ahmad & Slinkard 2004, p. 768; Thompson et al. 2011, p. 609; Kahraman et al. 2017, p. 3106.

Species composing the secondary genepool, namely *C. bijugum* K.H. Rech, *C. pinnatifidum* Juab & Spach and *C. judaicum* Boissier (Croser et al. 2003b, p. 436) are genetically more distant from domestic chickpea, and if successfully crossed with chickpea, regularly produce offspring that are partially sterile with low vigour because of reproductive barriers (Croser et al. 2003b, p. 436; Maxted et al. 2006, p. 2675). A new annual species named in 2021 by Toker et al. (p. 1) is *Cicer turcicum* Toker, Berger & Gokturk. This new species appears to be related to *C. reticulatum* and *C. echinospermum*, however, preliminary internal transcribed spacer sequencing has classified it into the secondary genepool,

although crossing studies are still underway to confirm this (Toker et al. 2021, p. 14).

The tertiary genepool, comprises *C. chorassanicum* (Bge) Popov, *C. cuneatum* Hochst and *C. yamashitae* Kitamura (Croser et al. 2003b; p. 437), which are even more distantly related to domestic chickpea than *Cicer* spp. from the secondary genepool. Specific breeding techniques such as embryo rescue, bridging crosses and tissue culture must be utilised for breeding with the tertiary genepool, and even if the cross succeeds the progeny are often sterile (Croser et al. 2003b, p. 436; Maxted et al. 2006, p. 2675; Kahraman et al. 2017, p. 3103).

### 2.6.1 *Cicer reticulatum* and *C. echinospermum*

The two wild annual species *C. reticulatum* and *C. echinospermum* are the focus of the research described in this thesis. Both species have a distinct morphology and seed coat. Foliage of *C. reticulatum* is described as semi-spreading and prostrate by Talip et al. 2018 (p. 956) with large leaflets which resemble *C. arietinum*. *Cicer echinospermum* is described as mostly prostate with smaller leaflets than *C. reticulatum* (Talip et al. 2018 p. 956). The difference can be clearly seen in Figures 7a and 8a, where the foliage of *C. reticulatum* grows more upright and clustered whereas *C. echinospermum* has a wider spread in its foliage that cascades away from the main growing point. *Cicer reticulatum* has a corrugated seed coat with seed similar to the size, shape, and colour of cultivated chickpea Desi seed (Figure 7b). *Cicer echinospermum* seed is smaller and darker, with a coarse, spiny seed coat (Figure 8b). Both species have small pods and purple flowers.

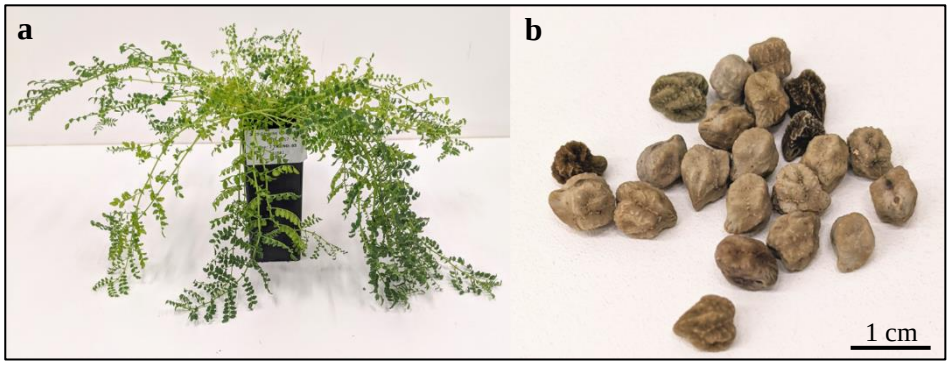


**Figure 7.** *Cicer reticulatum* (a) foliage and (b) seed. Bottom width of pot in (a) is 5 cm (H Rostad 2021, personal photographs).

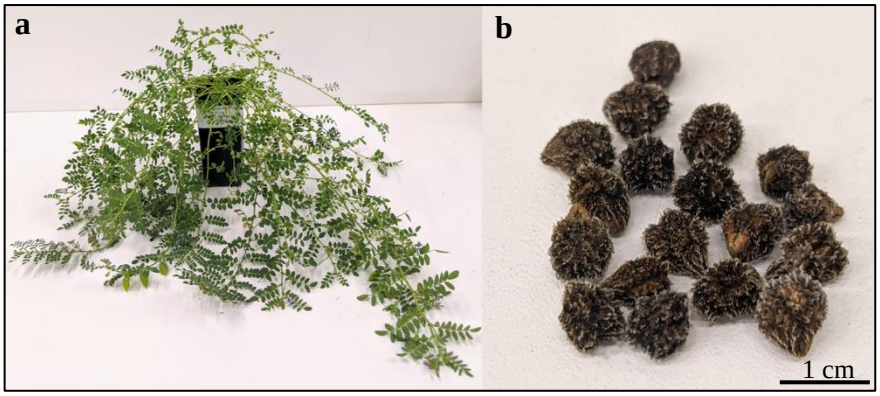


**Figure 8.** *Cicer echinospermum* (a) foliage and (b) seed. Bottom width of pot in (a) is 5 cm (H Rostad 2021, personal photographs).

Although, cultivated chickpea has superior agronomic traits compared to wild *Cicer* spp., there are important attributes that wild *Cicer* exhibit that are desirable for chickpea breeding. This includes canopy width, flower duration, number of pods per stem, plant height and resistance to major biotic constraints (Talip et al. 2018, p. 959; Singh et al. 2014, p. 238).

### 2.6.2 Benefits of using wild *Cicer* in commercial chickpea improvement

Sources of multiple resistance traits are advantageous in breeding programs with wild *Cicer* typically having multiple resistance genes, compared to that of cultivated chickpea (Croser et al. 2003b, p. 440–441). A recent study by Varshney et al. 2021 (p. 623) demonstrated chickpea experienced the strongest bottleneck in its history beginning around 10,000 years ago. Abbo et al. 2003 (p. 1082) has proposed cultivated chickpea has low genetic diversity because of four bottlenecks in its evolutionary history (Abbo et al. 2003, p. 1082). The first bottleneck occurred because of the progenitor chickpea *C. reticulatum* having a limited geographic range in southeast Turkey. The second bottleneck was due to domestication, which was brought about by continually selecting a small number of individuals for use and propagation. The third bottleneck developed from the change in sowing time from winter to spring, which has been theorized to be a way to minimise the effects of aschochyta blight (Kumar & Abbo 2001, p. 125). The fourth bottleneck was due to the replacement of landraces with cultivars produced through modern plant breeding, causing further lack of diversity within the crop (Abbo et al. 2003, p. 1082). Therefore, using wild *Cicer* in resistance breeding is critical to maintain crop productivity where genetic variability is low

(Rao et al. 2007, p. 1236), and overcome the complications brought about by these bottlenecks. Resistance has already been found in wild *Cicer* to major diseases and insect pests such as aschochyta blight (Collard et al. 2001, p. 272; Collard et al. 2003, p. 719; Newman et al. 2021, p. 373), botrytis grey mould (Pande et al. 1998, p. 13; Ramgopal et al. 2012, p. 3), phytophthora root rot (Knights et al. 2008, p. 384), fusarium wilt (Singh & Ocampo 1997, p. 420; Singh et al. 1998, p. 12), RLN *P. thornei* (Thompson et al. 2011 p. 606; Reen et al. 2019, p. 1277), and *P. neglectus* (Thompson et al. 2011 p. 606), chickpea cyst nematode (*Heterodera ciceri* Vovlas, Greco & Di Vito) (Di Vito et al. 1996 p. 103), pod borer (von Wettberg et al. 2018, p. 9) and leaf miner (Singh et al. 1998, p. 12; Talip et al. 2018, p. 961). It is clear that using wild relatives as a source of genetic diversity has already proved successful in sustaining productivity, managing diseases in chickpea and is vital when breeding for multiple disease resistance.

### 2.6.3 New wild *Cicer* collection

In 2000, nine out of 36 breeding lines in the Australian chickpea breeding program were derived from wild *C. echinospermum* (Berger et al. 2003, p. 1077). The importance of wild *Cicer* contributing resistance to biotic and abiotic stresses prompted a review of global *Cicer* germplasm by Berger et al. 2003 (p. 1077). The authors identified compromised genetic diversity in the world genebank collections with only 18 original accessions of *C. reticulatum* and 10 of *C. echinospermum* available for breeding. To address the gaps in the past wild *Cicer* collections, a group of scientists from Australia, the United States and Turkey conducted a series of comprehensive collection expeditions in southeast Turkey in 2013–2018 (von Wettberg et al. 2018, p. 3; Toker et al. 2021, p. 2). A total of 591 wild *Cicer* accessions were collected from 91 collection sites (Toker et al. 2021, p. 3). These collection missions attempted to cover the full geographic distribution of *C. reticulatum* and *C. echinospermum* (von Wettberg et al. 2018, p. 9).

The new wild *Cicer* accessions were collected from the geographic area of Anatolia with strategic trips into the northwest province of Eskişehir in Turkey, southern Armenia and central to west Georgia (Toker et al. 2021, p. 4). Annual

species identified in these collection missions were *C. reticulatum*, *C. echinospermum*, *C. bijugum* and *C. pinnatifidum*. Through later study of the accessions by DNA sequencing a new species of wild *Cicer* was discovered, *C. turcicum* (Toker et al. 2021, p. 1). This species has come from the Yedipinar collection site and is believed to be a sister species of *C. reticulatum* and *C. echinospermum* (Toker et al. 2021, p. 6). *Cicer turcicum* holds potential for chickpea improvement if its close relatedness supports interspecific hybridization with *C. arietinum* (Toker et al. 2021, p. 14). The discovery of a new *Cicer* species has increased breeding potential for chickpea improvement with crossing studies currently underway to see if successful breeding can be accomplished (Toker et al. 2021, p. 14).

By studying the new and wider collection of wild *Cicer* accessions von Wettberg et al. 2018 (p. 6) provided evidence for the genetic bottleneck that domestication has caused in chickpea and created an evolutionary timeline for wild *Cicer*. Through analysing site environments to determine elevation gradients, climatic and soil differences, it was identified that *C. echinospermum* occurs at slightly lower elevations of 740–1264 m compared to *C. reticulatum* that occurs at the higher elevational range of 915–1695 m (von Wettberg et al. 2018, p. 3). *Cicer reticulatum* was also found to occur in more fertile, alkaline soils and to better survive growing periods of infrequent rainfall than *C. echinospermum* (von Wettberg et al. 2018, p. 4). A model was also utilised from admixture graphs and residuals from maximum-likelihood analyses to estimate environmental impact on genetic structure and genetic spread of accessions, showing the variation between wild and cultivated accessions for agronomic properties (von Wettberg et al. 2018, p. 4).

Evidence of genetic variation present in the new collections of *C. reticulatum* and *C. echinospermum* has been shown in the following publications. These studies encompass abiotic and biotic stress resistance and study the agro-morphological traits of *C. reticulatum* and *C. echinospermum*. Talip et al. 2018 (p. 951) investigated the growing habits of 174 *C. reticulatum* and 47 *C. echinospermum* accessions from the 2013 collection. Both species of wild chickpea showed some more attractive morphological traits than *C. arietinum* such as increased canopy width and number of stems and pods per plant. The desirable morphological traits

in wild *Cicer* such as increased podding could be used in breeding programs to increase yield of *C. arietinum*. Resistant accessions to the abiotic constraints heat and cold were found in the new wild *Cicer* collection (Talip et al. 2018, p. 961; von Wettberg et al. 2018, p. 8; Toker et al. 2021, p. 1). Biotic stresses of leaf miner (Talip et al. 2018, p. 961), pod borer (von Wettberg et al. 2018, p. 8), RLN *P. thornei* (Reen et al. 2019 p. 1271), aschochyta blight (Newman et al. 2021, p. 373), bruchid beetle (*Callosobruchus chinensis* L.) (Toker et al. 2021, p. 1), and cottony soft rot were also evaluated, and superior resistance was found than in current commercial chickpea cultivars. These stresses reduce chickpea production on a global scale so research into improving yield, abiotic and biotic resistance is particularly important. These six publications on the new collection thus far have successfully shown it contains greater genetic diversity than current commercial chickpea cultivars and should be utilised in breeding programs to improve yield of *C. arietinum*.

To understand the genetic consequences of domestication, full genome sequences of 26 wild *Cicer* accessions, covering the biodiversity of the whole 2013 collection, were selected as parents to generate NAM populations with modern chickpea cultivars from major chickpea growing regions worldwide. From the genome sequencing of the NAM parents and modern chickpea cultivars it was estimated that 93.5–97.5% of the genetic variation of the wild *Cicer* accessions is absent from modern cultivated chickpea (von Wettberg et al. 2018, p. 6). The selected 26 NAM parents are important to the international scientific community to assist in cultivated chickpea improvement with approximately 10,000 segregating lineages being developed from the NAM parent accessions (von Wettberg et al. 2018, p. 6). In von Wettberg's et al. 2018 (p. 8) study, 23 of the 26 NAM parents produced lower host suitability indexes to pod borer than the tolerant check cultivars. Eleven of the 26 NAM parents produced lower RLN *P. thornei* population densities than the resistant reference *C. echinospermum* cv. ILWC 246 (Reen et al. 2019 p. 1274) and one NAM parent accession Gunas_062 showed moderate resistance to a cocktail of aschochyta blight isolates (Newman et al. 2021 p. 374). Therefore, it is clear that the NAM parent accessions have improved biotic stress resistance than current commercial chickpea cultivars and testing on this smaller number of accessions provides an immediate target for

introgression breeding. Large scale introgression which has been initiated through the development of segregating lines will provide many new chances to find superior resistance using wild alleles and increase genetic variation in cultivated chickpea.

### 2.7 Summary of and benefits of this research

*Pratylenchus neglectus* is a major pathogen in the Australian grain regions (Vanstone et al. 2008, p. 226) causing an estimated combined loss of 9.6M AUD/year to the Australian chickpea industry through yield loss and cost of control measures to prevent greater loss from this nematode (Murray & Brennan 2012). New sources of resistance against *P. neglectus,* will help reduce damage to crops by this pathogen. In this thesis, new accessions of *C. reticulatum* and *C. echinospermum* collected from Turkey in 2013 and 2014 have been compared with wild *Cicer* accessions of interest from the original global genebank collections, along with current commercial chickpea cultivars and breeding lines of chickpea to find new sources of resistance to *P. neglectus*. This new collection provides access to a significant increase in wild *Cicer* germplasm for resistance testing covering the full geographical range of *C. reticulatum* and *C. echinospermum*. Research conducted into testing these new accessions has provided the opportunity to broaden the genetic and adaptive diversity of chickpea by identifying sources of *P. neglectus* resistance to facilitate chickpea breeding in Australia and pave the way for providing growers with more resilient crops against this nematode. This research connects with genetic diversity studies of wild chickpeas and provides novel insights into *P. neglectus* resistance in chickpea, which is a global problem. Results from this study can be used in a genome wide association study to identify markers and candidate genes for *P. neglectus* resistance. To date, there is no published literature on chromosomal regions in chickpea associated with *P. neglectus* resistance. This is a novel study on phenotyping for *P. neglectus* resistance in chickpea and the first study to include wild *Cicer* accessions from the 2014 collection sites.

# CHAPTER 3: PUBLICATION

## Resistance to root-lesion nematode *Pratylenchus neglectus* identified in a new collection of two wild chickpea species (*Cicer reticulatum* and *C. echinospermum*) from Turkey.

This chapter is formatted for publication in the international Q1 journal Plant Pathology (Wiley-Blackwell Publishing, UK). Submitted 22/12/2021; accepted for publication 7/02/2022.

**Rostad HR, Reen, RA, Mumford, MH, Zwart RS & Thompson JP (2022) Resistance to root-lesion nematode *Pratylenchus neglectus* identified in a new collection of two wild chickpea species (*Cicer reticulatum* and *C. echinospermum*) from Turkey. (Submitted to Plant Pathology; Q1; Impact Factor: 2.59)**

### 3.1 Overview

This study characterised 329 new accessions of wild *Cicer* collected from 32 locations in southeast Turkey from 2013 and 2014 collection missions to identify resistance to the root-lesion nematode *Pratylenchus neglectus*. A summary of chickpea, the impact of *P. neglectus* in the chickpea industry within Australia and globally and previous research to identify resistance to *P. neglectus* and other biotic pressures are outlined. Phenotyping methodology and statistical analysis of data is reported. The paper discusses the findings in terms of response of wild *Cicer* accessions to *P. neglectus*, a comparison of resistance levels to current Australian chickpea cultivars and breeding lines, identification of accessions with possible multiple disease resistance through comparison reports in the published literature on the collection and how these findings can benefit the chickpea industry.

[Supplementary material associated with this chapter is attached in Appendix A.]

**Title**

**Resistance to root-lesion nematode *Pratylenchus neglectus* identified in a new collection of two wild chickpea species (*Cicer reticulatum* and *C. echinospermum*) from Turkey.**

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**Keywords:** *Pratylenchus neglectus*, root-lesion nematode, chickpea, *Cicer reticulatum*, *Cicer echinospermum*, crop wild relatives, resistance

## ABSTRACT

Chickpea (*Cicer arietinum*) is a major legume crop with Australia being the second largest producer worldwide. *Pratylenchus neglectus* is a root-lesion nematode that invades, feeds, and reproduces in roots of pulse and cereal crops. In Australia, chickpea, and wheat (*Triticum aestivum*) are commonly grown in rotation and annual damage by *P. neglectus* accounts for large economic losses to both crops. Cultivated chickpea has narrow genetic diversity which limits the potential for improvement in resistance breeding. New collections of wild chickpea species, *C. reticulatum* and *C. echinospermum*, have substantially increased the previously limited world collection of wild *Cicer* germplasm and offer potential to widen the genetic diversity of cultivated chickpea through the identification of accessions with good resistance.

This research assessed 243 *C. reticulatum* and 86 *C. echinospermum* accessions for response to *P. neglectus* in replicated experiments under controlled glasshouse

conditions from 2013 and 2014 collection missions which were received, tested, and analysed in two experimental sets. Multi-experiment analyses showed lower *P. neglectus* population densities in both sets of wild *Cicer* accessions tested than Australia's elite breeding cultivar PBA HatTrick at the significance level $P < 0.05$. Provisional resistance ratings were given to all genotypes tested in both experimental sets with *C. reticulatum* accessions CudiB_008B and Kayat_066 rated as resistant in both Set 1 and Set 2. New sources of resistance to *P. neglectus* observed in this study can be introgressed into commercial chickpea cultivars to improve their resistance to this nematode.

## INTRODUCTION

Chickpea (*Cicer arietinum*) is important in cereal-pulse cropping systems in Australia and globally, with global production of pulses increasing by over 20 million tonnes in the last decade (Belhassen et al. 2019). Chickpea plays an important role in feeding the populations of many countries while supplying a myriad of health benefits to consumers, such as high protein content, important nutrients for a balanced diet, and reduction of cholesterol and the risk of cardiovascular disease (Jukanti et al. 2012).

*Pratylenchus neglectus* is a migratory, endoparasitic, vermiform root-lesion nematode (RLN) that invades, feeds, and reproduces in the roots of many cereal and pulse crops, including chickpea. Symptoms of infestation include root necrosis and reduced root branching which results in the reduction of crop yield (Oldach et al. 2014). *Pratylenchus neglectus* reproduce via mitotic parthenogenesis with five life stages, egg, juvenile stages J2, J3 and J4, and adult with the full life cycle completed in 4–6 weeks depending on food source availability and optimum environmental conditions (Thompson et al. 2017). *Pratylenchus neglectus* is considered one of the most widespread *Pratylenchus* spp. worldwide (Behmand et al. 2019) due to its broad host range on the world's most economically important crops (Oldach et al. 2014). It is a major pathogen in the Australian grain regions where chickpea is commonly grown in rotation with wheat (Doughton et al. 1993; Thompson et al. 2011; Reen et al. 2019). *Pratylenchus neglectus* causes a loss in production valued at 73M AUD/year in wheat (Murray and Brennan 2009) and 9.6M AUD/year in chickpea (Murray & Brennan 2012). Nearly all current Australian commercial

chickpea cultivars are rated as moderately resistant to moderately susceptible (MR-MS) to *P. neglectus* (Matthews et al. 2021), where resistance is defined as the effects of host genes to prevent nematode multiplication in a host species (Trudgill 1991). Resistant plants will also reduce nematode reproduction to significantly less than that of its initial population. Limited variation in the resistance ratings of existing commercial chickpea cultivars is strong evidence that sources of good resistance and genetic diversity are needed.

Cultivated chickpea has low genetic diversity because of four genetic bottlenecks: (a) a limited centre of geographic origin, namely, an area spanning modern-day north Syria and southeast Turkey, (b) the founder effect associated with domestication from a limited number of plants, (c) a change early in the crop's history from winter to spring sowing and (d) a switch from diverse landraces to a narrower range of elite cultivars developed through modern plant breeding (Abbo et al. 2003). This makes genetic improvement of chickpea challenging. A study by von Wettberg et al. (2018) estimated that 93.5–97.5% of the genetic variation that is found in wild *Cicer* spp. is absent in modern cultivated chickpea.

Among the annual wild *Cicer* spp., the progenitor of cultivated chickpea, *C. reticulatum*, is wholly compatible with *C. arietinum* for breeding (Coyne et al. 2020). *Cicer echinospermum* is the only other annual wild *Cicer* sp. that is cross compatible with cultivated chickpea without the use of advanced hybridisation techniques (Croser et al. 2003). Therefore, these two wild *Cicer* spp. can be utilised in breeding programs to increase the genetic diversity of cultivated chickpea and maintain crop productivity (Rao et al. 2007). Resistance has been found in *C. reticulatum* and *C. echinospermum* to major biotic and abiotic constraints to chickpea, such as RLN *Pratylenchus thornei* (Thompson et al. 2011; Reen et al. 2019), Ascochyta blight (*Ascochyta rabei*) (Devadas et al. 2005; Newman et al. 2021), Botrytis grey mould (*Botrytis cinerea*) (Devadas et al. 2005), Phytophthora root rot (*Phytophthora medicaginis*) (Knights et al. 2008), pod borer (*Helicoverpa armigera*) (von Wettberg et al. 2018), leaf miner (*Liriomyza brassicae*), and tolerance of cold and drought (Talip et al. 2018). Multiple biotic resistance is desirable for crop breeding and provides protection from one disease dominating at sites where there are multiple biotic pressures (Wiesner-Hanks and Nelson 2016).

A 2011 study by Thompson et al. showed that *C. reticulatum* and *C. echinospermum* were largely more resistant to *P. neglectus*, *P. thornei* or had dual resistance to both species than commercial chickpea cultivars tested. However, only a small number of wild *Cicer* accessions were screened in that study because of the small numbers of original accessions of wild *Cicer* located in global genebanks at that time; 18 unique accessions of *C. reticulatum* and 10 of *C. echinospermum* (Berger et al. 2003). To address the limited number of wild *Cicer* accessions available in global genebanks, scientists from Australia, the United States of America and Turkey conducted a series of comprehensive collection expeditions in southeast Turkey spanning years 2013–18 (Toker et al. 2021). These recent collection missions attempted to cover the full geographic distribution of *C. reticulatum* and *C. echinospermum* in southeast Turkey. The 2013 collection mission focused on targeting areas with known latitudinal and longitudinal data from the original collection as a starting point for locating *Cicer* spp. in southeast Turkey. The 2014 collection mission largely focused on information gathered from local shepherds and finding similar growth habitats of already located wild *Cicer* in further eastern regions of Turkey (von Wettberg et al. 2018; Toker et al. 2021). These new collections are an integral resource for international research to identify new sources of genetic resistance in wild chickpea germplasm to biotic and abiotic constraints and facilitate cultivated chickpea improvement worldwide (Talip et al. 2018; von Wettberg et al. 2018; Reen et al. 2019).

Twenty-six accessions from this new collection, 20 *C. reticulatum* and six *C. echinospermum* were selected by von Wettberg et al. (2018) to represent the genetic and environmental diversity of the 2013 wild *Cicer* collection. Nested association mapping (NAM) populations have been developed by crossing these 26 wild *Cicer* accessions with a common elite parent from each of the major chickpea growing regions of the world, namely, Turkey, Canada, the United States of America, Ethiopia, India, Israel and Australia (von Wettberg et al. 2018). The elite cultivar chosen to be representative of Australian chickpea cultivars was moderately resistant cv. PBA HatTrick. At this time there are no Australian chickpea cultivars rated as fully resistant.

This study aims to screen a new and wider collection of wild *Cicer* accessions for *P. neglectus* resistance utilising final nematode population densities and known

resistance responses to commercial chickpea cultivars and current Australian breeding lines. New sources of *P. neglectus* resistance will widen the genetic base of cultivated chickpea, which in turn will reduce nematode populations in infested sites, improve yields and provide a resilient crop to strengthen rotation choices in Australia and globally.

## MATERIALS AND METHODS

**Plant material.** A total of 329 wild *Cicer* accessions (243 *C. reticulatum* and 86 *C. echinospermum*) were obtained from the Australian Grains Genebank (AGG) (Horsham, Victoria). Accessions tested in this study were collected from 32 sites within seven provinces of southeast Turkey (Figure 1; Table 1). Thirty-nine reference genotypes with known responses to *P. neglectus* were included in the experiments, comprising 19 Australian chickpea cultivars (Table 2), one Indian chickpea breeding line, ten Australian chickpea breeding lines derived from wild *Cicer*, six wild *Cicer* accessions from the original genebank collections (Table 3) and five reference hexaploid wheats (Table 2). The reference wheat cultivars, with known resistance and susceptibility ratings, were included in the experiments as confirmation that *P. neglectus* multiplied as expected.

**Experimental Design.** Glasshouse experiments were tested in two sets as wild *Cicer* accessions were released from quarantine after import into Australia. Each accession was tested at least twice across four experiments. In 2016, 174 accessions (133 *C. reticulatum* and 41 *C. echinospermum*) from the 2013 collection were received and tested as Set 1 (Experiments 1 and 2, conducted in 2016 and 2017, respectively) at the Leslie Research Facility, Toowoomba, QLD (latitude 27.56°S, longitude 151.95°E). The accessions were distributed across 21 collection sites in five Turkish provinces: Adiyaman, Diyarbakir, Mardin, Sirnak and Urfa. In 2018, an additional 155 accessions (110 *C. reticulatum* and 45 *C. echinospermum*) from the 2013 and 2014 collections were received and tested as Set 2 (Experiments 3 and 4, conducted in 2019 and 2020, respectively) at the University of Southern Queensland, Toowoomba, Queensland. These accessions were distributed across 32 collection sites in seven Turkish provinces: Adiyaman, Diyarbakir, Hakkari, Mardin, Siirt, Sirnak and Urfa. A sub-set of 11 *C. echinospermum* and 36 *C. reticulatum* accessions from Set 1, ranging in response to *P. neglectus*, were also included in Experiments 3

and 4. An unplanted control treatment was included in each experiment to establish a base line for *P. neglectus* population densities. All treatments were tested for response to *P. neglectus* with three replicates for each treatment, randomised using a row-column experimental design for each experiment.

**Glasshouse conditions**. Experiments were carried out in the Australian winter to spring period of July-November under controlled glasshouse conditions. Screening for resistance is ideally conducted under glasshouse conditions where environmental influences that affect nematode reproduction such as, temperature, soil moisture and inoculation rate can be controlled (Nombela & Romero 1999). Air temperature was maintained at ~20–25 °C and soil temperature was maintained at ~22 °C, which is optimum for *P. neglectus* reproduction (Vanstone & Nicol 1993), using under bench heating (Thompson et al. 2017). Plants were grown on a capillary matting bottom-watering system set at 6 cm water tension by a float-valve system to ensure optimum water supply during the growing period. This continuous supply of water to the soil allows for favourable nematode movement, feeding and reproduction (Castillo & Volvas 2007) which is important to obtain accurate phenotypic data in resistance testing. Plants were grown in square pots measuring 70 mm wide and 150 mm high, suitable for bottom watering (Garden City Pots, Woodridge, QLD, Australia). Pots contained 330 g (oven-dry (OD) equivalent) of black Vertosol soil (Isbell 1996) of the Waco series (Beckmann and Thompson 1960), which had been pasteurised at 85 °C for 45 min. Fertiliser in solution was added to 80% of the total soil volume providing 200 mg/kg nitrate nitrogen, 25 mg/kg phosphorus, 88 mg/kg potassium, 36 mg/kg sulphur and 5 mg/kg zinc.

Wild *Cicer* seed was scarified with a scalpel, removing a section of testa from the non-germ end to facilitate germination, and placed in moistened soil in the pots. A 1 mL slurry of Rhizobium Group N *Mesorhizobium ciceri* strain CC1192 (in a peat carrier) (Queensland Agricultural Seeds, Toowoomba, QLD, Australia) was inoculated into each pot at planting. A pure culture of *P. neglectus* was multiplied on susceptible wheat for 16 weeks in the glasshouse before extraction. Nematodes were extracted using the Whitehead tray method (Whitehead and Hemming 1965), by spreading the soil and roots evenly on a slightly raised grated basket lined with Kimwipes (KIMTECH; Kimberly-Clark Worldwide, INC.), sitting in a tray with 1L of water. For inoculum production, samples were left for up to 120 h at 22 °C. After

this period, nematodes were collected by pouring the water and nematodes at an angle through a 20-µm aperture sieve to obtain a suspension of nematodes in water, which was stored at 4 °C until microscopic enumeration. A 10 mL aliquant of the nematode suspension was pipetted around the seed at planting, supplying 3,300 *P. neglectus* per pot, equivalent to 10 *P. neglectus*/g soil. The remaining 20% soil volume was added to the pot as a cap after sowing and inoculation.

**Plant harvest, nematode extraction, and enumeration.** After 16 weeks, water supply to the plants was stopped and after a drying period of 2–3 days the plants were harvested. This drying period resulted in optimum soil moisture content (~45%) for processing of the soil and roots for nematode extraction. Soil from individual pots was manually processed, roots cut into ~10 mm pieces and the whole sample mixed to homogenize. Subsamples of 150 g for nematode extraction and 100 g soil for gravimetric moisture content were taken. Soil gravimetric moisture content was determined by drying in a forced draught oven for 48 h at 105 °C. Nematodes were extracted using the method described above, adjusted slightly for the smaller volume of soil being extracted. The 150 g subsample of soil and roots was spread evenly on a slightly raised grated basket lined with facial tissues (Kleenex, Australia), sitting in a tray with 1L of water for 48 h at 22 °C. After the 48 h period, nematodes were collected by pouring the water and nematodes at an angle through a 20-µm aperture sieve obtaining a ~15 mL water suspension of nematodes, which was stored in 30 mL vials at 4 °C until microscopic enumeration. Reen and Thompson (2009) demonstrated that the efficiency of this Whitehead tray method for 48 h and sieving with a 20-µm aperture sieve recovers 70% of *Pratylenchus* populations which is sufficient to show differences in population densities for resistance testing. *Pratylenchus neglectus* were counted in a 1-mL Peters slide (Peters 1952) (Chalex corporation, Portland Oregon, USA) under a compound microscope (40x) (Olympus BX53). Nematode population densities were expressed as number of *P. neglectus*/kg soil and roots and will be reported from here on as *P. neglectus*/kg.

**Statistical analyses.** The analysis of *P. neglectus* population densities (per kg) were performed using two multi-experiment analyses; the first consisting of Experiments 1 and 2, and the second consisting of Experiments 3 and 4. The decision to separate the analysis of the experiments in this way was made based on both practical and statistical considerations, discussed below.

The analysis of both sets of experiments was conducted using a linear mixed model framework, whereby *P. neglectus* population densities required a log transformation to meet the model assumption of homogeneity of variance; a commonplace transformation implemented in the analysis of RLN due to their exponential population growth (Proctor and Marks 1974). The general form of the linear mixed model for both sets of analyses is:

$$\log(y_{ijkl}) = c + s_k + d_j + sd_{jk} + u_{ijk} + b_{jl} + e_{ijkl},$$

where $y_{ijkl}$ is the *P. neglectus* population density (per kg) for genotype $i$ nested within species $k$, in experiment $j$, in replicate $l$; $c$ is the overall constant; $s_k$ is the fixed effect for the $k^{th}$ species, where species consisted of five levels (*T. aestivum*, *C. arietinum*, *C. echinospermum*, *C. reticulatum* and unplanted); $d_j$ is the fixed effect for the $j^{th}$ experiment; $sd_{jk}$ is the interaction effect between the $j^{th}$ experiment and $k^{th}$ species and $u_{ijk}$ is the genotype by experiment (G×E) interaction effect for the $i^{th}$ genotype nested within the $k^{th}$ species for the $j^{th}$ experiment. The replicate block effect for the $l^{th}$ replicate block within the $j^{th}$ experiment is represented by $b_{jl}$, and $e_{ijkl}$ is the error term for each individual pot.

Random effects were fitted for the genotype × experiment interaction, the replicate block effects and the error terms such that:

$$E(u_{ijk}) = E(b_{jl}) = E(e_{ijkl}) = 0;$$

$$\text{var}(u_{ijk}) = \sigma^2_{g_{jk}}; \quad \text{cov}(u_{ijk}, u_{imk}) = \sigma_{\text{g}_{jk,mk}}; \quad \text{cov}(u_{ijk}, u_{ijo}) = 0;$$

$$\text{var}(b_{jl}) = \sigma^2_{b_j}; \qquad \text{var}(e_{ijkl}) = \sigma^2_{e_j}.$$

All random effects were assumed to be normally distributed and independent, unless stated otherwise. The general model consisted of a heterogeneous genetic variance structure for each species × experiment combination (for species other than unplanted). For the wheat (*T. aestivum*) species, it was assumed that $\text{cov}(u_{ijk}, u_{imk}) = 0$ due to the low number of wheat cultivars present within each experiment. For the purposes of the analysis, the wild *Cicer* derivatives from crossing with *C. arietinum* cultivars were included under the *C. arietinum* species.

To simplify the general model, a second variance structure was considered, allowing for modelling of the G×E interaction effects on a "crop type" basis, where crop types are defined by grouping together species belonging to the same genus (*Cicer*, *Triticum* and unplanted). This model enabled the estimation of heterogeneous genetic variance for each crop type × experiment combination. In both instances, independence was assumed between the species or crop types, and between experiments within the respective species or crop types. The variance structures were compared using a residual maximum likelihood ratio test (REMLRT) to inform which modelling approach offered the most parsimonious solution for modelling the G×E interaction, for each set of experiments. Once the most parsimonious solution for modelling the G×E interaction at the species or crop type level was determined, the G×E variance structures were extended to enable the modelling of genetic covariance between experiments at a species or crop type level.

Wald tests with an approximate *F*-Statistic were used to assess statistical significance of the fixed effects. When terms were significant as per the Wald test, Fishers least significant difference (LSD) test was then used to perform multiple comparisons between treatment (e.g. species) levels. Estimates of the fixed effects were empirical best linear unbiased estimators (eBLUEs). Estimates of the random genotype effects for each experiment were empirical best linear unbiased predictors (eBLUPs). Spatial trends within each glasshouse experiment were adjusted for using the methods proposed in Gilmour et.al (1997). Back transformed standard errors of the eBLUEs and eBLUPs were calculated using the delta method.

When assessing whether a particular accession had a significantly lower *P. neglectus* population density than a reference cultivar, a one-sided $Z$-test was performed such that:

$$Z = \frac{u_{ijk} - u_{\text{check } jo}}{sed_{ijk,\text{check } jo}}$$

where $u_{ijk}$ is the eBLUP for accession $i$ in experiment $j$, $u_{\text{check } jo}$ is the eBLUP for the reference cultivar in experiment $j$, and $sed_{ijk,check\ jo}$ is the standard error of difference between accession $i$ and the reference cultivar in experiment $j$. If the genetic correlation between the two experiments being analysed was sufficiently

large, then the eBLUPs were averaged across the set of two experiments prior to performing the one-sided *Z*-test.

Provisional resistance ratings were given to all genotypes tested in both sets of experiments using the method described by Thompson et al. (2020). The range of $\log_e$ (*P. neglectus*/kg) as eBLUPs from the lowest genotype to the highest genotype (wheat cv. Gregory rated as S in consensus ratings) was subdivided into seven equal subranges. Genotypes within these subranges were assigned into seven ordinal categories as follows: resistant (R), resistant-moderately resistant (R-MR), moderately resistant (MR), moderately resistant-moderately susceptible (MR-MS), moderately susceptible (MS), moderately susceptible-susceptible (MS-S) and susceptible (S).

Practically, and as described previously, the accessions tested in Experiments 1 and 2 originated from the 2013 collection, while the accessions tested in Experiments 3 and 4 consisted of those obtained from both the 2013 and 2014 collection and a further array of *C. arietinum* breeding lines of interest. Furthermore, the two sets of experiments were conducted in different facilities, and although every caution was taken to ensure consistent experimental conduct, unmeasured and unforeseeable differences between the facilities could be expected. From a statistical perspective, exploratory data analysis identified that if all experiments were combined into a single analysis, it would be difficult to determine if the significant species × experiment interaction was due to differences in the genotypes present within each experiment as opposed to differences in genotypic performance across experiments. To avoid ambiguity in the interpretation of a significant species × experiment interaction, and since the primary focus was on differences in response to *P. neglectus* at the genotype level, a separate multi-experiment analysis was performed for (a) Set 1 (Experiments 1 and 2) and (b) Set 2 (Experiments 3 and 4).

All hypothesis testing was completed at the 5% significance level and all models were fit using the *ASReml-R* package in the *R* software environment, which implements residual maximum likelihood (REML, Patterson & Thompson 1971) estimation. All *P. neglectus* population densities discussed in the following results are back transformed means presented as *P. neglectus*/kg.

## RESULTS

### Set 1– Multi-experiment analysis of *P. neglectus* population densities.

The multi-experiment analysis of Experiments 1 and 2 found that the species × experiment interaction was not statistically significant ($P = 0.19$). There was a significant species main effect ($P < 0.001$) with the unplanted treatment having significantly *lower P. neglectus*/kg) than all *Cicer* species. There was no significant difference in the *P. neglectus* response level on average between *C. echinospermum* (6,565 *P. neglectus*/kg), *C. reticulatum* (7,790 *P. neglectus*/kg) and cultivated chickpea *C. arietinum* (8,128 *P. neglectus*/kg) when an LSD test was performed (Table 4).

At the genotype level, the REMLRT indicated there was no significant improvement in the model when fitting heterogenous genetic variance for each chickpea species within each experiment. Thus, homogenous genetic variance was fit across all chickpea species within an experiment. The genetic variance for wild *Cicer* accessions and cultivated chickpea was statistically significant for both Experiments 1 ($P < 0.001$) and 2 ($P < 0.001$), indicating that within each experiment, there was variation in response levels to *P. neglectus* between chickpea species.

There was strong genetic correlation between Experiments 1 and 2 ($\rho = 0.84$, Figure 2). Thus, predictions for chickpea genotypes were averaged across the two experiments (Figure 3, Supplementary Table 1). Wheat reference cultivars used to confirm multiplication of *P. neglectus,* performed as expected (population densities shown in Supplementary Table 1). The lowest *P. neglectus* population densities were in the unplanted treatment (1,591 *P. neglectus*/kg). *Pratylenchus neglectus* population densities for *C. reticulatum* ranged from 3,467 *P. neglectus*/kg for accession Oyali_073 to 14,138 *P. neglectus*/kg for accession Kalka_074, while population densities for *C. echinospermum* ranged from 4,598 *P. neglectus*/kg for accession Karab_082 to 12,321 *P. neglectus*/kg for accession S2Drd_062. The lowest *P. neglectus* population densities of Australian commercial chickpea cultivars tested was PBA Pistol (6,010 *P. neglectus*/kg) and PBA HatTrick (6,494 *P. neglectus*/kg). Of the Australian chickpea breeding lines tested, D05253>F3TMWR2AB001 had the lowest *P. neglectus* population densities of 5,639 *P. neglectus*/kg. D05253>F3TMWR2AB001 was derived from crosses with a *C. reticulatum*

accession from the original genebank collection, ILWC 140 (6,921 *P. neglectus*/kg) and the Australian commercial chickpea cv. Howzat (7,742 *P. neglectus*/kg).

One *C. reticulatum* accession Oyali_073, produced significantly ($P > 0.05$) lower *P. neglectus* population densities than Australia's elite chickpea cultivar and breeding parent PBA HatTrick (Supplementary Table 1). Two *C. reticulatum* accessions Oyali_073 and CudiB_008B produced lower (but non-significant, $P > 0.05$) *P. neglectus* population densities than the Australian breeding line D05253>F3TMWR2AB001. Using the method described by Thompson et al. (2020) provisional resistance ratings were given to all wild *Cicer* accessions tested. Twenty-four wild *Cicer* accessions (19 *C. reticulatum* and 5 *C. echinospermum*) were given a R rating. The eBLUPs and provisional resistance ratings for *P. neglectus* population densities for all accessions evaluated in Experiments 1 and 2 are given in Supplementary Table 1.

Wild *Cicer* accessions tested in Experiments 1 and 2 originated from 21 collection sites within five provinces of Turkey. The number of accessions varied between collection sites and within a collection site there was a range of *P. neglectus* population densities. This is shown using violin plots (Figure 4a and 4b) which were generated using the back transformed mean of accessions (Supplementary Table 1).

**Set 2– Multi-experiment analysis of *P. neglectus* population densities.**

Comparison of the species tested in Experiments 3 and 4 found that the species × experiment interaction was non-significant ($P = 0.11$). The species main effect between wild *Cicer* and cultivated chickpea *C. arietinum* was statistically significant ($P < 0.001$) with the LSD test indicating that on average there was significantly lower *P. neglectus* population densities in wild *Cicer*, *C. echinospermum* (3,413 *P. neglectus*/kg) and *C. reticulatum* (4,627 *P. neglectus*/kg) compared to cultivated chickpea *C. arietinum* (7,301 *P. neglectus*/kg) (Table 5).

Similar to the multi-experiment analysis for Set 1, a homogenous genetic variance was fit across all *Cicer* species as there was no evidence of genetic variance heterogeneity across the *Cicer* species as per the REMLRT. The genetic variance for the chickpea genotypes was also significant for Experiments 3 ($P < 0.001$) and 4 ($P < 0.001$) as per the REMLRT.

The genetic correlation between Experiments 3 and 4 was moderate ($\rho = 0.58$, Figure 5) indicating that a small to moderate amount of genotype × environment interaction may be present between Experiments 3 and 4. The genetic correlation was deemed strong enough to justify averaging predictions across Experiments 3 and 4, which is presented in Figure 6. Wheat reference cultivars used to confirm multiplication of *P. neglectus,* performed as expected (population densities shown in Supplementary Table 2).

The lowest *P. neglectus* population density was in the unplanted treatment (975 *P. neglectus*/kg). Values of *P. neglectus* population densities for *C. reticulatum* ranged from 1,945 *P. neglectus*/kg for accession Bari3_106D to 8,170 *P. neglectus*/kg for accession Dogan_033, while population densities for *C. echinospermum* ranged from 2,260 *P. neglectus*/kg for accession Isoha_025 to 7,090 *P. neglectus*/kg for accession Isoha_049. The lowest *P. neglectus* population density of Australian commercial chickpea cultivars tested was Moti at 2,492 *P. neglectus*/kg. A total of 34 wild *Cicer* accessions had significantly ($P < 0.05$) lower *P. neglectus* population densities than Australia's elite breeding cultivar PBA HatTrick at 5,628 *P. neglectus*/kg. Of the Australian chickpea breeding lines tested, 00283-1095-1002 (2,461 *P. neglectus*/kg) and D05253>F3TMWR2AB001 (3,152 *P. neglectus*/kg) had the lowest *P. neglectus* population densities, both derived from crosses with the *C. reticulatum* accession, ILWC 140 (3,224 *P. neglectus*/kg). The Australian commercial chickpea cultivars used to make these crosses were Jimbour (3,163 *P. neglectus*/kg) for 00283-1095-1002 and Howzat (3,099 *P. neglectus*/kg) for D05253>F3TMWR2AB001. Fourteen wild *Cicer* accessions (10 *C. reticulatum* and four *C. echinospermum*) were given a provisional R rating. The eBLUPs and provisional resistance ratings for *P. neglectus* population densities for all accessions evaluated in Experiments 3 and 4 are given in Supplementary Table 2.

Wild *Cicer* accessions tested in Experiments 3 and 4 originated from 26 and 32 collection sites respectively within seven provinces of Turkey. Like Experiments 1 and 2, the number of accessions varied between collection sites, and there was a range of *P. neglectus* population densities for accessions within collection sites for Experiments 3 and 4 as indicated by the violin plots (Figure 7a and 7b) using the back transformed mean of accessions (Supplementary Table 2).

**Nested Association Mapping Parents.** Analysis of the *P. neglectus* population densities for the 26 diverse wild *Cicer* NAM parent accessions from Experiments 3 and 4 revealed that 25 of the 26 produced lower population densities than the breeding parent PBA HatTrick (Figure 8). Of these 25 accessions, four accessions Bari3_106D (1,945 *P. neglectus*/kg), Kayat_077 (2,536 *P. neglectus*/kg), Oyali_084 (2,976 *P. neglectus*/kg) and CudiB_022C (3,020 *P. neglectus*/kg) produced significantly ($P < 0.05$) lower *P. neglectus* population densities than PBA HatTrick (Figure 8). Of the NAM parents, two accessions (Bari3_106D and Kayat_077) were rated R and seven accessions (Oyali_084, CudiB_022C, Derei_070, Besev_079, Bari2_072, Bari3_100 and Bari1_092) rated R-MR.

## DISCUSSION

This is the first known report on *P. neglectus* response in the new wild *Cicer* collection, assessing *C. reticulatum* and *C. echinospermum* accessions from both the 2013 and 2014 collection missions. This study has identified several wild *Cicer* accessions with better resistance to *P. neglectus* that can be utilised in future breeding programs and for targeted genetic exploration. Furthermore, the data provides newfound information in terms of *P. neglectus* resistance by characterising 329 of the 590 wild *Cicer* accessions acquired from southeast Turkey for the new collection (Toker et al. 2021). PBA HatTrick is used as a benchmark for *P. neglectus* response in this study as it is the elite Australian chickpea breeding parent for crossing and genetic studies for this wild *Cicer* collection. The results of this study showed only one accession with significantly lower *P. neglectus* population densities than PBA HatTrick in Experiments 1 and 2, while 34 wild accessions had significantly lower *P. neglectus* population densities than PBA HatTrick in Experiments 3 and 4 (Supplementary Tables 1 and 2). Twenty of these were new accessions received in Set 2 in 2018 demonstrating that expanding on and phenotyping accessions from different collection sites identified an increased number of accessions with improved resistance responses to *P. neglectus* and prevented this collection from being underrepresented.

An earlier study by Thompson et al. (2011) also showed greater *P. neglectus* resistance in a small number of original wild *Cicer* that included nine *C. reticulatum* and five *C. echinospermum* from the original genebank collection. This current study

has identified a range of *P. neglectus* responses in *C. reticulatum* and *C. echinospermum* and from the larger sample size has better represented the resistance status of both wild *Cicer* species compared to the Thompson et al. (2011) study. Thompson et al. (2011) highlighted ILWC 140 as an accession with greater resistance to *P. neglectus* than *C. arietinum*. It was then utilised in plant breeding as a parent to create many of the breeding lines tested in this study (Table 3). In this current study, accessions that produced significantly lower *P. neglectus* population densities than known resistant to moderately resistant Australian chickpea cultivars predominately consisted of *C. reticulatum* accessions. This is beneficial as *C. reticulatum* is directly cross compatible with *C. arietinum* making it a more desirable choice for breeders to use in breeding programs. Although *C. echinospermum* can be crossed with *C. arietinum*, there is greater chance of sterility and loss of vital genetic material (Croser et al. 2003).

To date, there is no published literature on chromosomal regions in chickpea associated with *P. neglectus* resistance. Channale et al. (2021) found that numerous genes participated in defence pathways to provide resistance against *P. thornei* in chickpea. This suggests that *P. thornei* resistance in chickpea is polygenic (Channale et al. 2021). It is also hypothesised that *P. neglectus* resistance in chickpea is polygenic, although no genetic studies have yet been performed with *P. neglectus*. In this study the Australian wild relative derived breeding line D05253>F3TMWR2AB001 performed better than its parent ILWC 140 for *P. neglectus* resistance. A future genome-wide association study will be important to identify if resistance in the wild *Cicer* accessions tested in this study differ in genomic regions compared with earlier accessions from the original genebank collection, such as ILWC 140. This information will also inform if greater genetic variance and resistance is already available in D05253>F3TMWR2AB001 or if there are novel sources of resistance that could be utilised from the new wild *Cicer* accessions for future breeding. Molecular markers derived from candidate genes for resistance will be useful tools for marker assisted selection to incorporate new sources of superior resistance into chickpea breeding programs.

In Australia and worldwide, greater resistance to multiple abiotic and biotic constraints in chickpea conveys maximum yield and profitability to the crop. Root-lesion nematode *P. thornei*, pod borer and Ascochyta blight are three major biotic

constraints to the chickpea industry worldwide. Under conducive disease/pest conditions, recorded yield losses in chickpea have been 20–50% from *P. thornei* (Di Vito et al. 1992; Reen et al. 2014), 80–90% from pod borer (Sehgal & Ujagir 1990, p. 30) and up to 100% from Ascochyta blight (Nene and Reddy 1987). Utilising data from previous studies published on the new wild *Cicer* collection, along with information from this study, multiple resistance has been identified in several of the wild *Cicer* accessions from the 2013 collection. Six *C. reticulatum* accessions that produced lower *P. neglectus* population densities than PBA HatTrick in both sets of experiments (Bari2_062, Bari3_106D, Kayat_061, Kayat_066, Oyali_073 and Oyali_084) also produced significantly ($P < 0.05$) lower *P. thornei* population densities than PBA HatTrick (Reen et al. 2019). The *C. echinospermum* NAM parent Gunas_062 produced lower *P. neglectus* population densities than PBA HatTrick in this study and also for *P. thornei* (Reen et al. 2019), and pod borer (von Wettberg et al. 2018), and was highly resistant to stem damage from a mixture of Ascochyta blight strains (Newman et al. 2021). The *C. reticulatum* accession CudiB_008B produced one of the lowest *P. neglectus* population densities in this study, and was also highly resistant to an Ascochyta blight mixture on the stem area and tolerant for the leaf area (Newman et al. 2021). However, it should be noted that unlike this current study, previously published studies on the new collection were focused only on the 2013 collection mission. These included the 26 NAM parent accessions tested for pod borer resistance (20 *C. reticulatum* and six *C. echinospermum*) in von Wettberg et al. (2018), 133 *C. reticulatum* and 41 *C. echinospermum* for *P. thornei* resistance in Reen et al. (2019) and 149 *C. reticulatum* and 48 *C. echinospermum* for Ascochyta blight resistance in Newman et al. (2021).

Accessions tested in this study were collected from 32 sites within seven provinces of southeast Turkey, which encompassed the full geographical range of the species (von Wettberg et al. 2018). von Wettberg et al. (2018) analysed site environments to determine elevation gradients, climatic and soil differences, which identified that *C. echinospermum* occurs at elevations of 740–1,264 m whereas *C. reticulatum* occurs at generally higher elevations of 915–1,695 m. This explained some genetic variation between the species (von Wettberg et al. 2018) however, there was no trend between *P. neglectus* response of accessions and collection site as there was a range of *P.*

*neglectus* population densities in collection sites that occurred at different geographical and elevational locations (Figures 4 and 7).

*Pratylenchus neglectus* reduce root branching and limited studies have shown wild *Cicer* spp also have smaller root systems than *C. arietinum* (Kashiwagi et al. 2005; Chen et al. 2016). This could imply low nematode population densities are the result of damaged or smaller root systems and not due to resistance. However, Reen et al. (2019) conducted correlation analyses between root biomass and final *P. thornei* population densities and found no significant relationship for *C. reticulatum*, *C. echinospermum* or *C. arietinum*. Similarly, the mean root biomass did not differ significantly among these *Cicer* spp. Lesions on the roots of infected plants is indicative of *P. neglectus* infestation, however, measuring lesions of infected roots is not recommended for determining levels of resistance (Ali & Ahmad 2000). This is due to the lesions being a symptom of infestation, rather than an indication of actual *P. neglectus* numbers present in the roots and soil which determines the resistance or susceptibility of a host. It is also important to note that initial planting inoculation density may not accurately reflect the number of *P. neglectus* that actually infects roots, with Vanstone & Nicol (1993) reporting that only 27-37% of *P. neglectus* successfully penetrated roots in a glasshouse experiment using 300ml pots. Therefore, to best categorize wild *Cicer* accessions for resistance response we have utilised the method in Thompson et al. (2020) to give a provisional resistance ranking to wild *Cicer* accessions and also provide probability scores of genotypes more resistant than PBA HatTrick at $P < 0.05$. Final nematode population densities for the same accession can vary between experiments (Kaplan, 1990) which also affects resistance ratings between experiments resulting in slight variation in relative ratings.

This study has demonstrated the diversity of *P. neglectus* response to over 300 wild *Cicer* accessions which can be utilised in future breeding programs by identifying wild *Cicer* accessions with greater resistance than current Australian commercial chickpea cultivars. The current Australian breeding line D05253>F3TMWR2AB001 is also still a strong candidate for proceeding as there were no wild *Cicer* accessions that produced significantly lower *P. neglectus* population densities than this line. Through this study and the bringing together of information from previous studies, it is clear there are wild *Cicer* accessions that have possible multiple resistance to *P.*

*neglectus*, *P. thornei*, Ascochyta blight and pod borer which would be highly advantageous in subsequent breeding programs. These results can also be utilised globally in countries where *P. neglectus* is a constraint to chickpea production.

Wild *Cicer* are integral for genetic disease improvement in cultivated chickpea with this research enabling breeders and industry to make informed decisions regarding *P. neglectus* resistance. The information provided here will support future development of more robust chickpea cultivars that will limit nematode reproduction in infested soils, resulting in greater yields and more profitable grains industries.

## ACKNOWLEDGMENTS

We would like to thank the Grains Research and Development Corporation (GRDC) for funding this research through Project USQ00017 and the Queensland Department of Agriculture and Fisheries (QDAF) for support under the Broadacre Cropping Initiative with the University of Southern Queensland. We acknowledge Professor Doug Cook, UC Davis, California, and Dr Jens Berger, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia for collection and importation of the wild *Cicer* accessions into Australia, and the Australian Grains Genebank for supplying the wild *Cicer* collection. Further we thank, the GRDC, New South Wales (NSW) Department of Primary Industries (DPI) and QDAF, Australia, as the co-owners of the chickpea breeding lines including D05253>F3TMWR2AB001 used in this study. We acknowledge the breeding by Mr Ted Knights, NSW DPI and thank Dr Kristy Hobson for providing seed for this study and Mr Luke Neale, Statistics for the Australian Grains Industry for statistical assistance.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## TABLES

Table 1. Passport information on the wild *Cicer* accessions tested for *Pratylenchus neglectus* resistance showing province and collection site, species, number of accessions at each collection site (*N*), prefix used with accession code number, and year of collection.

| Province, collection site | Species | *N* | Prefix | Accession code number | Year of collection |
|---|---|---|---|---|---|
| Adiyaman, Oyali | *C. reticulatum* | 11 | Oyali | 071, 073, 076, 081, 084, 085, 100, 101, 104, 105, 107 | 2013 |
| Diyarbakir, Cermik | *C. echinospermum* | 6 | Cermi | 061, 063, 071, 072, 073, 075 | 2013 |
| Egil | *C. reticulatum* | 7 | Egil | 063, 065, 066, 072, 073, 074, 075 | 2013 |
| Gunasan | *C. echinospermum* | 4 | Gunas | 061, 062, 100, 101 | 2013 |
| Kalkan | *C. reticulatum* | 7 | Kalka | 061, 064, 065, 066, 067, 070, 074, | 2013 |
| Kesentas | *C. reticulatum* | 12 | Kesen | 062, 065, 066, 067, 071, 072, 073, 074, 075, 077, 101, 104 | 2013 |
| Hakkari, Ayvalik | *C. reticulatum* | 1 | Olgun | 026 | 2014 |
| Mardin, Baristepe1 | *C. reticulatum* | 8 | Bari1 | 062, 063, 064, 068, 069, 091, 092, 093 | 2013 |
| Baristepe2 | *C. reticulatum* | 5 | Bari2 | 062, 064, 067, 072, 074 | 2013 |
| Baristepe3 | *C. reticulatum* | 18 | Bari3 | 064, 065, 067, 072C, 073, 074, 075, 079, 091, 092, 100, 101, 102, 103, 104, 106D, 110, 112 | 2013 |
| Beslever | *C. reticulatum* | 8 | Besev | 061, 062, 065, 066, 074, 075, 079, 083 | 2013 |
| Dereici | *C. reticulatum* | 10 | Derei | 062, 065, 066, 069, 070, 072, 073, 074, 075, 078 | 2013 |
| Kayatepe | *C. reticulatum* | 10 | Kayat | 061, 063, 064, 066, 067, 070, 071, 077, 080, 081 | 2013 |
| Sarikaya | *C. reticulatum* | 13 | Sarik | 061, 063, 064, 065, 066, 067, 072, 073, 074, 077, 078, 080, 081 | 2013 |
| Savur | *C. reticulatum* | 1 | Savur | 063 | 2013 |
| Siirt, Cukur | *C. reticulatum* | 1 | Golko | 001 | 2014 |
| Doganca | *C. reticulatum* | 18 | Dogan | 026, 027, 028, 029, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 042, 043, 044, 045 | 2014 |
| Ekinduzu | *C. reticulatum* | 17 | Ekind | 043, 044, 045, 047, 048, 049, 050, 051, 052, 053, 054, 055, | 2014 |

| | | | | | |
|---|---|---|---|---|---|
| | | | | 056, 057, 058, 059, 060, | |
| Erenkaya | *C. reticulatum* | 2 | Erenk | 001, 002 | 2014 |
| Golgelikonak | *C. reticulatum* | 9 | Golge | 026, 031, 032, 034, 035, 036, 037, 038, 039 | 2014 |
| Tasdibek | *C. reticulatum* | 1 | Tasdi | 025 | 2014 |
| Tuzcular | *C. reticulatum* | 5 | Tuzca | 032, 035, 038, 039, 044 | 2014 |
| Yanilmaz | *C. reticulatum* | 1 | Yanil | 013 | 2014 |
| Sirnak, | | | | | |
| CudiA | *C. reticulatum* | 18 | CudiA | 101A, 102, 103C, 104, 105, 107, 109, 122, 124, 125, 127, 128, 151, 152, 153, 154, 155, 221 | 2013 |
| CudiB | *C. reticulatum* | 14 | CudiB | 004, 005, 006, 008B, 009, 011, 012, 015, 016, 017, 018, 019, 022C, 023 | 2013 |
| Kaymakam Ceşmesi | *C. reticulatum* | 4 | Kayma | 005, 035, 039, 044 | 2014 |
| Sirnak | *C. reticulatum* | 42 | Sirna | 060, 061, 063, 064, 066, 067, 069, 070, 071C, 081B, 082, 083, 084, 085, 087, 088, 089B, 090, 101, 103, 104, 105, 110, 111A | 2013 |
| | | | | 030, 032, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 046, 047, 050, 051, 052 | 2014 |
| Urfa, | | | | | |
| Destek | *C. echinospermum* | 12 | Deste | 061, 063, 064, 066, 071, 072, 073, 075, 077, 078, 079, 080 | 2013 |
| Karabahce | *C. echinospermum* | 17 | Karab | 062, 063, 066A, 067, 081, 082, 084, 085C, 086, 091B, 092, 093, 162, 164, 171, 172, 174 | 2013 |
| Kargali | *C. echinospermum* | 33 | Isoha | 002, 010, 013, 018, 024, 025, 026, 027, 028, 030, 031, 032, 033, 034, 036, 037, 038, 039, 040, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055 | 2014 |
| Ortanca | *C. echinospermum* | 2 | Ortan | 061, 066 | 2013 |
| Siv-Diyar | *C. echinospermum* | 12 | S2Drd | 061, 062, 065, 100, 101, 102, 104, 105, 106, 107B, 108, 109 | 2013 |

**Table 2.** Commercial chickpea and wheat used as reference cultivars in Experiments.

| Species | Cultivar | Resistance rating[a] |
|---|---|---|
| *Cicer arietinum* | PBA Boundary | RMR |
| | Flipper | RMR |
| | Howzat | RMR |
| | PBA Pistol | RMR |
| | Sona | RMR |
| | Sonali | RMR |
| | Jimbour | MR |
| | PBA Drummond | MR |
| | PBA HatTrick | MR |
| | Yorker | MR |
| | Almaz | MR-MS |
| | Genesis 090 | MR-MS |
| | Kyabra | MR-MS |
| | Moti | MR-MS |
| | Neelam | MR-MS |
| | PBA Maiden | MR-MS |
| | PBA Seamer | MR-MS |
| | PBA Slasher | MR-MS |
| | PBA Striker | MR-MS |
| *Triticum aestivum* | Yenda | MR |
| | Wyalkatchem | MR-MS |
| | Machete | S |

[a]Resistance ratings: RMR= resistant-moderately resistant, MR= moderately resistant, MR-MS= moderately resistant- moderately susceptible, S= susceptible (J Sheedy pers. comm.; Matthews et al. 2021).

**Table 3**. Wild *Cicer* derived breeding lines and *Cicer reticulatum* and *Cicer echinospermum* accessions from the original genebank collection included in Experiments.

| Species | Line/Accession | Wild *Cicer* Derivation | Country |
|---|---|---|---|
| *Cicer arietinum* | CICA0709 | L204 *C. echinospermum* | Australia |
| | CICA1314 | ILWC246 *C. echinospermum* | Australia |
| | CICA1317 | ILWC104 *C. reticulatum* | Australia |
| | CICA1421 | ILWC104 *C. reticulatum* | Australia |
| | CICA1427 | ILWC246 *C. echinospermum* | Australia |
| | 00283-1095-1002 | ILWC140 *C. reticulatum* | Australia |
| | D05222>F3TMWR2AB001 | ILWC140 *C. reticulatum* | Australia |
| | D05253>F3TMWR2AB001 | ILWC140 *C. reticulatum* | Australia |
| | D05293>F3TMWR2AB002 | ILWC246 *C. echinospermum* | Australia |
| | D05295>F3TMWR2AB026 | ILWC246 *C. echinospermum* | Australia |
| | ICC11323 | landrace | India |
| *Cicer reticulatum* | ILWC 140 | | Turkey |
| | ILWC 127 | | Turkey |
| | ILWC 115 | | Turkey |
| *Cicer echinospermum* | PI 527932 | | Turkey |
| | ILWC 39 | | Turkey |
| | ILWC 180 | | Turkey |

**Table 4.** Empirical best linear unbiased estimates of *Pratylenchus neglectus* after 16 weeks growth for the *Cicer* species main effect in multi-experiment analysis of Experiments 1 and 2. There was no significant species by experiment interaction and thus *Cicer* species predictions were averaged across Experiments 1 and 2. Species with a letter in common are not significantly different as per an LSD test at the 5% level.

| Species | *N*[b] | *P. neglectus*/kg OD[a] soil | |
|---|---|---|---|
| | | $Log_e$ | BTM[c] |
| *C. arietinum* | 18 | 9.00 a | 8128 |
| *C. reticulatum* | 133 | 8.96 a | 7790 |
| *C. echinospermum* | 41 | 8.79 a | 6565 |

[a]OD= Oven dried
[b]*N* = Number of accessions
[c]BTM= Back transformed mean

**Table 5.** Empirical best linear unbiased estimates of *Pratylenchus neglectus* after 16 weeks growth for *Cicer* species main effect in multi-experiment analysis of Experiments 3 and 4. There was no significant species by experiment interaction and thus *Cicer* species predictions were averaged across Experiments 3 and 4. Species without a letter in common are significantly different as per an LSD test at the 5% level.

| Species | *N*[b] | *P. neglectus*/kg OD[a] soil | |
|---|---|---|---|
| | | $Log_e$ | BTM[c] |
| *Cicer arietinum* | 29 | 8.90 a | 7301 |
| *C. reticulatum* | 146 | 8.44 b | 4627 |
| *C. echinospermum* | 56 | 8.14 c | 3413 |

[a]OD= Oven dried
[b]*N* = number of accessions
[c]BTM= Back transformed mean

## FIGURES

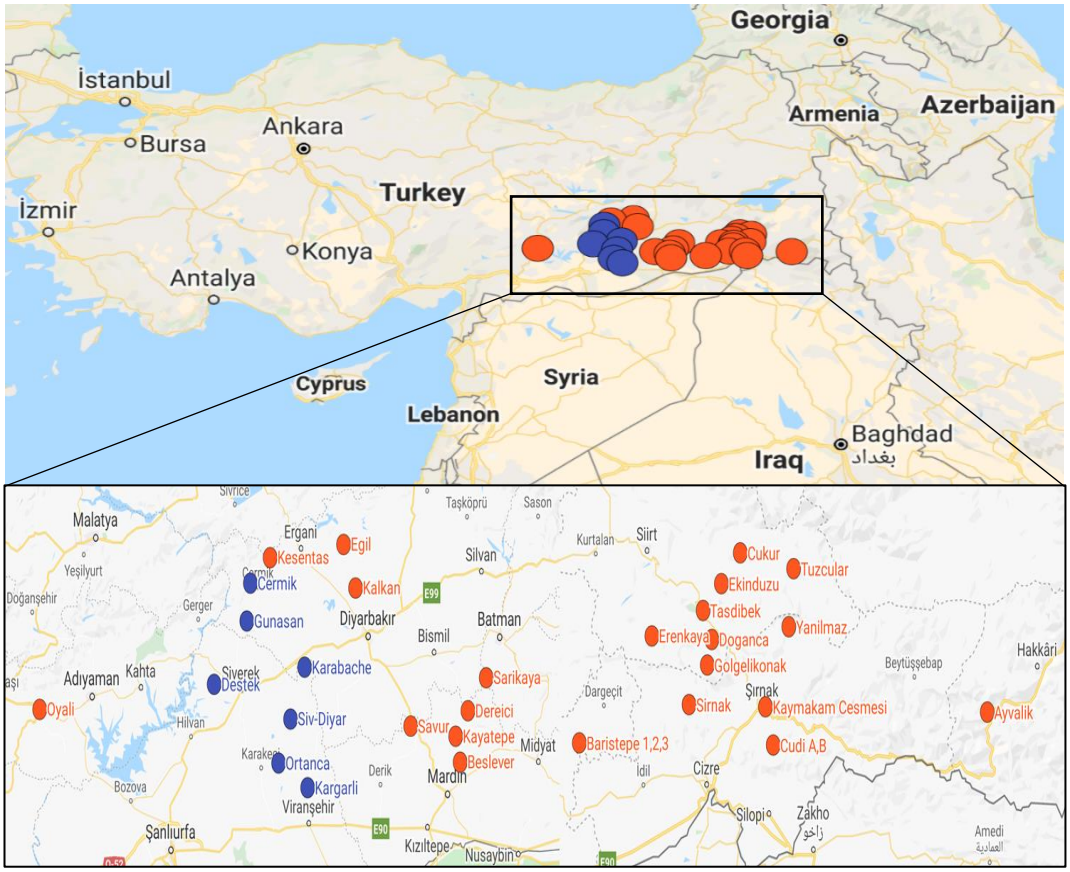


**Figure 1.** Map of southeast Turkey and the 32 collection sites where *Cicer reticulatum* and *Cicer echinospermum* accessions were collected (accessions from 21 collection sites tested in Experiments 1 and 2 plus an additional 11 collection sites tested in Experiments 3 and 4). Map data obtained from Map Maker 2021. Red marker= *Cicer reticulatum*; blue= *Cicer echinospermum*.

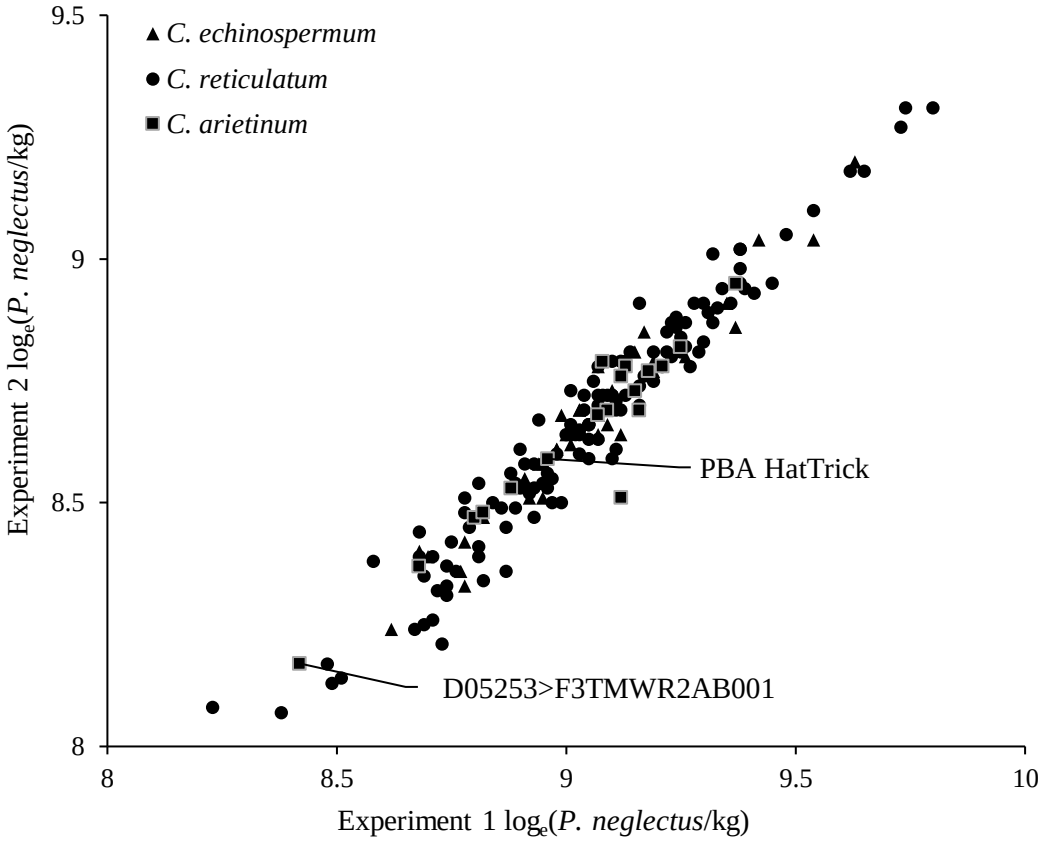


**Figure 2.** Empirical best linear unbiased predictions of *Pratylenchus neglectus* population densities for *Cicer* accessions calculated separately for each genotype from Experiments 1 and 2. The genetic correlation between the two experiments was strong ($\rho = 0.84$). PBA HatTrick is the elite chickpea cultivar chosen to represent Australia for the nested association mapping population produced from the wild *Cicer* collection and D05253>F3TMWRAB001 is Australia's current best breeding line with wild *Cicer* derivatives for *P. neglectus* resistance. *P. neglectus*/kg is based on extraction from soil and roots.

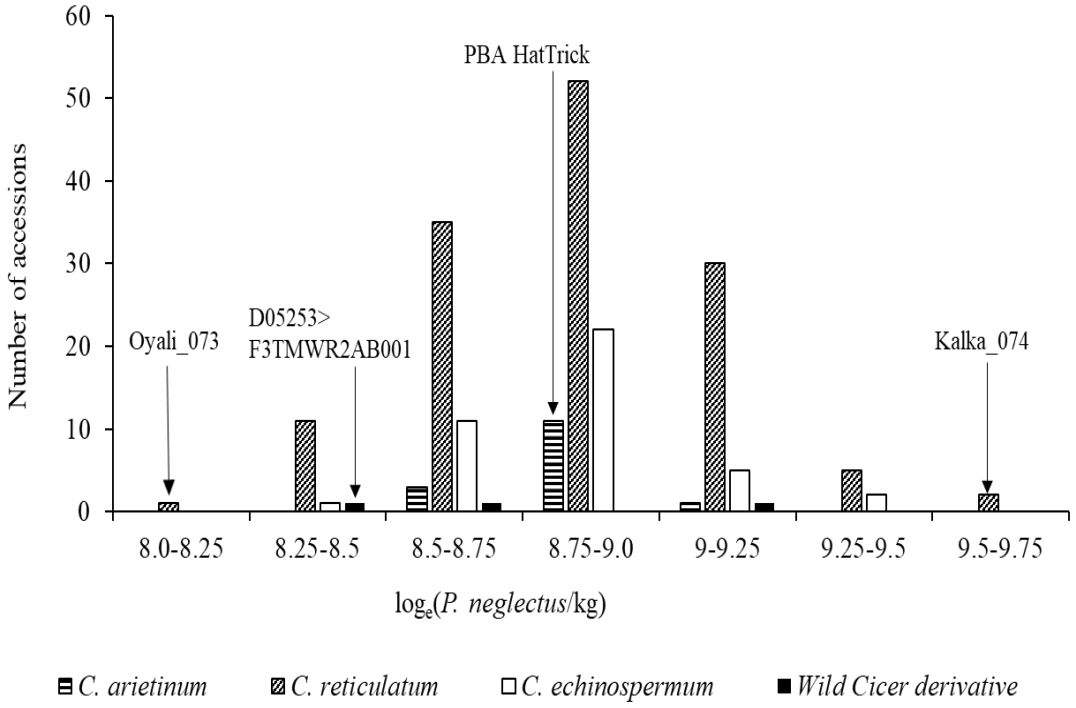


**Figure 3.** Frequency distributions of empirical best linear unbiased predictions of *Pratylenchus neglectus* population densities for cultivated chickpea and wild *Cicer* accessions after 16 weeks growth averaged across Experiments 1 and 2. Genotypes listed are the wild *Cicer* that had both the lowest and highest *P. neglectus*/kg. PBA HatTrick is the elite chickpea cultivar chosen to represent Australia for the nested association mapping population produced from the wild *Cicer* collection and D05253>F3TMWRAB001 is Australia's current best breeding line with wild *Cicer* derivatives for *P. neglectus* resistance. *P. neglectus*/kg is based on extraction from soil and roots.

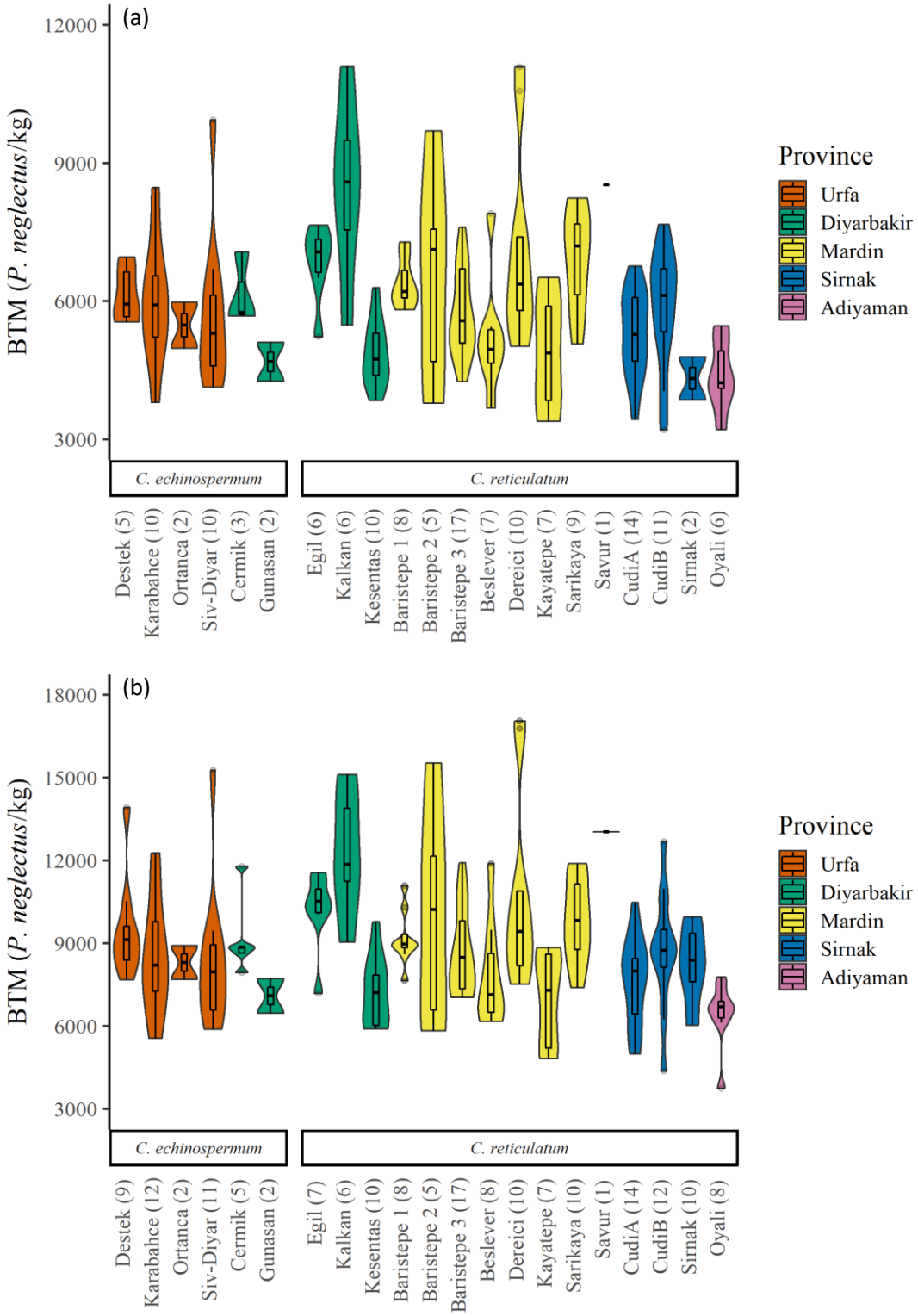


**Figure 4.** Violin plots with embedded boxplots showing the distribution of back transformed mean *Pratylenchus neglectus* population densities for 21 wild *Cicer* collection sites in (a) Experiment 1 and (b) Experiment 2. The number of accessions from each collection site are shown in parentheses. *P. neglectus*/kg is based on extraction from soil and roots.

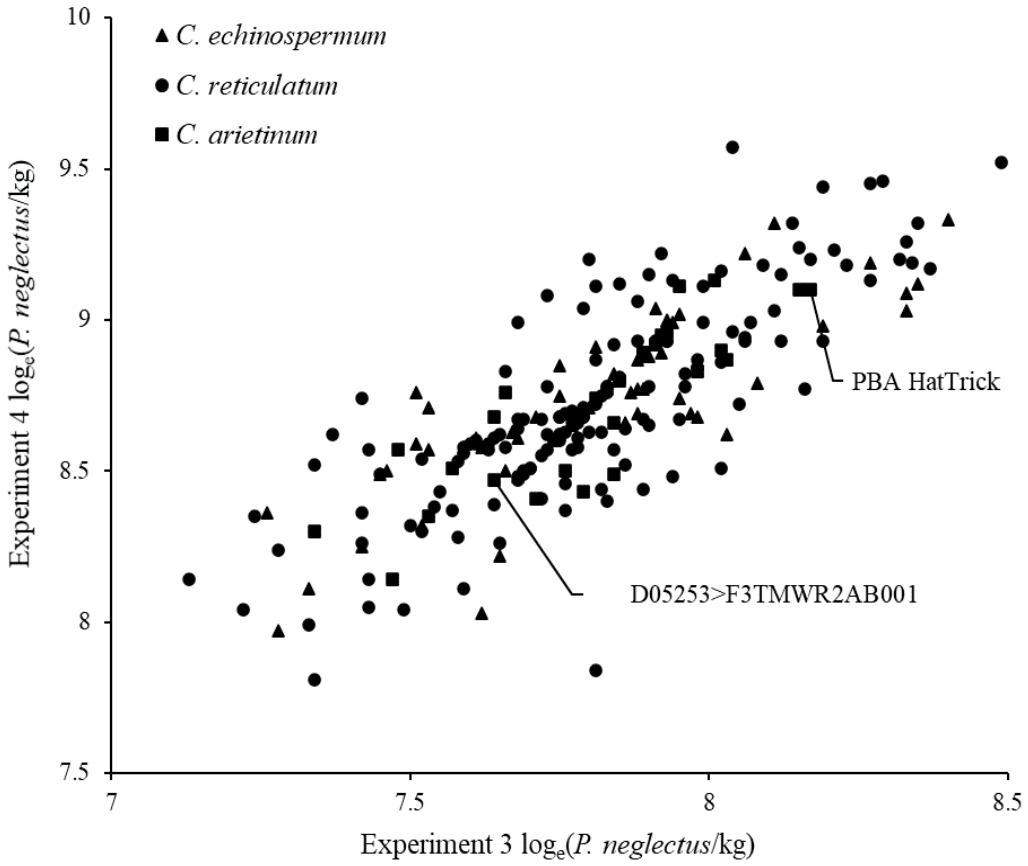


**Figure 5.** Empirical best linear unbiased predictions of *Pratylenchus neglectus* population densities for *Cicer* accessions calculated separately for each genotype from Experiments 3 and 4. The genetic correlation between the two experiments was moderate ($\rho = 0.58$). PBA HatTrick is the elite chickpea cultivar chosen to represent Australia for the nested association mapping population produced from the wild *Cicer* collection and D05253>F3TMWRAB001 is Australia's current best breeding line with wild *Cicer* derivatives for *P. neglectus* resistance. *P. neglectus*/kg is based on extraction from soil and roots.

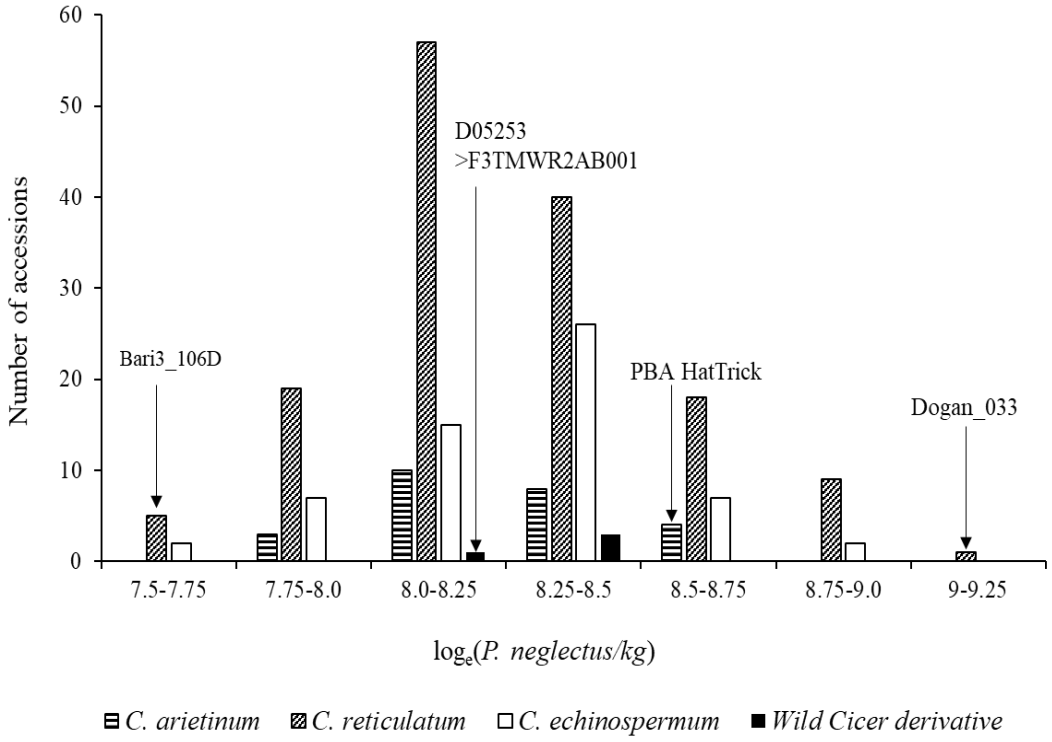


**Figure 6.** Frequency distributions of empirical best linear unbiased predictions of *Pratylenchus neglectus* population densities for cultivated chickpea and wild *Cicer* accessions after 16 weeks growth averaged across Experiments 3 and 4. Genotypes listed are the wild *Cicer* that had both the lowest and highest *P. neglectus*/kg. PBA HatTrick is the elite chickpea cultivar chosen to represent Australia for the nested association mapping population produced from the wild *Cicer* collection and D05253>F3TMWRAB001 is Australia's current best breeding line with wild *Cicer* derivatives for *P. neglectus* resistance. *P. neglectus*/kg is based on extraction from soil and roots.

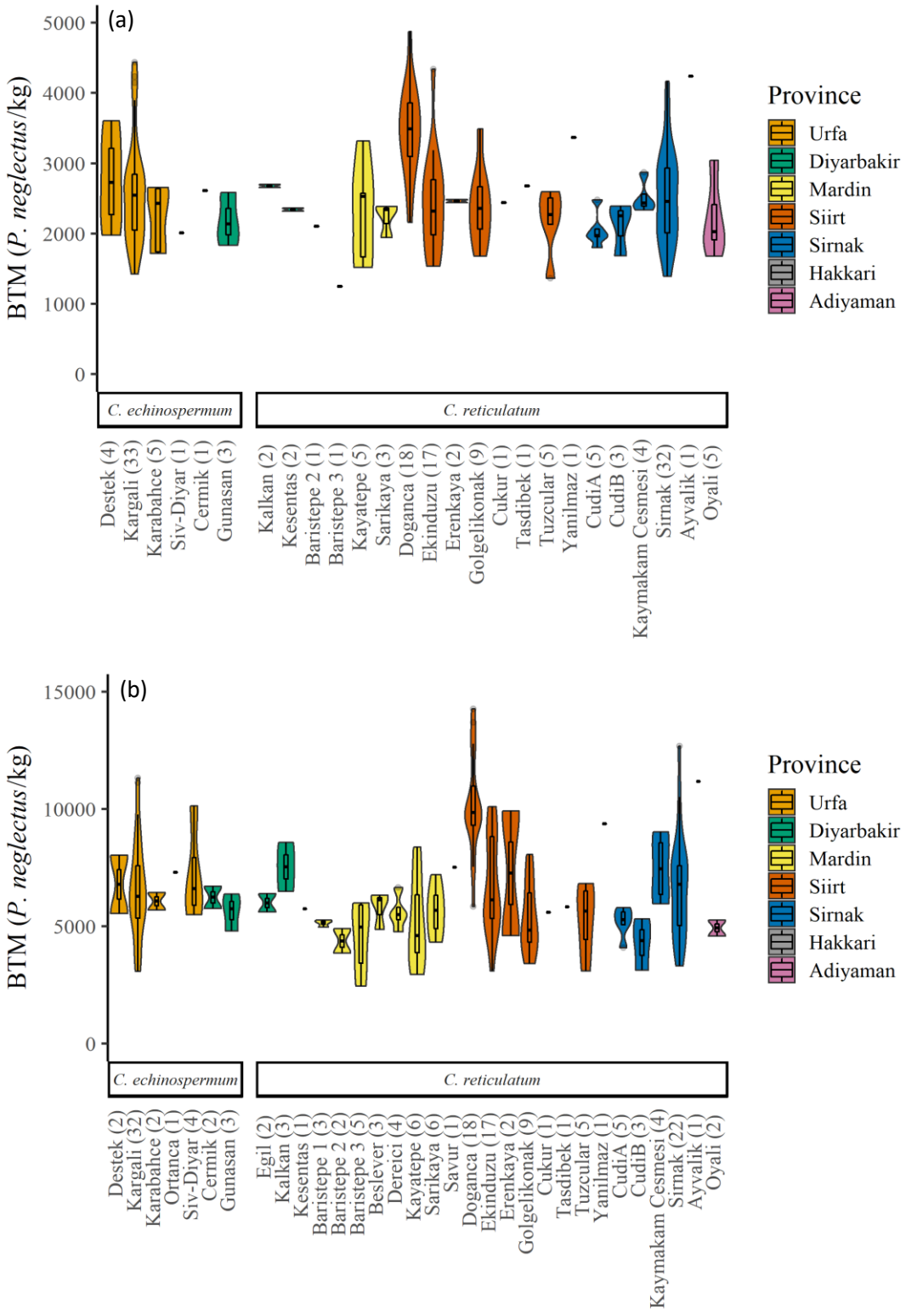


**Figure 7.** Violin plots with embedded boxplots showing the distribution of back transformed mean *Pratylenchus neglectus* population densities for (a) 26 wild *Cicer* collection sites in Experiment 3 and (b) 32 wild *Cicer* collection sites in Experiment 4. The number of accessions from each collection site are shown in parentheses. *P. neglectus*/kg is based on extraction from soil and roots.

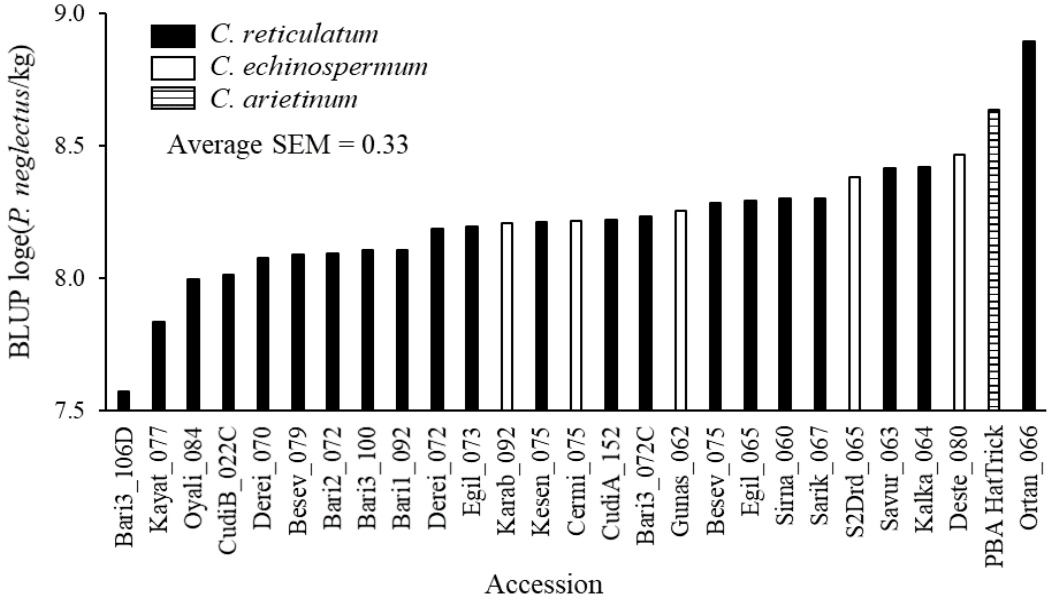


**Figure 8.** *Pratylenchus neglectus* empirical best linear unbiased predictions for the 26 nested association mapping wild *Cicer* parents (*Cicer reticulatum* shown in black; *Cicer echinospermum* shown in white) and the common parent PBA HatTrick (hashed) from multi-experiment analysis of Experiments 3 and 4. SEM = standard error of the mean. *P. neglectus*/kg is based on extraction from soil and roots.

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# CHAPTER 4: SUMMARY AND OUTLOOK FOR FUTURE INNOVATION

## 4.1 Summary

Research efforts to find resistance to abiotic and biotic constraints in chickpea have been extensive, but could only go so far with a previous limited world collection of wild *Cicer* germplasm. This has been greatly improved through recent collection missions, increasing the world wild *Cicer* germplasm collection by 21-fold for *C. reticulatum* and nine-fold for *C. echinospermum* (von Wettberg et al. 2018 p. 3).

Before this study, wild *Cicer* accessions phenotyped for *P. neglectus* resistance were identified by Thompson et al. 2011 (p. 607) who tested nine *C. reticulatum* and five *C. echinospermum* accessions from the original genebank collection. The purpose of this thesis has been to evaluate a new and wider collection of wild *Cicer* accessions (243 C. *reticulatum* and 86 *C. echinospermum*) that have been previously unavailable for testing. Research presented in this thesis highlights new sources of resistance to *P. neglectus* available within this collection, with wild *Cicer* showing significantly ($P < 0.05$) greater resistance to *P. neglectus* than Australia's elite breeding cv. PBA HatTrick. This research also offers valuable information for chickpea breeders worldwide with increased genetic diversity through identification of *P. neglectus* resistant germplasm. This is important to overcome the challenges posed by this pathogen, most importantly food security to human populations who rely on chickpea for their nutritional needs and are affected by the yield loss *P. neglectus* incurs. *Pratylenchus neglectus* resistance identified in the wild *Cicer* accessions phenotyped in this study provides a basis for resistance breeding efforts that are critical to provide a foundation in RLN resistance traits for future development of resilient and diverse chickpea cultivars.

## 4.2 Towards future innovation

### 4.2.1 Genetic studies

Breeding with wild relatives of chickpea has been advanced through genomic technologies such as marker assisted selection (MAS), whole genome sequencing, NAM populations, single-nucleotide polymorphism (SNP) which are used for

genome-wide association study (GWAS) (Roorkiwal et al. 2020, p. 1705-1716) and publication of the whole pan-genome in chickpea (Varshney et al. 2021, p. 623). Some examples of the leaps genomic resources have made to improve chickpea improvement include MAS being successfully used to introgress fusarium wilt and ascochyta blight resistance into elite chickpea cultivars through the targeted selection of molecular markers for backcrossing (Varshney et al. 2014, p. 4 & 5; Mannur et al. 2018, p. 5). Genome-wide association has been used to show drought and heat tolerance markers in chickpea. Thudi et al. 2014 (p. 2) used phenotypic data of drought response of 300 *Cicer* accessions to identify marker trait associations for drought and heat tolerance which can be utilised in molecular breeding for development of superior chickpea cultivars. Lastly, the pan-genome is a huge leap in knowledge and a significant resource for chickpea studies showing a number of novel genes in chickpea that have yet to be studied. The information in the pan genome can be used to identify gaps in breeding programs where superior haplotypes are absent, and also identifies lines that can be used to introduce superior genetic material into breeding programs using previously gathered phenotypic data and SNPs (Varshney et al. 2021, p. 625).

Genetic studies identifying nematode resistance genes have been explored extensively in other crops, however, little information is available on chromosomal regions in chickpea associated with PPN resistance (Zwart et al. 2019a, p. 10). Channale et al. 2021 (p.7) found that numerous genes participated in defence pathways to provide resistance against *P. thornei* in chickpea, suggesting that *P. thornei* resistance in chickpea is polygenic. Khoo et al. 2021 (p. 6) has also recently found one quantitative trait locus (QTL) on the Ca7 chromosome for *P. thornei* resistance in cv. PBA HatTrick. To date, there is no published literature on chromosomal regions in chickpea associated with *P. neglectus* resistance. Due to the absence of information concerning *P. neglectus* resistance mechanisms, future breeding of resistant chickpea cultivars requires a greater understanding of the genetic architecture of *P. neglectus* resistance in chickpea.

Genome-wide association studies are therefore the first step in identifying resistance gene markers allowing for educated choice of candidate genes (Korte and Farlow 2013, p. 2) which can also be used for *P. neglectus* resistance in chickpea. Zwart et al. 2019b (p. 51) conducted a preliminary analysis on existing *P. thornei* data (125 *C.*

*reticulatum*), however, the number of wild *Cicer* accessions phenotyped was insufficient for strong associations to be identified. Therefore a *P. neglectus* GWAS was not undertaken in 2019 as it was known that the number of accessions was insufficient to obtain meaningful marker trait associations using a GWAS. Together with the 243 C. *reticulatum* and 86 *C. echinospermum* evaluated in this thesis, 39 *C. echinospermum* accessions from the 2014 collection were also recently made available and phenotyping of these accessions is currently underway at the University of Southern Queensland. These forthcoming results will enable GWAS for *P. neglectus* resistance to be performed on an increased number of accessions, which is likely to improve the power of GWAS to detect associations for traits that are polygenic with small effect size (RS Zwart, personal communication, January 11, 2022). Utilising accurate phenotyping and molecular marker-based resources assists in the selection of nematode resistance, facilitates the pyramiding of resistance genes, and combines multiple resistance to biotic stresses, ensuring robust chickpea cultivars with greater *Pratylenchus* spp resistance (Zwart et al. 2019a, p. 9).

Development of NAM populations increase the accuracy of QTL mapping by combining the strengths of bi-parental mapping populations and association mapping (McCullen et al. 2009, p. 737; Roorkiwal et al. 2020, p. 1705). This efficiently signals alleles of interest, augmenting the possibility of accurately identifying novel resistance genes (Roorkiwal et al. 2020, p. 1705). Twenty-six NAM parent accessions were chosen out of the new wild *Cicer* collection and crossed with seven elite chickpea cultivars from the major chickpea production areas worldwide (Turkey, Israel, the United States, Canada, Ethiopia, India, and Australia) (von Wettberg et al. 2018, p.8). The populations developed from the NAM parent accessions are being developed between these multiple elite chickpea cultivars of diverse origin under a funnel crossing scheme leading to novel genotypic combinations (Roorkiwal et al. 2020, p. 1705). A small subset of accessions representing the genetic and environmental breadth of the wild collection crossed into cultivated accessions is the first step towards trait introgression (Shin et al. 2019, p. 123). This NAM population is expected to accelerate the efforts of identification, isolation, and transfer of key candidate genes to facilitate chickpea improvement and increase the genetic diversity of advanced lines. These populations have recently been genotyped by high resolution SNP chip at Agriculture Victoria and are now

stable at >F5 (JD Berger, personal communication, January 11, 2022). The populations are held at the Australian Grains Genebank ranging from 43 to 403 lines available of each population (S Norton, personal communication, January 24, 2022. Populations that will be useful to investigate for QTL mapping for *P. neglectus* resistance are HAT TRICK/POT874 Kayat_077 CR (222 lines), HAT TRICK/POT963 Bari3_106D CR (232 lines), HAT TRICK/POT931 Oyali_084 CR (139 lines) and HAT TRICK/POT964 CudiB_022C CR (294 lines) with the NAM accessions included in these crosses producing significantly ($P < 0.05$) lower *P. neglectus* population densities than PBA HatTrick in this study. These same NAM accessions also produced significantly ($P < 0.05$) lower *P. thornei* population densities than PBA HatTrick (Reen et al. 2019). Therefore, it would be advantageous to investigate these populations for resistance to both *Pratylenchus* spp.

### 4.2.2 Exploiting the full genetic diversity of the new wild *Cicer* collection through collaboration

The targeted collection of wild *Cicer* which dramatically increased world collections and sources of genetic diversity was a coordinated project spearheaded by GRDC. This Australian project was linked with international collaborators in Turkey, the USA, Canada, Ethiopia, and India for the purpose of a linked international phenotypic evaluation of priority traits for chickpea improvement (JD Berger, personal communication, January 27, 2020). This was important as the sharing of genotypic and phenotypic data was previously lacking among many breeding programs utilising crop wild relatives (Dempewolf et al. 2017, p. 10). Priority traits for consideration in this national project were flowering cold tolerance, terminal drought stress, phenology regulation, boron tolerance, high Aluminium tolerance and low pH tolerance, resistance to RLN (*P. neglectus* and *P. thornei*), resistance to ascochyta blight, resistance to sclerotinia and resistance to phytophthora root rot (JD Berger, personal communication, January 11, 2021). Table 1 summarises accessions of the NAM parents that have been identified with resistance and/or tolerance to the above priority traits. Nineteen of the 26 NAM parent accessions were found to have significant resistance and/or tolerance to the priority traits. Many of the accessions have tolerance and/or resistance to more than one priority trait, highlighting the importance of this collaboration for future breeding efforts to overcome multiple biotic and abiotic constraints in chickpea.

**Table 1.** Summary of tolerance and/or resistance of priority traits in NAM parent accessions.

| NAM Parent | Species | Abiotic and biotic resistance and/or tolerance |
|---|---|---|
| Bari1_092 | *C. reticulatum* | Flowering cold; phenology regulation |
| Bari2_072 | *C. reticulatum* | Flowering cold; terminal drought stress |
| Bari3_106D | *C. reticulatum* | Low pH; *P. neglectus* |
| Besev_075 | *C. reticulatum* | *P. thornei* |
| Besev_079 | *C. reticulatum* | Phenology regulation; low pH |
| CudiA_152 | *C. reticulatum* | Flowering cold; boron |
| CudiB_022C | *C. reticulatum* | Terminal drought stress; low pH; boron; *P. neglectus* |
| Derei_072 | *C. reticulatum* | Boron |
| Egil_065 | *C. reticulatum* | Low pH; boron |
| Gunas_062 | *C. echinospermum* | Phenology regulation; *P. thornei; P. neglectus*; ascochyta blight |
| Karab_092 | *C. echinospermum* | Flowering cold; phenology regulation |
| Kalka_064 | *C. reticulatum* | *P. thornei* |
| Kayat_077 | *C. reticulatum* | Boron; *P. thornei*; *P. neglectus* |
| Kesen_075 | *C. reticulatum* | Low pH; boron |
| Oyali_084 | *C. reticulatum* | Terminal drought stress; low pH; boron; *P. thornei; P. neglectus* |
| S2Drd_065 | *C. echinospermum* | Flowering cold; phenology regulation |
| Sarik_067 | *C. reticulatum* | Boron; *P. thornei* |
| Savur_063 | *C. reticulatum* | Low pH; boron |
| Sirna_060 | *C. reticulatum* | Terminal drought stress; boron |

(JD Berger, personal communication, January 27, 2020; JD Berger, personal communication, January 11, 2021; Reen et al. 2019; H Rostad unpublished data)

A database known as the Breeding Management System (https://bmspro.io/) was used to store all data for each trait being studied on the new wild *Cicer* collection and is accessible to all collaborators. Genotypic data from sequencing of wild *Cicer* accessions from the 2013 collection by the University of California Davis is available publicly through the National Centre for Biotechnology Information

database (von Wettberg et al. 2018, p. 12). Genotypic data of wild *Cicer* accessions by Agriculture Victoria (from the 2013–2018 and original collections) is also available to all GRDC pre-breeding and breeding programs (S Kaur, personal communication, November 18, 2021). Development of chickpea cultivars which will endure increased biotic and abiotic pressures can be accomplished through the utilisation of the information gathered by this collaboration. This comprehensive information is vital for the success of the chickpea industry in a future of changing and unknown climate extremes.

### 4.3 Conclusion

The advantages of increasing the world collection of wild *Cicer* and utilising their genetic diversity in breeding programs has clearly been demonstrated in this thesis. Improved *P. neglectus* resistance than currently present in Australian chickpea cultivars has been identified in the *C. reticulatum* and *C. echinospermum* accessions evaluated in this study. This information is useful for future breeding of *P. neglectus* resistance into cultivated chickpea *C. arietinum*. Exploiting these new sources of genetic diversity in chickpea will increase *P. neglectus* resistance in chickpea and ensure a resilient crop to be utilised in effective crop rotations. In Australia and globally, greater resistance to multiple abiotic and biotic constraints in chickpea will allow for the maximum yield and profitability of the crop.

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# APPENDIX A

## ELECTRONIC SUPPLEMENTARY INFORMATION OF CHAPTER 3

**Article title**

**Resistance to root-lesion nematode *Pratylenchus neglectus* identified in a new collection of two wild chickpea species (*Cicer reticulatum* and *C. echinospermum*) from Turkey.**

**Journal**

*Plant Pathology (Wiley-Blackwell Publishing, UK)*

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**Supplementary Table 1**. Final population densities of *Pratylenchus neglectus*/kg from the multi-experiment analysis of wild *Cicer* (*Cicer reticulatum* and *Cicer echinospermum*) and *Cicer arietinum* evaluated in Experiments 1 and 2. Empirical best linear unbiased predictions ($\log_e$(*Pratylenchus neglectus*/kg), back transformed means (BTM) and back transformed standard errors of the mean (BTSEM) are given. *Cicer* more resistant than PBA HatTrick are accessions with probability $P < 0.05$. Genotypes have been given a provisional resistance rating according to the method of Thompson et al. (2020). Inoculation rate of *Pratylenchus neglectus* was 10,000/kg.

| Genotype | Species | $\text{Log}_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating[a] |
|---|---|---|---|---|---|---|
| Unplanted | - | 7.370 | 1591 | 559 | - | - |
| Oyali_073 | *C. ret* | 8.151 | 3467 | 1058 | 0.038 | R |
| CudiB_008B | *C. ret* | 8.227 | 3740 | 1108 | 0.055 | R |
| D05253>F3TMWR2AB001 | *C. ari* | 8.297 | 4013 | 1330 | 0.097 | R |
| Kayat_066 | *C. ret* | 8.310 | 4065 | 1204 | 0.088 | R |
| Kayat_061 | *C. ret* | 8.325 | 4125 | 1222 | 0.095 | R |
| CudiA_153 | *C. ret* | 8.328 | 4138 | 1225 | 0.096 | R |
| ILWC 127 | *C. ret* | 8.400 | 4445 | 1316 | 0.136 | R |
| ILWC 39 | *C. ret* | 8.413 | 4506 | 1374 | 0.150 | R |
| Karab_082 | *C. ech* | 8.433 | 4598 | 1408 | 0.166 | R |
| Bari2_074 | *C. ret* | 8.455 | 4698 | 1435 | 0.180 | R |
| Besev_074 | *C. ret* | 8.470 | 4768 | 1456 | 0.191 | R |
| Kesen_077 | *C. ret* | 8.472 | 4778 | 1420 | 0.187 | R |
| CudiA_128 | *C. ret* | 8.482 | 4827 | 1473 | 0.201 | R |
| Sirna_060 | *C. ret* | 8.486 | 4846 | 1434 | 0.198 | R |
| Oyali_071 | *C. ret* | 8.518 | 5002 | 1484 | 0.224 | R |
| Kesen_071 | *C. ret* | 8.518 | 5006 | 1486 | 0.226 | R |
| CudiB_018 | *C. ret* | 8.523 | 5028 | 1491 | 0.229 | R |
| 00283-1095-1002 | *C. ari* | 8.525 | 5040 | 1671 | 0.247 | R |
| Kayat_064 | *C. ret* | 8.535 | 5091 | 1507 | 0.240 | R |
| Kesen_065 | *C. ret* | 8.536 | 5095 | 1510 | 0.242 | R |
| S2Drd_102 | *C. ech* | 8.542 | 5123 | 1524 | 0.247 | R |
| Karab_091B | *C. ech* | 8.549 | 5160 | 1706 | 0.270 | R |
| Sirna_085 | *C. ret* | 8.549 | 5163 | 1708 | 0.271 | R |
| S2Drd_107B | *C. ech* | 8.553 | 5180 | 1537 | 0.257 | R |
| CudiA_154 | *C. ret* | 8.559 | 5214 | 1593 | 0.267 | R |
| Oyali_107 | *C. ret* | 8.560 | 5216 | 1548 | 0.263 | R |
| CudiA_104 | *C. ret* | 8.562 | 5230 | 1551 | 0.266 | R |
| Gunas_100 | *C. ech* | 8.566 | 5250 | 1559 | 0.271 | R |
| Oyali_084 | *C. ret* | 8.577 | 5310 | 1576 | 0.280 | R-MR |
| Kesen_104 | *C. ret* | 8.588 | 5364 | 1590 | 0.290 | R-MR |
| Kesen_062 | *C. ret* | 8.597 | 5418 | 1606 | 0.300 | R-MR |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| S2Drd_105 | *C. ech* | 8.601 | 5440 | 1613 | 0.305 | R-MR |
| Besev_075 | *C. ret* | 8.609 | 5483 | 1624 | 0.312 | R-MR |
| Bari3_073 | *C. ret* | 8.613 | 5502 | 1632 | 0.316 | R-MR |
| Bari2_062 | *C. ret* | 8.622 | 5551 | 1700 | 0.328 | R-MR |
| Besev_062 | *C. ret* | 8.631 | 5603 | 1659 | 0.335 | R-MR |
| S2Drd_104 | *C. ech* | 8.636 | 5633 | 1674 | 0.341 | R-MR |
| D05293>F3TMWR2AB002 | *C. ari* | 8.637 | 5639 | 1948 | 0.357 | R-MR |
| Oyali_104 | *C. ret* | 8.642 | 5662 | 1957 | 0.362 | R-MR |
| Karab_171 | *C. ech* | 8.643 | 5668 | 1801 | 0.355 | R-MR |
| Besev_065 | *C. ret* | 8.644 | 5676 | 1680 | 0.348 | R-MR |
| PI 527932 | *C. ech* | 8.650 | 5711 | 2081 | 0.376 | R-MR |
| Bari3_106D | *C. ret* | 8.659 | 5760 | 1705 | 0.364 | R-MR |
| Bari3_091 | *C. ret* | 8.672 | 5839 | 1729 | 0.379 | R-MR |
| CudiA_103C | *C. ret* | 8.673 | 5843 | 1734 | 0.380 | R-MR |
| Oyali_100 | *C. ret* | 8.677 | 5866 | 1742 | 0.384 | R-MR |
| Kayat_063 | *C. ret* | 8.693 | 5962 | 1766 | 0.402 | R-MR |
| PBA Pistol | *C. ari* | 8.701 | 6010 | 2076 | 0.420 | R-MR |
| Sirna_064 | *C. ret* | 8.704 | 6029 | 1787 | 0.415 | R-MR |
| Bari3_067 | *C. ret* | 8.715 | 6095 | 1808 | 0.427 | R-MR |
| Bari3_100 | *C. ret* | 8.715 | 6095 | 1806 | 0.427 | R-MR |
| S2Drd_065 | *C. ech* | 8.718 | 6111 | 1814 | 0.431 | R-MR |
| Sarik_067 | *C. ret* | 8.720 | 6124 | 1875 | 0.433 | R-MR |
| Egil-_066 | *C. ret* | 8.720 | 6127 | 1813 | 0.435 | R-MR |
| Derei_069 | *C. ret* | 8.723 | 6144 | 1877 | 0.438 | R-MR |
| Ortan_061 | *C. ech* | 8.731 | 6190 | 1836 | 0.445 | R-MR |
| Bari3_092 | *C. ret* | 8.733 | 6202 | 1835 | 0.447 | R-MR |
| Karab_172 | *C. ech* | 8.734 | 6212 | 1844 | 0.449 | R-MR |
| Kesen_101 | *C. ret* | 8.738 | 6237 | 1847 | 0.453 | R-MR |
| CudiA_152 | *C. ret* | 8.742 | 6263 | 1858 | 0.458 | R-MR |
| Kesen_066 | *C. ret* | 8.744 | 6274 | 1857 | 0.460 | R-MR |
| Gunas_062 | *C. ech* | 8.745 | 6281 | 1862 | 0.462 | R-MR |
| CudiB_023 | *C. ret* | 8.747 | 6291 | 1862 | 0.463 | R-MR |
| CudiA_221 | *C. ret* | 8.748 | 6296 | 1864 | 0.464 | R-MR |
| Oyali_076 | *C. ret* | 8.753 | 6330 | 1933 | 0.471 | R-MR |
| Besev_079 | *C. ret* | 8.756 | 6346 | 1880 | 0.473 | R-MR |
| Bari3_075 | *C. ret* | 8.757 | 6356 | 1882 | 0.475 | R-MR |
| Karab_086 | *C. ech* | 8.760 | 6372 | 1890 | 0.478 | R-MR |
| Derei_078 | *C. ret* | 8.762 | 6385 | 2119 | 0.482 | R-MR |
| Deste_071 | *C. ech* | 8.764 | 6398 | 2116 | 0.484 | R-MR |
| Sirna_071C | *C. ret* | 8.766 | 6414 | 2119 | 0.487 | R-MR |
| Kesen_067 | *C. ret* | 8.773 | 6456 | 1913 | 0.493 | R-MR |
| Oyali_105 | *C. ret* | 8.774 | 6462 | 2135 | 0.495 | R-MR |
| PBA HatTrick | *C. ari* | 8.779 | 6494 | 1938 | - | R-MR |
| CudiA_127 | *C. ret* | 8.791 | 6574 | 1950 | 0.514 | R-MR |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| Cermi_071 | *C. ech* | 8.792 | 6582 | 2176 | 0.514 | R-MR |
| S2Drd_106 | *C. ech* | 8.796 | 6607 | 2184 | 0.518 | R-MR |
| Bari1_093 | *C. ret* | 8.805 | 6668 | 2035 | 0.530 | R-MR |
| Besev_083 | *C. ret* | 8.815 | 6733 | 1994 | 0.542 | R-MR |
| Jimbour | *C. ari* | 8.815 | 6735 | 2009 | 0.543 | R-MR |
| Deste_063 | *C. ech* | 8.818 | 6752 | 2003 | 0.545 | R-MR |
| Sarik_064 | *C. ret* | 8.818 | 6756 | 2000 | 0.546 | R-MR |
| S2Drd_100 | *C. ech* | 8.819 | 6758 | 2002 | 0.546 | R-MR |
| CudiB_011 | *C. ret* | 8.819 | 6761 | 2005 | 0.547 | R-MR |
| Bari3_064 | *C. ret* | 8.822 | 6785 | 2009 | 0.550 | R-MR |
| Sirna_104 | *C. ret* | 8.832 | 6847 | 2261 | 0.556 | R-MR |
| Derei_065 | *C. ret* | 8.834 | 6865 | 2033 | 0.564 | R-MR |
| Kayat_077 | *C. ret* | 8.835 | 6870 | 2036 | 0.565 | R-MR |
| Karab_162 | *C. ech* | 8.837 | 6881 | 2043 | 0.566 | R-MR |
| Kesen_075 | *C. ret* | 8.839 | 6897 | 2042 | 0.569 | R-MR |
| CudiB_022C | *C. ret* | 8.839 | 6900 | 2044 | 0.570 | R-MR |
| Bari3_074 | *C. ret* | 8.841 | 6913 | 2050 | 0.572 | R-MR |
| ILWC 140 | *C. ret* | 8.842 | 6921 | 2055 | 0.573 | R-MR |
| Bari3_103 | *C. ret* | 8.848 | 6964 | 2062 | 0.580 | R-MR |
| Bari3_110 | *C. ret* | 8.851 | 6983 | 2068 | 0.583 | R-MR |
| Sirna_084 | *C. ret* | 8.852 | 6986 | 2307 | 0.577 | R-MR |
| Sirna_105 | *C. ret* | 8.853 | 6992 | 2312 | 0.578 | R-MR |
| Cermi_072 | *C. ech* | 8.855 | 7009 | 2081 | 0.587 | R-MR |
| Kalka_066 | *C. ret* | 8.859 | 7039 | 2085 | 0.592 | R-MR |
| Deste_061 | *C. ech* | 8.861 | 7052 | 2090 | 0.594 | R-MR |
| Karab_092 | *C. ech* | 8.862 | 7058 | 2096 | 0.595 | R-MR |
| CudiA_122 | *C. ret* | 8.869 | 7110 | 2104 | 0.603 | R-MR |
| CudiA_105 | *C. ret* | 8.869 | 7111 | 2104 | 0.604 | R-MR |
| Cermi_075 | *C. ech* | 8.875 | 7149 | 2123 | 0.609 | R-MR |
| PBA Seamer | *C. ari* | 8.875 | 7150 | 2365 | 0.602 | R-MR |
| Deste_073 | *C. ech* | 8.875 | 7154 | 2372 | 0.602 | R-MR |
| CudiA_155 | *C. ret* | 8.878 | 7172 | 2123 | 0.613 | R-MR |
| Deste_080 | *C. ech* | 8.880 | 7186 | 2131 | 0.615 | R-MR |
| Bari3_101 | *C. ret* | 8.884 | 7216 | 2207 | 0.617 | R-MR |
| CudiB_017 | *C. ret* | 8.884 | 7218 | 2384 | 0.611 | R-MR |
| ICC11323 | *C. ari* | 8.886 | 7232 | 2397 | 0.614 | R-MR |
| Cermi_063 | *C. ech* | 8.890 | 7256 | 2403 | 0.616 | R-MR |
| Bari1_092 | *C. ret* | 8.892 | 7275 | 2153 | 0.629 | R-MR |
| CudiB_019 | *C. ret* | 8.894 | 7290 | 2159 | 0.631 | R-MR |
| S2Drd_061 | *C. ech* | 8.896 | 7300 | 2168 | 0.632 | R-MR |
| Ortan_066 | *C. ech* | 8.896 | 7302 | 2167 | 0.632 | R-MR |
| Derei_073 | *C. ret* | 8.897 | 7314 | 2167 | 0.634 | R-MR |
| Sarik_074 | *C. ret* | 8.901 | 7339 | 2247 | 0.635 | R-MR |
| Bari1_064 | *C. ret* | 8.903 | 7357 | 2191 | 0.641 | R-MR |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| Kayat_070 | *C. ret* | 8.903 | 7358 | 2177 | 0.641 | R-MR |
| Sarik_061 | *C. ret* | 8.908 | 7389 | 2190 | 0.630 | R-MR |
| CudiA_151 | *C. ret* | 8.908 | 7392 | 2554 | 0.646 | R-MR |
| Bari1_091 | *C. ret* | 8.910 | 7408 | 2195 | 0.649 | R-MR |
| Bari1_068 | *C. ret* | 8.911 | 7411 | 2195 | 0.649 | R-MR |
| S2Drd_109 | *C. ech* | 8.915 | 7443 | 2276 | 0.650 | R-MR |
| Karab_062 | *C. ech* | 8.922 | 7497 | 2236 | 0.661 | R-MR |
| Flipper | *C. ari* | 8.922 | 7498 | 2222 | 0.665 | R-MR |
| CudiA_101A | *C. ret* | 8.925 | 7518 | 2385 | 0.656 | R-MR |
| Kayat_080 | *C. ret* | 8.927 | 7529 | 2228 | 0.666 | R-MR |
| Deste_079 | *C. ech* | 8.932 | 7568 | 2503 | 0.658 | R-MR |
| Derei_074 | *C. ret* | 8.934 | 7585 | 2401 | 0.673 | R-MR |
| PBA Boundary | *C. ari* | 8.934 | 7588 | 2250 | 0.669 | R-MR |
| Sonali | *C. ari* | 8.943 | 7654 | 2284 | 0.687 | R-MR |
| Kyabra | *C. ari* | 8.944 | 7664 | 2287 | 0.688 | R-MR |
| Bari1_062 | *C. ret* | 8.945 | 7670 | 2270 | 0.685 | R-MR |
| Besev_061 | *C. ret* | 8.951 | 7712 | 2552 | 0.677 | R-MR |
| Howzat | *C. ari* | 8.954 | 7742 | 2313 | 0.699 | R-MR |
| CudiB_009 | *C. ret* | 8.957 | 7764 | 2300 | 0.698 | R-MR |
| CudiB_016 | *C. ret* | 8.960 | 7786 | 2304 | 0.700 | R-MR |
| Sirna_083 | *C. ret* | 8.965 | 7827 | 2585 | 0.691 | R-MR |
| Sirna_082 | *C. ret* | 8.966 | 7834 | 2589 | 0.691 | R-MR |
| Kesen_073 | *C. ret* | 8.968 | 7848 | 2325 | 0.708 | R-MR |
| Yorker | *C. ari* | 8.975 | 7902 | 2358 | 0.720 | R-MR |
| Derei_070 | *C. ret* | 8.976 | 7912 | 2417 | 0.712 | R-MR |
| Karab_084 | *C. ech* | 8.982 | 7958 | 2752 | 0.699 | R-MR |
| S2Drd_101 | *C. ech* | 8.982 | 7960 | 2361 | 0.721 | R-MR |
| Karab_063 | *C. ech* | 8.989 | 8011 | 2381 | 0.727 | R-MR |
| PBA Maiden | *C. ari* | 8.993 | 8044 | 2665 | 0.718 | R-MR |
| Sirna_081B | *C. ret* | 8.993 | 8047 | 2660 | 0.716 | R-MR |
| Bari3_072C | *C. ret* | 9.001 | 8113 | 2404 | 0.741 | MR |
| Deste_072 | *C. ech* | 9.010 | 8183 | 2425 | 0.747 | MR |
| Egil-_072 | *C. ret* | 9.012 | 8204 | 2710 | 0.733 | MR |
| Sarik_065 | *C. ret* | 9.018 | 8253 | 2445 | 0.757 | MR |
| Egil-_063 | *C. ret* | 9.023 | 8296 | 2459 | 0.761 | MR |
| Bari3_065 | *C. ret* | 9.028 | 8335 | 2468 | 0.765 | MR |
| Deste_064 | *C. ech* | 9.031 | 8359 | 2556 | 0.761 | MR |
| Egil-_074 | *C. ret* | 9.034 | 8384 | 2483 | 0.770 | MR |
| D05222>F3TMWR2AB001 | *C. ari* | 9.035 | 8392 | 2781 | 0.755 | MR |
| Sarik_078 | *C. ret* | 9.037 | 8405 | 2490 | 0.772 | MR |
| CudiA_124 | *C. ret* | 9.038 | 8420 | 2494 | 0.774 | MR |
| CudiB_005 | *C. ret* | 9.048 | 8504 | 2597 | 0.777 | MR |
| Bari3_112 | *C. ret* | 9.049 | 8513 | 2525 | 0.783 | MR |
| Bari2_072 | *C. ret* | 9.052 | 8535 | 2531 | 0.785 | MR |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| Bari1_069 | *C. ret* | 9.052 | 8538 | 2526 | 0.786 | MR |
| Sarik_073 | *C. ret* | 9.063 | 8629 | 2557 | 0.795 | MR |
| CudiB_004 | *C. ret* | 9.065 | 8648 | 2562 | 0.796 | MR |
| Egil-_065 | *C. ret* | 9.068 | 8670 | 2566 | 0.798 | MR |
| Derei_066 | *C. ret* | 9.093 | 8890 | 2632 | 0.818 | MR |
| Bari3_102 | *C. ret* | 9.095 | 8913 | 2641 | 0.821 | MR |
| Derei_075 | *C. ret* | 9.103 | 8982 | 2662 | 0.826 | MR |
| Bari1_063 | *C. ret* | 9.103 | 8986 | 2660 | 0.826 | MR |
| Kalka_067 | *C. ret* | 9.113 | 9073 | 2686 | 0.834 | MR |
| Cermi_073 | *C. ech* | 9.119 | 9129 | 2706 | 0.836 | MR |
| Karab_174 | *C. ech* | 9.132 | 9245 | 2741 | 0.847 | MR |
| Egil-_075 | *C. ret* | 9.133 | 9256 | 2742 | 0.847 | MR |
| Egil-_073 | *C. ret* | 9.139 | 9312 | 2756 | 0.851 | MR |
| Bari3_079 | *C. ret* | 9.161 | 9521 | 2820 | 0.866 | MR |
| Sona | *C. ari* | 9.162 | 9525 | 2842 | 0.872 | MR |
| Sarik_080 | *C. ret* | 9.164 | 9547 | 2913 | 0.862 | MR |
| Sarik_077 | *C. ret* | 9.165 | 9560 | 2923 | 0.863 | MR |
| Bari2_067 | *C. ret* | 9.168 | 9589 | 2927 | 0.865 | MR |
| Besev_066 | *C. ret* | 9.179 | 9693 | 2870 | 0.877 | MR |
| CudiB_006 | *C. ret* | 9.197 | 9866 | 2931 | 0.887 | MR |
| Sarik_066 | *C. ret* | 9.200 | 9897 | 2929 | 0.888 | MR |
| Kalka_061 | *C. ret* | 9.200 | 9898 | 2931 | 0.889 | MR |
| Karab_081 | *C. ech* | 9.230 | 10200 | 3023 | 0.903 | MR |
| Savur_063 | *C. ret* | 9.264 | 10549 | 3129 | 0.920 | MR |
| Deste_075 | *C. ech* | 9.291 | 10837 | 3586 | 0.913 | MR |
| Kalka_064 | *C. ret* | 9.317 | 11130 | 3296 | 0.940 | MR |
| Kalka_070 | *C. ret* | 9.401 | 12099 | 3586 | 0.964 | MR |
| Bari2_064 | *C. ret* | 9.415 | 12277 | 3636 | 0.968 | MR |
| S2Drd_062 | *C. ech* | 9.419 | 12321 | 3655 | 0.967 | MR |
| Derei_072 | *C. ret* | 9.497 | 13320 | 3946 | 0.981 | MR-MS |
| Derei_062 | *C. ret* | 9.529 | 13753 | 4071 | 0.985 | MR-MS |
| Kalka_074 | *C. ret* | 9.557 | 14138 | 4203 | 0.988 | MR-MS |
| Wyalkatchem | *T. aes* | 9.621 | 20359 | 8047 | - | MR-MS |
| Machete | *T. aes* | 10.585 | 39811 | 16511 | - | S |
| Gregory | *T. aes* | 11.120 | 73593 | 31909 | - | S |

[a]Provisional rating: R=resistant, R-MR= resistant to moderately resistant, MR= moderately resistant, MR-MS= moderately resistant to moderately susceptible, S= susceptible.

**Supplementary Table 2**. Final population densities of *Pratylenchus neglectus*/kg from the multi-experiment analysis of wild *Cicer* (*Cicer reticulatum* and *Cicer echinospermum*) and *Cicer arietinum* evaluated in Experiments 3 and 4. Empirical best linear unbiased predictions ($\log_e$(*Pratylenchus neglectus*/kg), back transformed means (BTM) and back transformed standard errors of the mean (BTSEM) are given. *Cicer* more resistant than PBA HatTrick are accessions with probability $P < 0.05$. Genotypes have been given a provisional resistance rating according to the method of Thompson et al. (2020). Inoculation rate of *Pratylenchus neglectus* was 10,000/kg.

| Genotype | Species | $\log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating[a] |
|---|---|---|---|---|---|---|
| Unplanted | - | 6.880 | 975 | 343 | - | - |
| Bari3_106D | *C. ret* | 7.573 | 1945 | 644 | 0.002 | R |
| PI 527932 | *C. ech* | 7.628 | 2054 | 612 | 0.002 | R |
| Tuzca_035 | *C. ret* | 7.629 | 2057 | 610 | 0.002 | R |
| Bari3_104 | *C. ret* | 7.637 | 2074 | 616 | 0.002 | R |
| Kayat_066 | *C. ret* | 7.659 | 2120 | 630 | 0.002 | R |
| Isoha_025 | *C. ech* | 7.723 | 2260 | 673 | 0.004 | R |
| CudiB_008B | *C. ret* | 7.740 | 2299 | 683 | 0.005 | R |
| Sirna_042 | *C. ret* | 7.761 | 2346 | 697 | 0.006 | R |
| Ekind_047 | *C. ret* | 7.763 | 2352 | 699 | 0.006 | R |
| Golge_032 | *C. ret* | 7.781 | 2395 | 711 | 0.007 | R |
| Sirna_103 | *C. ret* | 7.793 | 2425 | 807 | 0.013 | R |
| 00283-1095-1002 | *C. ari* | 7.808 | 2461 | 735 | 0.008 | R |
| Isoha_018 | *C. ech* | 7.814 | 2476 | 888 | 0.021 | R |
| Moti | *C. ari* | 7.821 | 2492 | 744 | 0.009 | R |
| Isoha_048 | *C. ech* | 7.827 | 2507 | 747 | 0.010 | R |
| ILWC 115 | *C. ret* | 7.828 | 2511 | 746 | 0.010 | R |
| Kayat_077 | *C. ret* | 7.838 | 2536 | 753 | 0.010 | R |
| Isoha_010 | *C. ech* | 7.839 | 2538 | 756 | 0.011 | R |
| Sirna_035 | *C. ret* | 7.847 | 2557 | 759 | 0.012 | R-MR |
| Sirna_067 | *C. ret* | 7.891 | 2674 | 748 | 0.012 | R-MR |
| CudiA_109 | *C. ret* | 7.906 | 2713 | 842 | 0.021 | R-MR |
| Golge_035 | *C. ret* | 7.908 | 2720 | 845 | 0.021 | R-MR |
| Isoha_043 | *C. ech* | 7.920 | 2753 | 819 | 0.020 | R-MR |
| Ekind_052 | *C. ret* | 7.929 | 2776 | 824 | 0.021 | R-MR |
| Kayat_061 | *C. ret* | 7.929 | 2777 | 918 | 0.030 | R-MR |
| Isoha_033 | *C. ech* | 7.936 | 2796 | 832 | 0.022 | R-MR |
| Sonali | *C. ari* | 7.940 | 2806 | 837 | 0.021 | R-MR |
| Bari2_062 | *C. ret* | 7.954 | 2848 | 846 | 0.025 | R-MR |
| Sirna_088 | *C. ret* | 7.962 | 2871 | 854 | 0.026 | R-MR |
| Karab_066A | *C. ech* | 7.968 | 2886 | 1036 | 0.048 | R-MR |
| Sirna_061 | *C. ret* | 7.969 | 2891 | 1036 | 0.048 | R-MR |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| Sarik_081 | *C. ret* | 7.974 | 2904 | 863 | 0.029 | R-MR |
| Karab_164 | *C. ech* | 7.979 | 2920 | 1047 | 0.051 | R-MR |
| Oyali_073 | *C. ret* | 7.994 | 2963 | 880 | 0.032 | R-MR |
| Ekind_051 | *C. ret* | 7.995 | 2965 | 883 | 0.033 | R-MR |
| Oyali_084 | *C. ret* | 7.998 | 2976 | 884 | 0.033 | R-MR |
| CudiB_022C | *C. ret* | 8.013 | 3020 | 1000 | 0.049 | R-MR |
| PBA Drummond | *C. ari* | 8.029 | 3067 | 915 | 0.038 | R-MR |
| Sirna_070 | *C. ret* | 8.031 | 3073 | 1102 | 0.066 | R-MR |
| Howzat | *C. ari* | 8.039 | 3099 | 924 | 0.040 | R-MR |
| Isoha_038 | *C. ech* | 8.047 | 3124 | 930 | 0.046 | R-MR |
| Isoha_026 | *C. ech* | 8.048 | 3126 | 930 | 0.046 | R-MR |
| CudiA_102 | *C. ret* | 8.056 | 3152 | 937 | 0.045 | R-MR |
| D05253>F3TMWR2AB001 | *C. ari* | 8.056 | 3151 | 940 | 0.047 | R-MR |
| Jimbour | *C. ari* | 8.059 | 3163 | 970 | 0.049 | R-MR |
| Sirna_087 | *C. ret* | 8.068 | 3190 | 948 | 0.051 | R-MR |
| Golge_031 | *C. ret* | 8.069 | 3194 | 949 | 0.051 | R-MR |
| Derei_070 | *C. ret* | 8.076 | 3217 | 1063 | 0.054 | R-MR |
| Ekind_056 | *C. ret* | 8.076 | 3215 | 956 | 0.068 | R-MR |
| ILWC 140 | *C. ret* | 8.078 | 3224 | 1067 | 0.070 | R-MR |
| Ekind_053 | *C. ret* | 8.080 | 3229 | 959 | 0.055 | R-MR |
| Gunas_100 | *C. ech* | 8.081 | 3231 | 1160 | 0.056 | R-MR |
| Isoha_032 | *C. ech* | 8.081 | 3232 | 1071 | 0.071 | R-MR |
| CudiA_153 | *C. ret* | 8.081 | 3232 | 962 | 0.083 | R-MR |
| Deste_078 | *C. ech* | 8.085 | 3245 | 1165 | 0.085 | R-MR |
| Besev_079 | *C. ret* | 8.090 | 3262 | 1079 | 0.072 | R-MR |
| Sirna_110 | *C. ret* | 8.092 | 3267 | 1085 | 0.075 | R-MR |
| Bari2_072 | *C. ret* | 8.096 | 3280 | 1084 | 0.076 | R-MR |
| Sirna_089B | *C. ret* | 8.101 | 3297 | 1095 | 0.078 | R-MR |
| CudiA_125 | *C. ret* | 8.103 | 3303 | 982 | 0.063 | R-MR |
| Isoha_036 | *C. ech* | 8.104 | 3307 | 985 | 0.064 | R-MR |
| Oyali_101 | *C. ret* | 8.105 | 3311 | 1187 | 0.093 | R-MR |
| Bari1_092 | *C. ret* | 8.106 | 3315 | 1100 | 0.080 | R-MR |
| Bari3_100 | *C. ret* | 8.106 | 3314 | 1097 | 0.080 | R-MR |
| PBA Pistol | *C. ari* | 8.107 | 3318 | 991 | 0.061 | R-MR |
| S2Drd_108 | *C. ech* | 8.109 | 3325 | 989 | 0.066 | R-MR |
| Sarik_063 | *C. ret* | 8.110 | 3328 | 990 | 0.065 | R-MR |
| Golge_034 | *C. ret* | 8.113 | 3339 | 993 | 0.066 | R-MR |
| Tuzca_032 | *C. ret* | 8.113 | 3339 | 992 | 0.066 | R-MR |
| Isoha_013 | *C. ech* | 8.121 | 3365 | 1000 | 0.069 | MR |
| Ekind_045 | *C. ret* | 8.121 | 3363 | 1002 | 0.070 | MR |
| Sirna_090 | *C. ret* | 8.126 | 3381 | 1124 | 0.089 | MR |
| Erenk_001 | *C. ret* | 8.127 | 3383 | 1005 | 0.071 | MR |
| Sona | *C. ari* | 8.130 | 3395 | 1014 | 0.070 | MR |
| Sirna_066 | *C. ret* | 8.136 | 3415 | 1135 | 0.093 | MR |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| Gunas_061 | *C. ech* | 8.137 | 3420 | 1018 | 0.077 | MR |
| Bari1_063 | *C. ret* | 8.138 | 3422 | 1134 | 0.093 | MR |
| Isoha_051 | *C. ech* | 8.141 | 3432 | 1021 | 0.078 | MR |
| Gunas_101 | *C. ech* | 8.148 | 3457 | 1241 | 0.112 | MR |
| Bari1_062 | *C. ret* | 8.149 | 3459 | 1144 | 0.098 | MR |
| Kyabra | *C. ari* | 8.157 | 3488 | 1042 | 0.081 | MR |
| Sirna_101 | *C. ret* | 8.162 | 3505 | 1165 | 0.105 | MR |
| Golge_036 | *C. ret* | 8.164 | 3513 | 1044 | 0.088 | MR |
| PBA Slasher | *C. ari* | 8.165 | 3516 | 1049 | 0.084 | MR |
| Yorker | *C. ari* | 8.171 | 3537 | 1051 | 0.091 | MR |
| Sirna_036 | *C. ret* | 8.171 | 3537 | 1173 | 0.107 | MR |
| Sirna_034 | *C. ret* | 8.172 | 3541 | 1053 | 0.091 | MR |
| Derei_062 | *C. ret* | 8.176 | 3554 | 1177 | 0.093 | MR |
| Dogan_045 | *C. ret* | 8.176 | 3553 | 1056 | 0.111 | MR |
| Ekind_049 | *C. ret* | 8.177 | 3558 | 1059 | 0.094 | MR |
| CudiB_012 | *C. ret* | 8.179 | 3565 | 1059 | 0.094 | MR |
| Derei_072 | *C. ret* | 8.186 | 3591 | 1188 | 0.116 | MR |
| Ekind_059 | *C. ret* | 8.187 | 3593 | 1069 | 0.098 | MR |
| Isoha_052 | *C. ech* | 8.191 | 3609 | 1072 | 0.101 | MR |
| Ekind_043 | *C. ret* | 8.191 | 3609 | 1075 | 0.101 | MR |
| Sarik_072 | *C. ret* | 8.191 | 3609 | 1072 | 0.101 | MR |
| CudiB_015 | *C. ret* | 8.194 | 3618 | 1298 | 0.135 | MR |
| Egil-_073 | *C. ret* | 8.196 | 3627 | 1200 | 0.122 | MR |
| Kayat_067 | *C. ret* | 8.202 | 3649 | 1087 | 0.106 | MR |
| Karab_092 | *C. ech* | 8.209 | 3674 | 1218 | 0.130 | MR |
| PBA Seamer | *C. ari* | 8.210 | 3678 | 1099 | 0.107 | MR |
| Golge_039 | *C. ret* | 8.213 | 3689 | 1096 | 0.112 | MR |
| Golko_001 | *C. ret* | 8.214 | 3693 | 1099 | 0.113 | MR |
| Sirna_111A | *C. ret* | 8.214 | 3693 | 1325 | 0.147 | MR |
| Kesen_075 | *C. ret* | 8.215 | 3695 | 1223 | 0.132 | MR |
| Cermi_075 | *C. ech* | 8.217 | 3705 | 1227 | 0.134 | MR |
| Kesen_072 | *C. ret* | 8.217 | 3704 | 1328 | 0.149 | MR |
| CudiA_152 | *C. ret* | 8.220 | 3716 | 1229 | 0.135 | MR |
| Kayma_005 | *C. ret* | 8.224 | 3730 | 1111 | 0.119 | MR |
| CudiA_107 | *C. ret* | 8.225 | 3735 | 1111 | 0.119 | MR |
| Kesen_074 | *C. ret* | 8.232 | 3759 | 1348 | 0.157 | MR |
| Deste_066 | *C. ech* | 8.233 | 3763 | 1350 | 0.143 | MR |
| Sarik_078 | *C. ret* | 8.233 | 3764 | 1247 | 0.158 | MR |
| Bari3_072C | *C. ret* | 8.235 | 3771 | 1247 | 0.144 | MR |
| Bari3_091 | *C. ret* | 8.246 | 3812 | 1262 | 0.131 | MR |
| Tuzca_038 | *C. ret* | 8.246 | 3813 | 1134 | 0.151 | MR |
| Oyali_081 | *C. ret* | 8.249 | 3824 | 1371 | 0.168 | MR |
| PBA Boundary | *C. ari* | 8.250 | 3828 | 1143 | 0.130 | MR |
| Tuzca_039 | *C. ret* | 8.250 | 3829 | 1141 | 0.134 | MR |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| S2Drd_100 | *C. ech* | 8.252 | 3836 | 1271 | 0.155 | MR |
| Isoha_037 | *C. ech* | 8.253 | 3839 | 1146 | 0.137 | MR |
| Karab_067 | *C. ech* | 8.255 | 3847 | 1380 | 0.137 | MR |
| Tuzca_044 | *C. ret* | 8.255 | 3845 | 1143 | 0.171 | MR |
| Gunas_062 | *C. ech* | 8.257 | 3854 | 1148 | 0.139 | MR |
| Besev_066 | *C. ret* | 8.262 | 3875 | 1283 | 0.161 | MR |
| Sirna_043 | *C. ret* | 8.264 | 3882 | 1153 | 0.142 | MR |
| Sirna_030 | *C. ret* | 8.276 | 3930 | 1304 | 0.151 | MR |
| D05222>F3TMWR2AB001 | *C. ari* | 8.276 | 3930 | 1173 | 0.168 | MR |
| Tasdi_025 | *C. ret* | 8.282 | 3952 | 1208 | 0.160 | MR |
| Isoha_042 | *C. ech* | 8.287 | 3971 | 1317 | 0.159 | MR |
| Besev_075 | *C. ret* | 8.287 | 3974 | 1182 | 0.178 | MR |
| Egil-_065 | *C. ret* | 8.295 | 4006 | 1325 | 0.183 | MR |
| Isoha_002 | *C. ech* | 8.299 | 4019 | 1197 | 0.168 | MR |
| Karab_174 | *C. ech* | 8.300 | 4025 | 1335 | 0.187 | MR |
| Sarik_067 | *C. ret* | 8.304 | 4039 | 1337 | 0.188 | MR |
| Sirna_060 | *C. ret* | 8.304 | 4041 | 1336 | 0.189 | MR |
| Kalka_067 | *C. ret* | 8.306 | 4050 | 1339 | 0.191 | MR |
| Sirna_032 | *C. ret* | 8.313 | 4076 | 1211 | 0.177 | MR |
| Cermi_061 | *C. ech* | 8.316 | 4089 | 1467 | 0.213 | MR |
| Wyalkatchem | *T. aes* | 8.322 | 4567 | 1943 | - | MR |
| CICA1317 | *C. ari* | 8.324 | 4120 | 1370 | 0.202 | MR |
| Karab_085C | *C. ech* | 8.324 | 4123 | 1479 | 0.219 | MR |
| Derei_073 | *C. ret* | 8.326 | 4129 | 1365 | 0.205 | MR |
| Deste_064 | *C. ech* | 8.328 | 4137 | 1231 | 0.189 | MR |
| Isoha_047 | *C. ech* | 8.329 | 4141 | 1233 | 0.190 | MR |
| Isoha_034 | *C. ech* | 8.330 | 4145 | 1235 | 0.190 | MR |
| Karab_093 | *C. ech* | 8.330 | 4146 | 1488 | 0.224 | MR |
| Cermi_073 | *C. ech* | 8.332 | 4154 | 1376 | 0.211 | MR |
| Isoha_039 | *C. ech* | 8.333 | 4157 | 1238 | 0.192 | MR |
| Kayat_081 | *C. ret* | 8.333 | 4160 | 1235 | 0.193 | MR |
| Golge_037 | *C. ret* | 8.340 | 4187 | 1244 | 0.197 | MR |
| Golge_038 | *C. ret* | 8.342 | 4195 | 1246 | 0.199 | MR |
| Kalka_065 | *C. ret* | 8.342 | 4197 | 1562 | 0.239 | MR |
| Isoha_055 | *C. ech* | 8.344 | 4203 | 1254 | 0.201 | MR |
| Isoha_050 | *C. ech* | 8.359 | 4268 | 1272 | 0.214 | MR |
| Kayma_035 | *C. ret* | 8.369 | 4313 | 1281 | 0.222 | MR |
| Isoha_046 | *C. ech* | 8.373 | 4327 | 1293 | 0.226 | MR |
| Sirna_038 | *C. ret* | 8.378 | 4348 | 1292 | 0.227 | MR |
| D05295>F3TMWR2AB026 | *C. ari* | 8.383 | 4374 | 1452 | 0.250 | MR |
| S2Drd_065 | *C. ech* | 8.384 | 4378 | 1305 | 0.235 | MR |
| Ekind_044 | *C. ret* | 8.384 | 4376 | 1451 | 0.253 | MR |
| Sarik_077 | *C. ret* | 8.386 | 4384 | 1450 | 0.253 | MR |
| CICA1427 | *C. ari* | 8.387 | 4391 | 1455 | 0.251 | MR |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| CICA1421 | *C. ari* | 8.389 | 4399 | 1460 | 0.254 | MR |
| Isoha_030 | *C. ech* | 8.390 | 4403 | 1311 | 0.241 | MR |
| Sirna_063 | *C. ret* | 8.390 | 4404 | 1580 | 0.270 | MR |
| Genesis 090 | *C. ari* | 8.403 | 4461 | 1325 | 0.248 | MR-MS |
| Ekind_055 | *C. ret* | 8.403 | 4458 | 1332 | 0.251 | MR-MS |
| Ekind_057 | *C. ret* | 8.405 | 4471 | 1328 | 0.254 | MR-MS |
| Isoha_024 | *C. ech* | 8.406 | 4475 | 1334 | 0.256 | MR-MS |
| Sirna_069 | *C. ret* | 8.411 | 4495 | 1525 | 0.279 | MR-MS |
| Kayma_039 | *C. ret* | 8.412 | 4500 | 1337 | 0.259 | MR-MS |
| CICA1314 | *C. ari* | 8.417 | 4522 | 1500 | 0.279 | MR-MS |
| Savur_063 | *C. ret* | 8.417 | 4523 | 1496 | 0.281 | MR-MS |
| Dogan_037 | *C. ret* | 8.420 | 4537 | 1347 | 0.268 | MR-MS |
| Kalka_064 | *C. ret* | 8.420 | 4535 | 1502 | 0.283 | MR-MS |
| Sirna_046 | *C. ret* | 8.425 | 4560 | 1354 | 0.272 | MR-MS |
| Sirna_044 | *C. ret* | 8.428 | 4572 | 1359 | 0.275 | MR-MS |
| Sirna_051 | *C. ret* | 8.430 | 4583 | 1362 | 0.277 | MR-MS |
| Isoha_044 | *C. ech* | 8.432 | 4591 | 1367 | 0.280 | MR-MS |
| CICA0709 | *C. ari* | 8.439 | 4626 | 1535 | 0.300 | MR-MS |
| Oyali_085 | *C. ret* | 8.442 | 4640 | 1664 | 0.315 | MR-MS |
| D05293>F3TMWR2AB002 | *C. ari* | 8.443 | 4641 | 1538 | 0.303 | MR-MS |
| PBA Striker | *C. ari* | 8.450 | 4675 | 1397 | 0.294 | MR-MS |
| Kayma_044 | *C. ret* | 8.457 | 4709 | 1401 | 0.304 | MR-MS |
| ICC11323 | *C. ari* | 8.462 | 4730 | 1450 | 0.309 | MR-MS |
| Golge_026 | *C. ret* | 8.463 | 4735 | 1407 | 0.309 | MR-MS |
| Deste_080 | *C. ech* | 8.467 | 4755 | 1576 | 0.315 | MR-MS |
| Isoha_027 | *C. ech* | 8.467 | 4758 | 1415 | 0.328 | MR-MS |
| Kalka_074 | *C. ret* | 8.470 | 4771 | 1417 | 0.317 | MR-MS |
| Isoha_040 | *C. ech* | 8.475 | 4793 | 1428 | 0.323 | MR-MS |
| Isoha_031 | *C. ech* | 8.484 | 4837 | 1439 | 0.332 | MR-MS |
| Dogan_032 | *C. ret* | 8.488 | 4856 | 1442 | 0.335 | MR-MS |
| Sirna_050 | *C. ret* | 8.488 | 4858 | 1446 | 0.336 | MR-MS |
| Sirna_052 | *C. ret* | 8.495 | 4892 | 1455 | 0.343 | MR-MS |
| Sirna_041 | *C. ret* | 8.497 | 4901 | 1457 | 0.345 | MR-MS |
| Dogan_034 | *C. ret* | 8.499 | 4908 | 1461 | 0.347 | MR-MS |
| Erenk_002 | *C. ret* | 8.501 | 4921 | 1464 | 0.350 | MR-MS |
| ILWC 127 | *C. ret* | 8.524 | 5034 | 1805 | 0.390 | MR-MS |
| Ekind_048 | *C. ret* | 8.528 | 5052 | 1501 | 0.378 | MR-MS |
| Ekind_054 | *C. ret* | 8.528 | 5055 | 1503 | 0.379 | MR-MS |
| Almaz | *C. ari* | 8.529 | 5058 | 1582 | 0.381 | MR-MS |
| Ekind_058 | *C. ret* | 8.534 | 5082 | 1509 | 0.385 | MR-MS |
| Sirna_047 | *C. ret* | 8.550 | 5166 | 1535 | 0.403 | MR-MS |
| Sirna_039 | *C. ret* | 8.561 | 5226 | 1552 | 0.416 | MR-MS |
| Kayat_071 | *C. ret* | 8.570 | 5272 | 1567 | 0.425 | MR-MS |
| PBA Maiden | *C. ari* | 8.572 | 5284 | 1570 | 0.428 | MR-MS |

| Genotype | Species | $Log_e$ (*Pn*/kg) | BTM | BTSEM | Pr< PBA HatTrick | Provisional Rating |
|---|---|---|---|---|---|---|
| Ekind_050 | *C. ret* | 8.572 | 5284 | 1842 | 0.435 | MR-MS |
| Deste_077 | *C. ech* | 8.583 | 5341 | 1918 | 0.448 | MR-MS |
| Dogan_036 | *C. ret* | 8.589 | 5372 | 1650 | 0.448 | MR-MS |
| Neelam | *C. ari* | 8.623 | 5560 | 1731 | 0.486 | MR-MS |
| Yenda | *T. aes* | 8.629 | 5652 | 2377 | - | MR-MS |
| Yanil_013 | *C. ret* | 8.633 | 5616 | 1668 | 0.498 | MR-MS |
| PBA HatTrick | *C. ari* | 8.635 | 5628 | 1679 | - | MR-MS |
| Dogan_043 | *C. ret* | 8.638 | 5642 | 1676 | 0.503 | MR-MS |
| S2Drd_061 | *C. ech* | 8.642 | 5664 | 1878 | 0.507 | MR-MS |
| ILWC 180 | *C. ech* | 8.683 | 5902 | 1758 | 0.554 | MS |
| Dogan_035 | *C. ret* | 8.685 | 5912 | 1757 | 0.556 | MS |
| Dogan_039 | *C. ret* | 8.693 | 5961 | 1773 | 0.566 | MS |
| Dogan_026 | *C. ret* | 8.700 | 6006 | 1784 | 0.574 | MS |
| Dogan_042 | *C. ret* | 8.701 | 6010 | 1789 | 0.575 | MS |
| Isoha_045 | *C. ech* | 8.707 | 6046 | 1802 | 0.581 | MS |
| Isoha_028 | *C. ech* | 8.716 | 6097 | 1817 | 0.591 | MS |
| Dogan_044 | *C. ret* | 8.722 | 6135 | 1825 | 0.598 | MS |
| Isoha_054 | *C. ech* | 8.728 | 6172 | 1838 | 0.604 | MS |
| Dogan_031 | *C. ret* | 8.730 | 6188 | 1839 | 0.608 | MS |
| Isoha_053 | *C. ech* | 8.736 | 6224 | 1854 | 0.614 | MS |
| Dogan_029 | *C. ret* | 8.758 | 6363 | 1893 | 0.638 | MS |
| Dogan_028 | *C. ret* | 8.764 | 6401 | 1903 | 0.645 | MS |
| Ekind_060 | *C. ret* | 8.775 | 6468 | 1921 | 0.656 | MS |
| Sirna_037 | *C. ret* | 8.797 | 6617 | 1968 | 0.680 | MS |
| Dogan_040 | *C. ret* | 8.803 | 6653 | 1976 | 0.685 | MS |
| Dogan_027 | *C. ret* | 8.813 | 6723 | 1998 | 0.696 | MS |
| Olgun_026 | *C. ret* | 8.837 | 6882 | 2137 | 0.713 | MS |
| Sirna_040 | *C. ret* | 8.861 | 7051 | 2094 | 0.742 | MS |
| Isoha_049 | *C. ech* | 8.866 | 7090 | 2110 | 0.746 | MS |
| Dogan_038 | *C. ret* | 8.875 | 7148 | 2128 | 0.754 | MS |
| Ortan_066 | *C. ech* | 8.896 | 7302 | 2168 | 0.632 | MS |
| Dogan_033 | *C. ret* | 9.008 | 8170 | 2427 | 0.858 | MS-S |
| Brookton | *T. aes* | 9.442 | 14962 | 6315 | - | S |
| Gregory | *T. aes* | 9.487 | 15073 | 6224 | - | S |

[a]Provisional rating: R=resistant, R-MR= resistant to moderately resistant, MR= moderately resistant, MR-MS= moderately resistant to moderately susceptible, MS= moderately susceptible, MS-S= moderately susceptible to susceptible, S= susceptible.

# APPENDIX B

## List of publications

### Poster presentations

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/>.

**Rostad, HE**, Reen, RA, Mumford, M, Thompson, JP, Zwart, RS 2021 'Wild chickpea, the basis for genetic improvement to root lesion nematode (*Pratylenchus neglectus)*', *Stay Connected for Plant Health 2021 Australasian Plant Pathology Society Biennial Conference*, Hobart, Australia, 2021, 23–26th November, p. 245. Available at: <https://appsconference.com.au/wp-content/uploads/2021/11/2021-APPS-Conference-Handbook-1.pdf>. (Awarded ePoster Presentation Prize)

### Oral presentations

**Rostad, HE**, Reen, RA, Mumford, M, Thompson, JP, Zwart, RS 'Resistance of wild relatives (*Cicer reticulatum* and *C. echinospermum*) of chickpea (*C. arietinum*) to the root-lesion nematode *Pratylenchus neglectus'*, Centre for Crop Health, University of Southern Queensland HDR Seminar, Toowoomba, 2021, 19th August. (Awarded Presentation Prize)