THE IDENTIFICATION OF NOVEL ROOT-LESION NEMATODE (*Pratylenchus thornei* & *P. neglectus*) RESISTANCES IN IRANIAN LANDRACE WHEAT (*Triticum aestivum*) AND EINKORN (*T. monococcum*) AND THEIR INTROGRESSION INTO AUSTRALIAN WHEAT CULTIVARS

A Thesis submitted by

Jason Glen Sheedy

B.App.Sci (Rural Tech) (Hons), MPhil (Ag Sci)

For the award of

Doctor of Philosophy

2022

ABSTRACT

The root-lesion nematodes (RLN) *Pratylenchus thornei* and *P. neglectus* are globally important pathogens of wheat that can reduce yields by up to 60% and 20%, respectively. They often occur together in mixed populations that are best managed through the incorporation of genetic tolerance and resistance into adapted wheat genotypes. A genome-wide association study (GWAS) was conducted on a collection of 245 Iranian landrace wheats (ILWs) to identify quantitative trait loci (QTL) associated with *P. thornei* resistance, which were then validated using seven ILW-derived BC_1F_4 populations. A *P. thornei*-resistant subset of 91 ILWs were characterised for their *P. neglectus* resistance and were genetically analysed to determine if any carried the primary *P. neglectus* resistance genes used in Australia. ILW-derived breeding populations were selected for resistance to *P. thornei* and/or *P. neglectus*. Four einkorn-derived recombinant inbred line (RIL) populations were produced and evaluated for resistance to *P. thornei* to identify resistant RILs and to estimate the effective number of resistance genes using segregation and quantitative genetics analyses. Five novel QTL located on chromosomes 2B (x2), 3B, 5B and 7B were identified in the GWAS. Individual ILWs carried up to six QTL and final *P. thornei* population density decreased exponentially as QTL number per genotype increased. Ten KASP markers were validated in the BC_1F_4 populations, which carried two to five *P. thornei*-resistance QTL. Seven ILW accessions were identified as resistant to *P. neglectus* with five carrying novel *P. neglectus* resistance. Six ILW-derived breeding lines with resistance to both *P. thornei* and *P. neglectus* were developed. Evaluation of four einkorn-derived RIL populations identified 26 RILs that were resistant to *P. thornei*. Both segregation and quantitative genetics analyses indicated that one to two genes controlled the resistance in all populations. The ILWs are a valuable source of novel resistances to both *P. thornei* and *P. neglectus*. Analysing existing phenotypic data in a GWAS effectively identified *P. thornei* resistance QTL without the need to develop biparental populations. This is the first report of *P. thornei* resistance being transferred from einkorn to wheat. The five novel *P. thornei* resistance QTL and the 43 wheat breeding lines with resistance to *P. thornei*, *P. neglectus* or both *P. thornei* and *P. neglectus* will increase diversity in the genetic management of RLN and deliver new gene combinations to increase the overall level of resistance available to plant breeders.

CERTIFICATION OF THESIS

I, Jason Glen Sheedy, declare that the PhD thesis entitled 'The identification of novel root-lesion nematode (*Pratylenchus thornei* & *P. neglectus*) resistances in Iranian landrace wheat (*Triticum aestivum*) and einkorn (*T. monococcum*) and their introgression into Australian wheat cultivars' is not more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references, and footnotes.

This thesis is the work of Jason Glen Sheedy except where otherwise acknowledged, with the majority of the contribution to the papers 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.

Date: 29 November 2022

Endorsed by:

Professor John Thompson

Principal Supervisor

Dr Mandy Christopher

Associate Supervisor

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

STATEMENT OF CONTRIBUTION

Paper 1:

Introgression into advanced breeding lines of novel root-lesion nematode (*Pratylenchus thornei*) resistance QTL identified in a genome-wide association study of Iranian landrace wheats (*Triticum aestivum*). Jason G Sheedy, Raj K Pasam, Matthew J Hayden, Kerrie L Forrest and John P Thompson. This chapter was prepared according to the instructions to authors given by *Molecular Breeding*.

Jason Sheedy contributed 60% to this paper. Collectively Raj K Pasam, Matthew J Hayden, Kerrie L Forrest and John P Thompson contributed the remainder.

Paper 2

Discovery of resistance to *Pratylenchus neglectus* among *P. thornei*-resistant Iranian landrace wheats and the introgression of both resistances into advanced breeding lines. JG Sheedy, J Lin and JP Thompson. 2022. *Plant Pathology*, 71, 2017-2028. https://doi.org/10.1111/ppa.13616

Jason Sheedy contributed 70% to this paper. Collectively Jing Lin and John P Thompson contributed the remainder.

Paper 3:

The first transfer of resistance to the root-lesion nematode (*Pratylenchus thornei*) from diploid einkorn (*Triticum monococcum*) to hexaploid wheat (*T. aestivum*). Jason G Sheedy, Jing Lin, Mandy Christopher and John P Thompson. This chapter has been submitted to *Crop Science* and is currently under review.

Jason Sheedy contributed 70% to this paper. Collectively Jing Lin, Mandy Christopher and John P Thompson contributed the remainder.

ACKNOWLEDGEMENTS

First and foremost, I want to thank my wife Angel and daughters Marni and Isabelle for their unwavering support during my PhD candidature. I would not have had any chance of success without your support. I love you all very much!

Sincere thanks to my supervisors Prof. John Thompson and Dr Mandy Christopher. I have appreciated your support, guidance and expertise throughout my PhD. You have both helped me tremendously with the planning and implementation of experiments, data analyses and drafting of this thesis and the journal articles it contains. I appreciate your ongoing commitment to my PhD studies.

I would like to thank all of my co-authors and collaborators for their contributions to the research presented in this thesis. In particular, the USQ Crop Nematology team without whom I could not have produced all of the breeding populations and conducted the glasshouse experiments.

I thank all of my colleagues and the students who have participated in the USQ Crop Nematology Action Learning Group and the CCH manuscript advisory group. Your constructive and valuable feedback on the manuscripts that have contributed to this thesis has helped me immensely.

This research has been supported by the Australian Government Research Training Program Scholarship and through research projects funded by the Grains Research and Development Corporation, the State of Queensland through its Department of Agriculture and Fisheries and the University of Southern Queensland.

TABLE OF CONTENTS

ABBREVIATIONS

ABL, advanced breeding line;

AR1, autoregressive structure of order 1;

AU, Australian;

AWBMMP, Australian Wheat and Barley Molecular Marker Program;

BLUE, best linear unbiased estimate;

BLUP, best linear unbiased predictor;

CIMMYT, International Maize and Wheat Improvement Center;

DBCP, 1,2-dibromo-3-chloropropane;

DD, 1,3-dichloropropene, 1,2-dichloropropane;

DH, doubled-haploid

EDB, ethylene dibromide;

GWAS, genome-wide association study

GS50a, a wheat selection from the cultivar Gatcher

h^2, heritability;

ILW, Iranian landrace wheats

ITMI, International Triticeae Mapping Initiative;

KASP, Kompetitive allele specific PCR

LMM, linear mixed model;

M, million;

MR, moderately resistant

MRMS, moderately resistant-moderately susceptible

PCR, Polymerase chain reaction

REML, restricted maximum likelihood;

RF; reproduction factor (final population [P_f] ÷ initial population [P_i])

RIL, recombinant inbred line;

RILP, recombinant inbred line population;

RLN, root-lesion nematode;

RMR, resistant-moderately resistant

SHW, synthetic hexaploid wheat

SNP, single nucleotide polymorphism;

SSD, single seed descent;

UTCAN, University of Tehran, College of Agriculture & Natural Resources

QTL, quantitative trait locus or loci.

V_e, Environmental variation;

V_g, Genetic variation;

V_{gxe}, Genetic x environmental variation;

WANA, West Asia and North Africa.

CHAPTER 1: INTRODUCTION

Root-lesion nematodes (RLNs; *Pratylenchus* spp.) are an important group of plant-parasitic nematodes that can reduce the yield and/or quality of many crop species including tree crops, ornamental plants, horticultural and field crops (Fortuner 1977). In particular, *Pratylenchus thornei* and *P. neglectus* are plant parasites of global significance (Castillo and Vovlas 2007) that commonly occur together in farmers' fields (Thompson et al. 2010). They are widespread and have the capacity to significantly reduce the yields of many crops, primarily cereals and legumes (Reen et al. 2014; Thompson et al. 2021). As a result, significant resources have been dedicated to the management of these phytoparasitic nematodes through both host genetic resistance and tolerance (Thompson et al. 2020; Thompson et al. 2021) and identifying effective crop sequences (Owen et al. 2022; Taylor et al. 2000) to manage their populations in soil.

Despite these efforts, only one commercial wheat (*Triticum aestivum*) variety recommended for the Australian northern grains region is moderately resistant to *P. neglectus* and none resistant to *P. thornei* (GRDC 2021; Matthews et al. 2022). Consequently, there are no cultivars that have resistance to both species and therefore can effectively manage mixed *P. thornei* and *P. neglectus* populations. Research has shown that the incorporation of RLN tolerance and resistance into wheat genotypes can significantly increase their yields when compared with intolerant genotypes grown in RLN-infested soil (Thompson et al. 2001; Vanstone et al. 1998). This project offers the opportunity to identify genotypes that carry novel *P. thornei* and/or *P. neglectus* resistance, increase the diversity of genetic resistance available, create new trait combinations, and produce advanced breeding lines suitable for use by plant breeding programs through three primary objectives.

The first objective was to 1) analyse *P. thornei*-resistance data from a collection of 245 Iranian landrace wheats (ILW) (Sheedy and Thompson 2009) in a genome-wide association study (GWAS) framework, using linear mixed model (LMM) and BayesR methods (Pasam et al. 2017), to identify quantitative trait loci (QTL) associated with resistance, 2) validate the QTL in ILW-derived BC_1F_4 breeding populations and 3) develop ILW-derived advanced breeding lines with *P. thornei* resistance levels similar to, or exceeding, the best levels commercially available.

The second objective was to 1) characterise the *P. thornei*-resistant ILW accessions for their resistance to *P. neglectus* phenotypically, 2) identify genotypes with resistance to both *P. thornei* and *P. neglectus*, 3) determine if any genotypes carry the previously identified *Rlnn1* gene and/or *QRlnn.lrc-2B* QTL and 4) develop advanced breeding lines (ABL) with resistance to both *P. thornei* and *P. neglectus* through crosses between RLN-resistant ILW and Australian commercial cultivars.

The third objective was to 1) produce recombinant inbred line (RIL) wheat populations (RILPs) after wide-crossing of *P. thornei*-resistant einkorn accessions with adapted wheat genotypes, 2) phenotypically characterise the RILPs in the BC_1F_2, BC_1F_6, BC_3F_5 and/or BC_3F_6 generations for their resistance to *P. thornei* and 3) study the genetic basis of the resistance by estimating the effective number of resistance genes in each population using segregation and quantitative genetic analyses.

CHAPTER 2: LITERATURE REVIEW

Nematodes are among the most diverse taxa on earth and oftentimes are beneficial organisms that accelerate soil nutrient cycling, provide biological control of phytoparasitic nematodes and insects and are useful as indicators of ecosystem health. Alternatively, nematodes can cause adverse effects on the health of humans, animals and plants, and affect human economies in many ways including loss of agricultural production, damage to pasture and turf, and invasion of forest trees. Plant parasitic nematodes are estimated to cause USD 157 billion per year in damage to crops (Mendoza-de Gives 2022; Yeates 2010).

Wheat is a crop of global importance. More than 730 million tonnes are produced annually on a land area greater than any other commercial crop (FAO 2021) and its global trading is forecast to reach 197 million tonnes during 2022/23 (FAO 2022; FAO 2023) at a value of nearly 52 billion USD (wheat priced at 262 USD/t). Wheat is often considered as a primary source of dietary carbohydrate, however, it is also a source of fibre, vitamins and minerals (Shewry and Hey 2015), and contributes about 20% of the total dietary calories and proteins worldwide (Shiferaw et al. 2013).

2.1 Wheat genetic resources

Wheat is an allohexaploid (6x; 2n=42) composed of three related genomes (A, B and D) that were originally derived from three diploid (2x; 2n=14) species within the tribe *Triticeae*. The plant species donors of these respective genomes are considered to be (i) *Triticum urartu* (A-genome), (ii) a currently unidentified (or extinct) species closely related to *Aegilops speltoides* (S-genome that can substitute for chromosomes on the B-genome), and (iii) *Aegilops tauschii* (D-genome) (Marcussen et al. 2014). The initial hybridisation between *T. urartu* and the species closely related to *A. speltoides* produced the wild tetraploid wheat *T. turgidum* ssp. *dicoccoides* and the primitive cultivated form of this species, *T. turgidium* ssp. *dicoccon*, which is the direct progenitor of modern durum wheat (*T. turgidium* ssp. *durum*). A spontaneous hybridisation between *T. turgidum* ssp. *dicoccon* and *A. tauschii*, the two progenitors of hexaploid wheat, probably took place in a farmer's field in western Iran about 8000 years ago when the cultivated tetraploid wheat was brought into the area of the wild diploid wheat (Feldman and Sears 1981).

A high strategic priority for practical cereal improvement worldwide is to enrich the cultivated gene pools by incorporating favourable alleles, genes or gene complexes from wild relatives (Feuillet et al. 2007). The crop wild relatives have been grouped into three gene pools based on their genetic similarity as determined by the ease of genetic transfer through hybridisation with cultivated crop species. The primary gene pool corresponds to the traditional concept of the biological species and its subordinate races where crossing and gene transfer are easy. The secondary gene pool includes all biological species that will cross with the target crop but where biological barriers exist that will cause defects in the progeny, including sterility, poor chromosome pairing or low vigour. The tertiary gene pool encompasses all other plant species that can be crossed with the target crop and where specialised plant breeding techniques including embryo culture, doubling chromosome number or using bridging species to obtain some fertility are required (Harlan and de Wet 1971).

Traditionally, wheat breeders have preferred to use germplasm that is well adapted to the domestic environment. When unadapted parents must be used to provide the desired type and level of genetic variation, the order of preference is i) landraces (primary gene pool), ii) closely related species (secondary gene pool) and iii) more distantly related species or genera in the tertiary gene pool (Cox 1991).

Wheat landraces, the principal source of genetic diversity in the primary gene pool, have been defined as cultivated, genetically heterogeneous varieties that have evolved in certain ecogeographical areas and are therefore adapted to those edaphic and climatic conditions and to their traditional management and uses (Casanas et al. 2017). Landraces are not the unchanging embodiment of ancient germplasm, but rather the outcomes of imperfect and iterative choices regarding the qualities judged useful or attractive to a grower at a particular point in time. They are not conserved for the sake of conservation but rather are continually compared to and enriched by new materials of both local and foreign origin (Tripp 1996). More recently, landraces have been defined as plant materials consisting of cultivated varieties that have evolved and may continue evolving, using conventional or modern breeding techniques, in traditional or new agricultural environments within a defined ecogeographical area and under the influence of local human culture (Casanas et al. 2017). It is important to note that the breeding techniques used in the evolution of landraces can be via both formal breeding programs and informal farmer selections.

The development of synthetic hexaploid wheats (SHWs), where the original hybridisation between tetraploid *T. turgidum* (BBAA) and diploid *A. tauschii* (DD) is recreated, has helped overcome the limited genetic diversity of modern wheat (BBAADD) (Ogbonnaya et al. 2008). This has been achieved primarily through the increased availability of secondary gene pool species by transferring their genetic diversity into the primary gene pool. During the development of SHWs, the use of improved tetraploid wheat was important to success, as two out of the three genomes had already been selected for desirable traits. Crosses with wild tetraploid wheat species have usually led to tall SHWs with very undesirable agronomic properties (Rosyara et al. 2019).

2.2 Iranian landrace wheats

Wheat has been domesticated in Iran for about the last 10,000 years (~7,500 B.C.) and remains one of that country's most important crops (Ramshini et al. 2016; Saidi 2001). The Iranian School of Agriculture, now known as the University of Tehran, College of Agriculture and Natural Resources (UTCAN), initiated wheat research efforts in 1930, where its initial activities included the collection of wheat and barley landraces (Saidi 2001). In 1935, UTCAN undertook a program of collecting ILWs from farmers' fields and provincial markets in wheat-producing regions. In all, more than 11,000 ILW accessions were collected and used for wheat improvement in Iran. Seed from these accessions was transferred to the Genetic Resources Conservation Program, University of California at Davis, USA in 1986–1987 with ~8,000 ILWs surviving subsequent seed production phases. Of these surviving ILWs, ~6,800 were submitted to the gene banks of the USDA National Small Grains Collection, Aberdeen, Idaho and to CIMMYT, Mexico (Vikram et al. 2020).

These ILWs have proven to be a genetically diverse source of traits that have been useful for wheat improvement (Alipour et al. 2017). These beneficial traits include tolerance to drought and heat stress (Ehdaie et al. 1988) and cover a wide diversity in starch physical properties that could be useful in breeding for improved quality of specific end-use products. These qualities include i) pasting characteristics considered desirable for high-quality Japanese-style white-salted noodles, ii) exceptionally high hot-paste and cool-paste viscosities, and iii) starch with unusually high resistance to shear-thinning (Bhattacharya et al. 1997). The ILWs have also been valuable sources of resistance to pests and disease, including resistances to stem rust (*Puccinia graminis*

f. sp. *tritici*) (Saremirad et al. 2020) and to Russian wheat aphid (RWA, *Diuraphis noxia*), contributing the resistance genes *Dn1* and *Dn6* (Ehdaie and Baker 1999).

Thompson et al. (1999) screened landrace wheats from West Asia and North Africa (WANA) for resistance to *P. thornei*. Resistance at the level of the wheat line Gatcher selection 50a (GS50a, a source of partial resistance) or better was detected in bread wheats originally collected in Morocco (2 accessions), Iran (8 accessions) and Iraq (2 accessions). A collection of modern West Asia and North Africa (WANA) wheats was also tested and two bread wheats from Sudan and Iran had resistance levels comparable to GS50a. Notably, a collection of 274 ILW that were selected for spring growth habit by the late Dr Bent Skovmand from the larger collection held by CIMMYT, were characterised for their resistance to *P. thornei* with 46% proving to be at least moderately resistant (Sheedy and Thompson 2009). Interestingly, eight *Pratylenchus* spp. have been reported from cereal-producing regions of Iran (Mokrini et al. 2018) with *P. thornei* and *P. neglectus* being the most common species (Pourjam et al. 1999), suggesting that resistance to the other *Pratylenchus* spp. may be available among ILW accessions.

2.3 Einkorn (*Triticum monococcum*)

Triticum monococcum, commonly known as einkorn or small spelt wheat, was once considered the A-genome progenitor of polyploid wheats. *Triticum urartu* is now thought to have played that role; however, *T. monococcum* is still considered the first wheat widely domesticated and used in ancient agriculture (Yanushevich et al. 1989). The *T. monococcum* species consists of two sub-species: the wild form *T. monococcum* ssp. *aegilopoides* and the cultivated form *T. monococcum* ssp. *monococcum* (van Slageren 1994). Both species have proven to be valuable sources of desirable traits and genetic diversity for wheat improvement (Adhikari et al. 2022).

Pratylenchus thornei-resistant accessions have been identified in both sub-species of einkorn (Sheedy et al. 2012). However, there is only one report of phytoparasitic nematode resistance being transferred from einkorn to wheat. That study indicated that cereal cyst nematode (*Heterodera avenae*) resistance was controlled by two genes for which QTL were identified on chromosomes 1AS and 2AS (Singh et al. 2010).

Einkorn's value for wheat improvement extends to many other biotic, abiotic and grain quality traits. Accessions of *T. monococcum* have been found to be resistant to stripe

rust (*Puccinia striformis*) (Tomar et al. 1988; Valkoun et al. 1982), stem rust (*Puccinia graminis* f. sp. *tritici*) (Anker et al. 2001; Valkoun et al. 1989) and leaf rust (*Puccinia triticina*) (Anker et al. 2001; Hussein et al. 1998). Resistances to powdery mildew (*Blumeria graminis* f. sp. *tritici*) (Shi et al. 1998; Valkoun et al. 1982), septoria nodorum blotch (*Septoria nodorum*) (Ma and Hughes 1993) and eyespot (*Pseudocercosporella herpotrichoides*) (Cadle et al. 1997) have also been identified in einkorn.

Although einkorn has often been used in breeding programs to transfer genes for resistance to fungal pathogens to polyploid wheats, a number of insect pest resistances have also been identified. These include cereal leaf beetle (*Oulema melanopus*) (Kolarov 1988), Hessian fly (*Mayetiola destructor*) (El Bouhssini et al. 1998; Sharma et al. 1992) and various aphid species including Russian wheat aphid (*Diuraphis noxia*) (Potgieter et al. 1991; Quick et al. 1991), English grain aphid (*Sitobion avenae*) (Di Pietro et al. 1998; Sotherton and Lee 1988), rose-grain aphid (*Metopolophium dirhodum*) (Spiller and Llewellyn 1986; Sotherton and Lee 1988) and bird cherry-oat aphid (*Rhopalosiphum padi*) (Spiller and Llewellyn 1986).

Several valuable traits for abiotic stress resistance and bread-making quality have been identified in einkorn accessions. Most notably, heat tolerance, particularly during the grain-filling period (Khanna-Chopra and Viswanathan 1999), salt tolerance through the K+/Na+ discrimination trait (Gorham 1990; Gorham et al. 1991), complete resistance to the herbicide isoproturon at twice the recommended rate (Gill et al. 1986) and high grain protein and lysine contents (Vallega 1992; Waines et al. 1987). Additionally, D'Egidio et al. (1993) found that the carotene content of einkorn accessions was about three times higher than in polyploid wheats. High carotene content is favoured in semolina of durum and some wheat market classes (D'Egidio et al. 1993). High carotene content is also important in poultry feed to improve bird production performance and health and to enhance the quality of eggs and meat (Nabi et al. 2020), and in swine feed to increase sow immune status and litter weight (Chen et al. 2021).

2.4 Importance of root-lesion nematodes

Since Sher and Allen (1953) revised the genus *Pratylenchus*, it has attracted the attention of numerous researchers and many observations have been published on

distribution, damage, hosts, biology and control. The genus *Pratylenchus* now comprises around 70 nominal species (Castillo and Vovlas 2007), many of which cause characteristic lesions on roots of plant hosts, leading to the common name 'root-lesion nematode' (Corbett 1982). Their wide distribution and host range have contributed to their economic impact on agriculture, leading to them being considered the third most damaging group of phytoparasitic nematodes after root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp. and *Globodera* spp.) (Jones et al. 2013). However, in some cropping systems, like Australian rain-fed wheat (*Triticum aestivum*) systems, they can be the most economically important phytoparasitic nematode (Jones and Fosu-Nyarko 2014; Murray and Brennan 2009).

The RLNs *Pratylenchus thornei* and *P. neglectus* are migratory endoparasites that feed and reproduce in the cortex of crop roots, particularly cereals and pulses, and are found in all major Australian wheat-growing regions (Thompson et al. 2008; Vanstone et al. 2008). They are also widely distributed internationally and have been associated with crops from Asia (Bucki et al. 2020; Fatemah et al. 2012; Khan et al. 2010) Africa (Fourie et al. 2001; Mokrini et al. 2016), Europe (Castillo et al. 1995; Keil et al. 2009), North America (Koenning et al. 1999; Yu 2008) and South America (Aballay et al. 2009; da Luz 1982).

Pratylenchus thornei and *P. neglectus* have been reported to reduce the grain yields of intolerant wheat cultivars by up to 60% (Thompson et al. 2021) and 20% (Taylor et al. 1999), respectively. In the north-eastern Australian grain producing region, also known as the northern grains region, *P. thornei* or *P. neglectus* are present in at least 73% of fields. *Pratylenchus thornei* is the dominant species, occurring in 67% of fields compared with 32% for *P. neglectus*. Importantly, both species occur together in at least 26% of fields, which requires them to be managed simultaneously (Thompson et al. 2010).

2.5 Management of root-lesion nematodes

Without appropriate management, *P. thornei* and *P. neglectus* have the potential to cost the Australian wheat industry AU$591 M annually (wheat priced at AU$326/t; modified from Murray and Brennan 2009). The four main methods used to manage nematode populations are genetic tolerance and resistance, crop sequencing and other agronomic practices, and biological and chemical control.

2.5.1 Genetic tolerance and resistance

Tolerance and its opposite, intolerance, are used to describe the ability of the plant to withstand nematode infection. Tolerant cultivars grow and yield well when sown into soil with a high density of nematodes even though the nematode can multiply in the roots of the tolerant cultivar (Roberts 2002). The development of cultivars tolerant of *P. thornei* has been a long-standing objective of wheat breeding programs in the northern grains region (Thompson et al. 1999). Pelsart was the first wheat cultivar to be released that was specifically bred for tolerance to *P. thornei* (Brennan et al. 1994). Subsequently, many more *P. thornei*-tolerant cultivars have been released so that now 27 of the 57 (47%) wheat cultivars recommended for the northern grains region are at least moderately tolerant of *P. thornei* (GRDC 2021; Matthews et al. 2022). Notably, there are no reports of targeted breeding efforts to incorporate *P. neglectus* tolerance into Australian wheat cultivars. Consequently, only 14 (25%) wheat cultivars are at least moderately tolerant of *P. neglectus* and seven (12%) to both RLN. Although these cultivars have reduced yield losses caused by RLN, they have not necessarily reduced RLN populations in northern farming systems because the majority of them are at least moderately susceptible to *P. thornei* and/or *P. neglectus* (GRDC 2021; Matthews et al. 2022). Where the RLN population densities are maintained above the economic damage threshold, estimated as 2,000 *P. thornei*/kg of soil for wheat in the north-eastern grain-producing region of Australia (Thompson et al. 2010), subsequently grown intolerant crop cultivars are still at risk of substantial yield losses.

Resistance is the ability of a plant cultivar to retard nematode multiplication in its roots (Rhode 1972). A completely or highly resistant plant allows virtually no nematode reproduction. Partially or moderately resistant plants allow some intermediate amounts of reproduction. Susceptibility, the opposite of resistance, allows greater nematode reproduction to take place and the expression of any associated symptoms (Roberts 2002).

Resistance and tolerance can be under separate genetic control in some plant-nematode interactions (France and Brodie 1995, 1996) and therefore are not always coupled. Resistance may confer tolerance if it decreases the intensity of nematode attack (Trudgill 1991) in the resistant host compared to a susceptible host, allowing the resistant host to yield closer to its potential. In wheat, breeding lines that combined *P. thornei* tolerance and resistance have out-yielded tolerant commercial cultivars by up

to 17%, while reducing *P. thornei* populations (Thompson et al. 2001). Similarly, Excalibur, a commercial wheat cultivar that combined *P. neglectus* tolerance and resistance, out-yielded other commercial cultivars by up to 33% and produced lower final population densities when grown in *P. neglectus*-infested soil (Vanstone et al. 1998). Effectively, when resistance and tolerance are combined the crop is 'self-protected'. This combination presents the best opportunity to maximise yields while reducing RLN populations, regardless of the cropping system. Of the 59 wheat cultivars currently recommended for the northern grains region, no cultivars are classified as moderately resistant for *P. thornei* and only one (Coota) for *P. neglectus* (GRDC 2021; Matthews et al. 2022). The identification of additional *P. thornei* and *P. neglectus* resistance genes and their transfer to germplasm adapted to the northern grains region will facilitate the development of commercial wheat cultivars that minimise both yield loss and RLN population densities by combining tolerance and resistance.

2.5.2 Crop sequence

Crop sequences, including host-free fallow, are one of the oldest and most important approaches to control phytoparasitic nematodes in an integrated cropping system. The value of varying a crop sequence was often recognised long before its effect upon nematode populations and communities were considered (Nusbaum and Ferris 1973). Both *P. thornei* and *P. neglectus* can parasitise a wide range of economically important cereal, legume, cruciferous and tree crops (Castillo and Vovlas 2007; Fortuner 1977; Townshend and Anderson 1976) and survive extended fallow periods (Meagher 1970; Whish et al. 2017).

Recent research has shown that growing at least two consecutive resistant crops is critical in reducing RLN populations (Fanning et al. 2018; Owen et al. 2022). This strategy can also improve crop yields, for example, when the intolerant wheat cv. Strzelecki was grown after two susceptible crops, it yielded 62% less than when grown after two resistant crops (Owen et al. 2014). Similarly, the intolerant wheat cv. Timgalen produced its highest yield after long fallow and a *P. thornei*-resistant sorghum cultivar when compared with Timgalen grown in the three successive seasons (Thompson et al. 2012).

Importantly, resistance to one nematode species does not necessarily confer resistance to the other (Sheedy et al. 2022; Thompson and Seymour 2011). This can hinder the development of effective crop sequences to reduce RLN population densities, particularly when *P. thornei* and *P. neglectus* occur together in mixed populations. In this situation, crop genotypes that are resistant to both *P. thornei* and *P. neglectus* are essential to manage on-farm RLN population densities effectively.

2.5.3 Biological control

Biological control agents are organisms, commonly grouped as parasites and predators, which maintain nematode population densities at a lower average level than would occur in their absence (Stirling 1991). The most likely predators of phytoparasitic nematodes are predacious nematodes, mites, collembola and symphylans (Stirling 2014). Recent research has confirmed that while these organisms were able to feed on phytoparasitic nematodes they did not do so exclusively but were generally omnivorous (Cabos et al. 2013; Stirling 2014) and their diet may be partially or entirely organisms other than nematodes (Norton 1978).

Viruses, bacteria and fungi are common parasites of animals. Phytoparasitic nematodes have often been found to vector plant viruses (Holeva et al. 2006; Martin et al. 2009), however, there is limited data on viruses that infect nematodes. To date, soybean cyst nematode (*Heterodera glycines*) is the only phytoparasitic nematode in which nematode-associated viruses have been recovered (Bekal et al. 2014) but they were not nematicidal.

Bacteria are numerically the most abundant organisms in field soil (Siddique and Mahmood 1999). They are commonly found in the rhizosphere and several genera, including *Pasteuria* (Cho et al. 2005; Stirling 2014), *Bacillus* (Aballay et al. 2012; Rao et al. 2017) and *Pseudomonas* (Aballay et al. 2012; Khan et al. 2016) have been associated with the suppression of phytoparasitic nematode populations. *Pasteuria thornei* is the only species to be identified as a parasite of root-lesion nematodes (Sturhan et al. 2005) and its presence has not been reported as being associated with improved crop yields.

Nematophagous fungi have often reduced nematode populations in pot and small-scale field trials (as reviewed by Siddique and Mahmood 1996; Stirling 2014). However, the scarcity of commercial biocontrol agents suitable for broadacre cropping systems

has meant that the high level of control required in modern agriculture has not been consistently achieved on a field scale.

2.5.4 Chemical Control

The discovery of the nematicidal properties of DD (1,3-dichloropropene, 1,2-dichloropropane), ethylene dibromide (EDB) and DBCP (1,2-dibromo-3-chloropropane) during the 1940s and 1950s revolutionised the management of phytoparasitic nematodes. For the first time, nematicides became economically viable in many horticultural, vegetable and ornamental crops, leading to their use becoming standard practice. This continued in the 1960s with the development of systemic organophosphate and carbamate nematicides that controlled both nematodes and insects, leading to a belief that nematode problems in high-value crops could be solved largely with nematicides (Stirling 1991).

Extensive evaluation, particularly in lower-value field crops, has shown that nematicides, although generally effective, can be inconsistent in their control of nematode populations, particularly in dry seasons (Thompson et al. 2012a) or when populations are distributed deeper than 0.3 m in the soil profile (Doyle et al. 1987; Reen et al. 2014). This inconsistency coupled with high product prices, the need for specialised application equipment, continually restricted availability of these highly toxic compounds and risk of environmental contamination has rendered chemical control of phytoparasitic nematodes economically and socially unviable.

2.5.5 Value of effective root-lesion nematode management

These management approaches have been very effective in reducing the economic losses caused by *P. thornei* and *P. neglectus* and have been estimated to be worth AU$423 M annually to Australian wheat producers (wheat priced at AU$326/t; modified from Murray and Brennan 2009). Although, these management strategies have reduced losses from *P. thornei* and *P. neglectus* by 72%, those RLN still cost wheat growers AU$168 M annually. The most effective pathway to minimising the remaining losses is the development of commercial wheat cultivars that combine resistance and tolerance to both *P. thornei* and *P. neglectus*. The identification of novel resistances against *P. thornei* and *P. neglectus* and their incorporation into breeding lines suitable for use by commercial plant breeding programs, will facilitate that process.

2.6 Qualitative and quantitative genetics

Genetic variations are commonly described as qualitative or quantitative. Both categories follow the same laws of inheritance (Stoskopf et al. 1993). Qualitative genetic traits are controlled by a few genes, possibly one to three, that express major phenotypic effect and quantitative genetic traits are controlled by many genes whose individual effects on a trait are small in comparison with the total variation (Bos and Caligari 1995; Sleper and Poehlman 2006; Stoskopf et al. 1993).

Quantitative traits are controlled by many genes at various loci, resulting in a continuous distribution of phenotypic expression where variation can only be observed for groups or populations, but not for individuals, because the effect of individual genes is generally too small to be recognised (Sleper and Poehlman 2006; Stoskopf et al. 1993). Four types of gene action, namely additive, dominance, epistasis and overdominance, are recognised in the phenotypic expression of quantitative traits. Additive effects proportionally enhance the expression of a trait for each additional gene and are sufficiently stable to select superior genotypes. Dominance effects are deviations from additivity so that the heterozygote and one homozygote have equal effects. Epistatic effects are the result of the interaction of genes at different loci. Both effects are difficult to isolate and fix in superior genotypes. Overdominance effects occur when each allele contributes an effect but when combined the alleles have greater effect than additive gene action and are generally only expressed in F_1 hybrids (Sleper and Poehlman 2006).

Phenotypic variation can be partitioned into three components: genetic variation (V_g), environmental variation (V_e) and their interaction (V_{gxe}) (Stoskopf et al. 1993). Typically, the environment plays a minor role in the phenotypic expression of qualitative traits and given the clear dominance and recessive inheritance relationships, segregating generations can be grouped into a small number of distinct classes, unless there is dominance gene action (Sleper and Poehlman 2006; Stoskopf et al. 1993). Additionally, non-genetic variation (V_e) is truly continuous and can result in a trait phenotypically appearing to have a continuous distribution due to the increased variation within the classes. The expression of quantitative traits is more likely to be influenced by, and interact with, the environment. Therefore, the distinction between qualitative and quantitative traits often lies in the magnitude of their effects relative to other sources of variation (Falconer and Mackay 1996).

To distinguish between genetic and environmental effects, a generalised measure of heritability (h^2) can be calculated (Cullis et al. 2006). Heritability is the proportion of the observed variation in progeny that is inherited (Sleper and Poehlman 2006) and its most important function is expressing the reliability of the phenotypic value (Falconer and Mackay 1996). If the proportion of genetic variation is relatively high compared with environmental variation, as h^2 approaches 1, trait heritability will be high and selection will be more effective than when environmental variation is high (h^2 approaches 0) (Sleper and Poehlman 2006).

2.7 Sources of resistance to *Pratylenchus thornei* and *P. neglectus*

To date there have been no wheat accessions identified that have complete resistance to either *P. thornei* or *P. neglectus*. Consequently, Australian wheat-breeding programs use parents with varying levels of partial resistance. Many quantitative trait loci (QTL) associated with either *P. thornei* or *P. neglectus* resistance have been reported and are summarised in Table 1. GS50a has been the primary source of partial resistance to *P. thornei* and has been used in backcrossing programs with domestic wheat cultivars (Thompson et al. 1999). Early QTL analysis of GS50a-derived populations suggested *P. thornei*-resistance loci may be present on chromosomes 6B and 6D (Viccars et al. 1999), however, more recent studies conducted by the Australian Wheat and Barley Molecular Marker Program (AWBMMP) University of Adelaide http://www.markers.net.au/, have identified a major QTL only on chromosome 6D (D. Mather pers. comm.).

The only catalogued wheat gene (McIntosh et al. 2020) conferring resistance to *P. neglectus* is *Rlnn1*, which is located on chromosome 7A (Williams et al. 2002). It has been widely used in Australian wheat germplasm and effectively controls *P. neglectus* populations (Vanstone et al. 1998). *Rlnn1* can be readily detected using the kompetitive allele specific PCR (KASP) markers *uat128* and *uat129* (AWBMMP), however, this gene is strongly linked with yellow flour colour (Jayatilake et al. 2013). Generally, cultivars with white flour are selected in wheat breeding programs because yellow pigments are considered a detrimental quality factor for bread making (Zhang and Dubcovsky 2008). The *phytoene synthase 1* (*Psy1*) gene contributes to yellow flour colour variation in wheat, with the *Psy-A1t* ('very yellow') allele strongly influencing yellow flour colour in Australian germplasm (Crawford et al. 2011). Jayatilake et al. (2013) hypothesised that the linkage between *Psy-A1t* and *Rlnn1*

appeared unlikely to be broken because genotypes with *Rlnn1* resistance carried a chromosome rearrangement on 7AL that suppressed genetic recombination in that region. Identifying genotypes that carry alternative *P. neglectus* resistance QTL will be necessary to produce wheat genotypes that are resistant to *P. neglectus*, but do not carry the yellow flour colour quality defect.

Alternate sources of partial resistance to *P. thornei* have since been identified in wild relatives of wheat (Sheedy et al. 2012; Thompson and Haak 1997), Middle Eastern landraces (Sheedy and Thompson 2009; Thompson et al. 2009) and synthetic hexaploids (Ogbonnaya et al. 2008; Thompson 2008). Importantly, several of the synthetic hexaploids reported to be partially resistant to *P. thornei* were also partially resistant to *P. neglectus*. These are the only wheat genotypes reported to be resistant to both RLN species.

Genetic analysis of the International Triticeae Mapping Initiative (ITMI) population found that *P. thornei* resistance was controlled by a few loci with relatively large effects. Two QTL that were located on chromosomes 2BS and 6DS were detected and explained up to 19% and 23% of phenotypic variation, respectively (Zwart et al. 2006). Doubled-haploid (DH) populations derived from several of the accessions from the aforementioned collections have also undergone genetic and QTL analysis. Evaluation of five populations derived from resistant synthetic hexaploid wheats crossed with the susceptible wheat cultivar Janz has determined that their *P. thornei* resistance was polygenic, controlled by three to six genes, and additive (Thompson et al. 2012b) with all synthetic hexaploid lines, but particularly CPI133872, having better general combing ability than GS50a (Zwart et al. 2004). Genetic analyses of the CPI133872/Janz DH population identified major QTL for *P. thornei* resistance on chromosomes 2B (*QRlnt.lrc-2B*) and 6D (*QRlnt.lrc-6D.1*; *QRlnt.lrc-6D.2*) (Zwart et al. 2005; 2010). Genetic analyses of other populations (Kumar et al. 2021; Linsell et al. 2014; Rahman et al. 2019; Schmidt et al. 2005; Toktay et al. 2006; Zwart et al. 2006) have also identified QTL that were considered similar to those reported by Zwart et al. (2005; 2010). A QTL associated with *P. neglectus* resistance was also identified on chromosome 2B (*QRlnn.lrc-2B*) of CPI133872 and was closely linked with the *P. thornei* resistance QTL *QRlnt.lrc-2B*.

Similarly, studies of *P. thornei*-resistant West Asia and North Africa (WANA) wheats crossed with Janz have found that the minimum number of effective *P. thornei*-

resistance genes ranged from three to six for four WANA wheats and two for GS50a (Thompson and Seymour 2011). QTL analyses of the landrace-derived populations have identified novel putative *P. thornei*-resistance loci on chromosome 3B and a susceptibility locus on 1B (Schmidt et al. 2005) and *P. thornei*-resistance loci on chromosomes 2B, 7B and 6D (Thompson et al. 2015). A Genome-wide association study of 126 wheat breeding lines developed by the International Maize and Wheat Improvement Center (CIMMYT) was used to identify novel single nucleotide polymorphisms (SNPs) associations with *P. thornei* resistance on chromosomes 1D, 2A and 5B and SNPs associated with *P. neglectus* resistance on chromosomes 1A, 1B, 3A, 3B, 6B, 7A and 7D. The 1D and 3B SNPs explained 10.8% and 10.2% of the phenotypic variation, respectively, of their traits with the remaining SNPs each explaining 4.0% to 5.7% of the phenotypic variation (Dababat et al. 2016). Evaluation of 143 Indian wheat genotypes using four GWAS methods identified seven novel SNP associations with *P. thornei* resistance on chromosomes 1B (two SNPs), 1D (two SNPs), 5A, 6B and 7A. Only two of the SNPs, one each on chromosomes 1B and 1D, were associated with *P. thornei* resistance by two or more of the GWAS methods (Kumar et al. 2021). Further associations with *P. neglectus* resistance have been identified using a GWAS approach in a synthetic hexaploid wheat collection by Mulki et al. (2013), who reported novel SNP associations on chromosomes 4A, 5B and 7B that explained between 4 and 5% of the phenotypic variation.

Virtually all of the reported major QTL for *P. thornei* resistance have been identified on the B and D-genomes. That is probably not surprising given that evaluation of 21 accessions of *A. speltoides* and 244 *A. tauschii* accessions for *P. thornei* resistance has found that 52% (Sheedy et al. 2012) and 16% (Thompson and Haak 1997), respectively, were at least moderately resistant. Interestingly, evaluation of 21 accessions of *T. urartu* and 23 accessions of *T. monococcum* for *P. thornei* resistance found 24% and 43% of accessions of the A-genome diploids, respectively, were at least moderately resistant (Sheedy et al. 2012). Since the proportion of *P. thornei* resistant accessions of species that can substitute chromosomes on the B- and D-genomes of wheat is comparable with that of the A-genome relatives of wheat, it is curious that major QTL for *P. thornei* resistance on the A-genome have not been detected in the polyploid wheats.

Table 1. Previously reported quantitative trait loci (QTL) associated with resistance or susceptibility to the root-lesion nematodes *Pratylenchus thornei* and *P. neglectus*. Cultivars contributing resistance QTL are bolded. QTL associated with susceptibility are denoted by (S).

Population	**RLN**	**Chromosomal Location**	**Ref**
ABL-derived			
GS50a/Janz DH	Pt	6D	1
GS50a/Janz F3; **GS50a**/Batavia DH	Pt	6B 6D	2
Tammin/**Excalibur** DH	Pn	7A	3
Excalibur/Kukri DH	Pn	7A	4
Indian wheat GWAS	Pt	1B x 2 1D 2B 3B 5A 6B 7A	8
CIMMYT ABL GWAS	Pt	1D 2A 5B	12
CIMMYT ABL GWAS	Pn	1A 1B 3A 3B 6B 7A 7D	12
Landrace-derived			
Morocco426/Janz DH	Pt	2B 3B	9
Morocco426/Janz DH	Pt	2B 7B	11
Iraq43/Janz DH	Pt	3B	9
Iraq43/Janz DH	Pt	6D 7B	11
Iraq43/**Janz** DH	Pt	1B (S)	9
AUS49307.2/Pastor RIL	Pt	1B 2B 6D	10
SHW-derived			
W-7984/Opata RILs (ITMI population)	Pt	2B 6D	5
CPI133872/Janz DH	Pt	6D	6
CPI133872/Janz DH	Pn	4D 6D	6

CPI133872/Janz DH	Pt			2B							6D x 2		7
CPI133872/Janz DH	Pn			2B							6D		7
Croc_1/Ae.sq224//Opata x Pastor RIL	Pt					3B							10
CIMMYT SHW GWAS	Pn						4A		5B			7B	13
Sokoll/Krichauff DH	Pt	2A	2B x 3		2D					5D	6D x 2		14
Sokoll-derived RIL	Pt			2B							6D		15

Note: ABL = Advanced breeding line (includes released cultivars); DH = double haploid; GWAS = Genome-wide association study; RIL = Recombinant inbred line; RLN = Root-lesion nematode; Pn = *Pratylenchus neglectus*; Pt = *Pratylenchus thornei*; SHW = Synthetic hexaploid wheat. References: 1. D. Mather pers comm 2013; 2. Viccars et al. 1999; 3. Williams et al. 2002; 4. Jayatilake et al. 2013; 5. Zwart et al. 2006; 6. Zwart et al. 2005; 7. Zwart et al. 2010; 8. Kumar et al. 2021; 9. Schmidt et al. 2005; 10. Toktay et al. 2006; 11. Thompson et al. 2015; 12. Dababat et al. 2016; 13.Mulki et al. 2013; 14. Linsell et al. 2014; 15. Rahman et al. 2019.

2.8 Transfer of *Pratylenchus thornei* resistance via interspecific hybridisation

Despite *T. monococcum* belonging to the secondary gene pool of wheat (Harlan and de Wet 1971), gene transfer can be achieved by direct hybridization with adapted durum (*Triticum turgidum* ssp. *durum*) and/or wheat mutants that allow homoeologous chromosome pairing. Several authors report that crosses between *T. monococcum* and common or durum wheats produced viable F_1 hybrids that were male sterile and partially female sterile (Johnson and Dhaliwal 1976; Ma and Hughes 1993). F_1 hybrids produced using a durum parent generally recovered full fertility after the first backcross (Valkoun 2001), whereas F_1 hybrids derived from wheat generally required several additional backcrosses before the progeny were self-fertile (Singh and Sharma 1997; Schmolke et al. 2012). Suppression of the resistance gene(s) or dilution of its products may result in a reduction of expressed disease resistance when transferred from a species of a lower level of ploidy to one of a higher level (Cox 1991; Gill et al. 1986; Kerber and Green 1980; Potgieter et al. 1991). Nonetheless, many genes conferring effective levels of resistance to diseases and pests have been transferred using direct hybridisation (Cox 1991; Cox 1998; Friebe et al. 1996), including cereal cyst nematode resistance from *T. monococcum* to durum and wheat cultivars (Singh et al. 2010).

CHAPTER 3: PAPER 1 – INTROGRESSION INTO ADVANCED BREEDING LINES OF NOVEL ROOT-LESION NEMATODE (*Pratylenchus thornei*) RESISTANCE QTL IDENTIFIED IN A GENOME-WIDE ASSOCIATION STUDY OF IRANIAN LANDRACE WHEATS (*Triticum aestivum*).

Introgression into advanced breeding lines of novel root-lesion nematode (*Pratylenchus thornei*) resistance QTL identified in a genome-wide association study of Iranian landrace wheats (*Triticum aestivum*). Jason G Sheedy, Raj K Pasam, Matthew J Hayden, Kerrie L Forrest and John P Thompson. This chapter was prepared according to the instructions to authors given by the journal *Molecular Breeding*.

For this investigation, a genome wide association study was performed using *P. thornei* resistance data collected from 245 ILWs to identify resistance QTL. Those QTL were then validated in ILW-derived BC_1F_4 breeding populations and used to examine the relationship between the number of QTL per accession and *P. thornei* reproduction factor. Advanced breeding lines were produced with novel QTL combinations conferring superior resistance to *P. thornei* compared with current commercial cultivars and which are suitable for use by commercial plant breeding programs.

Supplementary information referenced in this chapter is documented in Appendix A.

Introgression into advanced breeding lines of novel root-lesion nematode (*Pratylenchus thornei*) resistance QTL identified in a genome-wide association study of Iranian landrace wheats (*Triticum aestivum*).

Jason G Sheedy[AC], Raj K Pasam[B], Matthew J Hayden[B], Kerrie L Forrest[B], John P Thompson[A]

[A] University of Southern Queensland, Centre for Crop Health, Toowoomba, QLD, Australia

[B] Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, VIC, Australia

[C] Corresponding author jason.sheedy@usq.edu.au

Telephone: +61 7 46311185

ORCID ID

JG Sheedy: 0000-0001-6569-5016

RK Pasam: 0000-0003-0908-1882

MJ Hayden: 0000-0003-3117-5500

KL Forrest: 0000-0001-6529-8678

JP Thompson: 0000-0002-2097-5695

Abstract

The root-lesion nematode *Pratylenchus thornei* is widely distributed in global grain producing regions and can reduce the yield of intolerant wheat cultivars by up to 65%. The incorporation of *P. thornei* tolerance and resistance into wheat cultivars has proven the most effective strategy to minimise these losses. A collection of 245 Iranian landrace wheats (ILW) was characterised for resistance to *P. thornei* in two glasshouse experiments. Six *P. thornei*-resistant landraces were selected and crossed with wheat cultivars adapted to the Australian northern grain region to produce seven BC_1 populations. A genome-wide association study (GWAS) of the ILW identified eight putative quantitative trait loci (QTL) on the B- and D-genomes that were associated with *P. thornei* resistance, five of which (2B [x2], 3B, 5B and 7B) were novel. Ten kompetitive allele specific PCR (KASP) markers for SNPs were validated on parental genotypes and ILW-derived BC_1F_4 populations. Selection lines from six of the populations combined up to three *P. thornei*-resistance QTL. One population, carrying only the chromosome 1B susceptibility QTL, produced the highest final *P. thornei* population densities. These ILW are a valuable but largely untapped source of genetic diversity. The five novel QTL for *P. thornei* resistance offer the opportunity both to increase diversity in the genetic management of *P. thornei* and to develop new gene combinations to increase the overall level of resistance available to wheat improvement programs.

Key Words

Iranian landrace wheat; Root-lesion nematode; *Pratylenchus thornei*; Genome-wide association study; GWAS; Breeding for resistance; Genetic diversity; Crop improvement.

Introduction

Phytoparasitic nematodes have been estimated to cost global agriculture 80 to 118 billion USD annually (Bernard et al. 2017). Of these, root-lesion nematodes (RLN; *Pratylenchus* spp.) are the third most damaging genus behind root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp. and *Globodera* spp.) (Castillo and Volvas 2007). For cereal production, *Pratylenchus thornei* is the most damaging of the RLN globally and it can reduce yield of intolerant wheat (*Triticum aestivum*) cultivars by as much as 65% (May et al. 2016; Nicol and Ortiz-Monasterio 2004; Thompson et al. 1999). In addition to its pathogenicity, *P. thornei* has a cosmopolitan distribution and has been reported in cereal and legume crops in major wheat-growing regions in Australia (Thompson et al. 2008; Vanstone et al. 2008), Europe (Castillo et al. 1995; Keil et al. 2009), West Asia and North Africa (WANA) (Fatemah et al. 2012; Greco et al. 1988; Mokrini et al. 2016), South Asia (Mishra and Gupta 1988; Subramaniyan and Sivakumar 1991) and North America (Koenning et al. 1999; Nicol and Ortiz-Monasterio 2004; Yu 1997).

Wheat is a crop of global importance. More than 730 million tonnes are produced annually on a land area greater than any other commercial crop (FAO 2021) and its global trading is greater than for all other crops combined (Curtis 2002). Wheat is often considered as a primary source of dietary carbohydrate, however, it is also a source of fibre, vitamins and minerals (Shewry and Hey 2015), and supplies 20% of the daily protein for 4.5 billion people (Lucas 2012). By 2050, the demand for wheat is expected to rise by 60%. To meet this demand, annual wheat yield increases must rise from the current <1% to at least 1.6%. Increased resistance and/or tolerance to biotic and abiotic stresses, improved input use efficiency and agronomic practices are the most likely avenues to achieve the necessary increase in production (Lucas 2012).

Wheat breeding lines that combined *P. thornei* tolerance, the ability of a plant to grow and yield well under high nematode pressure, and resistance, the ability of a plant to restrict nematode multiplication, have out-yielded tolerant commercial cultivars by up to 17%, while limiting *P. thornei* population development (Thompson et al. 2001). Effectively, when resistance and tolerance are combined the crop is 'self-protected' against these pests. This combination presents the best opportunity to maximise yields while reducing *P. thornei* populations, regardless of the cropping system. Therefore, the identification of novel *P. thornei*-resistance genes and their transfer to germplasm suitable for use by plant breeders will facilitate the development of commercial wheat cultivars that minimise both yield loss and *P. thornei* populations by combining tolerance and resistance.

Wheat is an allohexaploid (6x; 2n=42) composed of three related genomes (A, B and D) that were originally derived from three diploid (2x; 2n=14) species within the tribe *Triticeae*. The plant species donors of these respective genomes are considered to be (i) *Triticum urartu* (A-genome), (ii) a currently unidentified (or extinct) species closely related to *Aegilops speltoides* (S-genome that can substitute for chromosomes on the B-genome), and (iii) *A. tauschii* (D-genome) (Marcussen et al. 2014). Marker-assisted selection (MAS) is one of the preferred methods of introducing new traits into a breeding program. For this approach to be possible, genetic markers closely-linked to the trait need to be identified. Traditionally, quantitative trait loci (QTL) linked to traits of interest have been identified in biparental recombinant inbred line (RIL) or doubled-haploid (DH) populations and then validated in breeding populations or other biparental populations.

A genome-wide association study (GWAS) provides the opportunity to streamline this process by detecting associations between genetic variants and traits in samples from diverse existing populations (Visscher et al. 2017) that are assessed in the trait discovery phase of germplasm improvement. The data may be collected specifically for a GWAS, or as in the

93 case of this research, a GWAS can add value to existing phenotypic data. This is particularly
94 useful where resistant accessions identified during the phenotyping process were used to
95 produce advanced breeding lines. High-density single nucleotide polymorphism (SNP) data
96 are widely used to detect marker–trait associations in QTL mapping experiments and GWAS
97 (Wang et al. 2014). Population-based association analysis using linear mixed models (LMM)
98 is a common approach to analysing SNP data, primarily due to its ability to prevent false
99 positive associations which arise due to inherent population structure in natural populations
100 (Yang et al. 2014). Bayesian models have the advantage of fitting markers simultaneously
101 into the model when estimating marker effects to increase resolution when mapping causal
102 SNPs associated with traits of interest. They also allow the inclusion of all SNPs in the model
103 to eliminate the need to fit a separate covariate matrix (Pasam et al. 2017). Despite the
104 differences in model assumptions between LMM and Bayesian multilocus (BayesR) models,
105 both approaches were shown to give similar results when identifying QTL for disease
106 resistance in wheat (Pasam et al. 2017). Given that LMM and BayesR have different
107 underlying statistical assumptions, comparison of their outputs facilitates the identification of
108 loci stably associated with the target trait(s) and consistent candidates for validation.

109 During the last two decades, GWAS was initially used to identify disease susceptibility genes
110 in humans and has now been applied to other fields, particularly agrigenomics (Scherer and
111 Christensen 2016). To identify resistance to phytoparasitic nematodes in wheat, GWAS has
112 been applied to a collection of synthetic hexaploid wheat (Mulki et al. 2013), breeding lines
113 produced by the International Wheat and Maize Improvement Centre (CIMMYT, Mexico)
114 (Dababat et al. 2016), and Indian wheat genotypes (Kumar et al. 2021) where novel QTL
115 associated with resistance to RLN (*P. thornei* and/or *P. neglectus*) and cereal cyst nematode
116 (CCN; *H. avenae*) were reported.

117 Landraces harbour unique and greater allelic diversity than modern highly selected crop
118 cultivars that can be easily introgressed into elite germplasm. Landraces have been
119 successfully used to identify QTL for abiotic and biotic stress tolerance (Vikram et al. 2019)
120 in wheat and in other crops. There have been no reports of studies assessing Iranian landrace
121 wheat (ILW) collections for novel resistance QTL to RLN. Given that ILW have substantial
122 genetic diversity, are genetically diverse from breeding lines (Alipour et al. 2017) and have
123 phenotypic resistance to *P. thornei* (Sheedy and Thompson 2009), they are a likely source of
124 novel *P. thornei* resistance QTL of value to wheat improvement programs.

125 Virtually all of the reported major QTL for *P. thornei* resistance in wheat have been
126 identified on the B and D-genomes. Early QTL analysis of Gatcher selection 50a (GS50a), an
127 important source of partial resistance to *P. thornei* identified in Australia and subsequently
128 used in domestic and international breeding programs (Thompson et al. 1999), suggested *P.*
129 *thornei*-resistance loci may be present on 6B and 6D (Viccars et al. 1999). However, recent
130 studies conducted by the Australian Wheat and Barley Molecular Marker Project
131 (AWBMMP, University of Adelaide, http://www.markers.net.au/) have identified a major
132 QTL only on chromosome 6D in GS50a. Genetic analysis of the International Triticeae
133 Mapping Initiative (ITMI) population detected two QTL on chromosomes 2B and 6D
134 contributed by the synthetic parent (Zwart et al. 2006), as did studies on two other synthetic
135 hexaploid-derived DH populations (Linsell et al. 2014; Zwart et al. 2005; 2010) and a
136 landrace-derived population (Toktay et al. 2006). Toktay et al. (2006) also reported a
137 synthetic hexaploid-derived *P. thornei*-resistance QTL located on chromosome 3B. Studies of
138 DH populations derived from *P. thornei*-resistant landrace wheats from Morocco and Iraq
139 initially identified putative *P. thornei*-resistance loci on chromosomes 2B, 3B and 6D and a
140 susceptibility locus on 1B (Schmidt et al. 2005) but subsequent higher density mapping
141 identified *P. thornei*-resistance loci on chromosomes 2B, 7B and 6D (Thompson et al. 2015).

Recent GWAS of CIMMYT advanced breeding lines identified novel QTL associated with *P. thornei* resistance on chromosomes 1D, 2A and 5B (Dababat et al. 2016) and of Indian wheat genotypes on 1D, 1B and 2B (Kumar et al. 2021).

The purpose of this study was to analyse *P. thornei*-resistance data from a collection of 245 ILW (Sheedy and Thompson 2009) in a GWAS framework, using LMM and BayesR methods, to identify novel QTL associated with resistance. The five putative QTL were then validated using seven ILW-derived backcross populations (BC_1F_4) developed to deliver novel *P. thornei* resistance genes to wheat improvement programs.

Materials and methods

Germplasm

A set of 245 landrace wheats originating from 14 provinces of Iran (Fig. 1) selected for spring growth habit from a larger collection held by CIMMYT (Vikram et al. 2019) were used in this GWAS. From that set, six *P. thornei*-resistant genotypes (AUS28369, AUS28372, AUS28451, AUS28470, AUS28647, AUS28677) (Sheedy and Thompson 2009) were crossed with the Australian commercial wheat cultivars Gregory and/or Wylie to produce seven backcross (BC_1) populations. Both recurrent parents have been characterised as moderately susceptible to susceptible (MSS) and moderately tolerant to tolerant (TMT) to *P. thornei* (Thompson et al. 2020; Thompson et al. 2021).

Prior to this study, the BC_1F_4 populations were selected phenotypically for resistance to *P. thornei* in glasshouse assays in the BC_1F_1 and BC_1F_2 generations and further selected in the BC_1F_3 generation for *P. thornei* tolerance. Tolerance was measured as grain yield (Thompson et al. 2021) when grown on a rain-fed field site near Formartin, Australia (27.4676S, 151.42554E) with a damaging *P. thornei* population density (>2000 *P. thornei*/kg soil). In

each generation, resistant or tolerant single plant selections (SPS) were progressed for evaluation in the subsequent generation.

In the BC_1F_4 generation, 16 to 17 lines per SPS progressed from the BC_1F_3 generation were evaluated for resistance to *P. thornei* in glasshouse assays. Advanced breeding lines selected from these populations were characterised twice in replicated glasshouse resistance experiments using the procedures reported by Thompson et al. (2020).

Pratylenchus thornei **resistance phenotyping**

The 245 ILW were phenotyped in each of two years by Sheedy and Thompson (2009) and the reproduction factor (RF; final *P. thornei* population [P_f] ÷ initial *P. thornei* population [P_i]) data were reanalysed using the GWAS framework.

The ILW-derived BC_1F_4 populations were assessed for resistance to *P. thornei* in two experiments grown in glasshouses located at the Queensland Department of Agriculture and Fisheries Leslie Research Facility (27.534°S, 151.936°E) in Toowoomba, Australia. Experiment 1 tested four ILW-derived populations along with 17 standard cultivars ranging from resistant to very susceptible and an inoculated unplanted treatment (data not presented), while Experiment 2 tested three ILW-derived populations along with 19 standard cultivars and an inoculated unplanted treatment (data not presented). The entries were grown in three (Experiment 1) or five (Experiment 2) randomised blocks with each block comprising a complete set of standard cultivars and an equal fraction of the total number of segregating individuals being characterised.

For both experiments, plants were grown in 1.4 L (150 mm-diameter) plastic pots suitable for bottom watering (P140ECOX; Garden City Plastics, Brisbane, Australia) containing 850 g (oven-dry equivalent) of a vertosolic soil of the Irving clay soil association (Thompson and

190 Beckman 1959). The soil had been sieved to remove aggregates >1 cm and then pasteurised
191 using aerated-steam at 85°C for 40 min (modified from Thompson 1990) and fertilised with
192 2.5 g of Osmocote® Plus Trace Elements Landscape Formula (21:2:6 NPK) slow-release
193 fertiliser (Scotts Australia Pty Ltd., Baulkham Hills, Australia). The experiments were
194 conducted on benches fitted with a bottom-watering system regulated by a float valve set to a
195 water tension of 2 cm. Two seeds of each cultivar, or single seeds from segregating
196 populations, were placed on a base layer of soil (70% of total soil weight). In Experiment 1,
197 each pot was inoculated with 4,250 each of *P. thornei* and *P. neglectus*. The inocula were
198 applied separately in 10 mL water suspensions. In Experiment 2, *P. thornei* alone were
199 applied in a 10 mL water suspension at the rate of 4,250 per pot. After inoculation, the
200 remaining soil (30% of total soil weight) was placed over the seed. Excess plants were
201 removed after emergence by cutting below the seed with a scalpel (leaving the roots behind)
202 to leave one plant per pot. Soil and air temperatures were maintained between 20 and 25°C,
203 the optimum temperature for *P. thornei* reproduction (Thompson et al. 2014), by under-bench
204 heating and by using shade cloth (as required) and evaporative coolers. Plants were sprayed
205 as required with 1 mL/L of Tilt® 250 EC (250 g/L Propiconazole; Syngenta Australia, North
206 Ryde, Australia) to control powdery mildew and with 0.5 g/L of Pirimor WG Aphicide® (500
207 g/kg pirimicarb; Syngenta Australia, North Ryde, Australia) to control aphids.

208 Growth stage (Zadoks et al. 1974), tiller number and plant height were recorded at harvest
209 after 16 weeks of plant growth. Resistance was determined by counting the final number of
210 *Pratylenchus* spp. in the roots and soil of each cultivar. The soil and roots from each pot were
211 thoroughly mixed and the roots cut into ~1 cm lengths. A 100 g subsample of the processed
212 soil and roots was dried at 105°C for 48 h in a forced-draught oven to determine gravimetric
213 moisture content. *Pratylenchus* spp. were extracted from a 150 g subsample at 22°C for 48 h
214 using the Whitehead tray method (Whitehead and Hemming 1965) and nematodes were

collected on a 20-µm aperture sieve. Samples were stored in 30 mL vials at 4°C. Nematodes extracted from soil and roots were counted once using a 1 mL capacity 24-cell nematode counting slide (Chalex Corporation, Portland, USA) under a compound microscope (x40). In Experiment 1, *P. thornei* and *P. neglectus* adults were differentiated morphometrically (Townshend and Anderson 1976; Fortuner 1977) and juveniles were allocated in the proportions observed for the adults.

Nematode population density (number/kg oven-dry soil) was calculated and the data statistically analysed for each individual experiment using a linear mixed model framework. A natural log transformation was applied to the population density to ensure homoscedastic variance over the range of fitted values. The overall experiment mean and crop type were fitted as fixed effects, while terms for replicate and potential spatial variation using an AR1 by AR1 structure (AR1 = autoregressive structure of order 1) were fitted as random. The main effect of genotype was modelled as random and in order to incorporate the pedigree information of the BC_1F_4 lines, the genotype effects were partitioned into additive and non-additive effects (Oakey et al. 2007). The mixed model was fitted using the ASReml-R package (Butler et al. 2017) in R (R Core Team 2017), where all variance components were estimated using residual maximum likelihood (REML) and the best linear unbiased predictions (BLUPs) were obtained.

DNA extraction and genotyping

Genomic DNA was extracted from two crushed seeds of the ILW and from leaf material of the BC_1F_4 lines using the cety trimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al. 1984). The 245 ILW were genotyped using the Illumina Infinium iSelect 90k wheat SNP array following the protocol of Wang et al. (2014). SNPs with >10% missing

values were excluded. Beagle version 5.0 (Browning et al. 2018) was used for imputing missing values for the remaining SNPs. SNPs with a minor allele frequency of <0.03 were excluded from the analysis. The final dataset comprised 245 lines with 25,084 genotyped SNPs.

Linkage disequilibrium

HaploView (Barrett 2009) was used to calculate the extent of linkage between markers from the squared allele frequency correlation (r^2) estimates and to visualise linkage disequilibrium (LD) patterns. SNPs having LD r^2>0.25 were considered linked and corresponding to the same QTL region.

Genome-wide association analysis

The kinship matrix was calculated using the VanRaden method (VanRaden 2008) implemented in GAPIT (Lipka et al. 2012). A principal components analysis (PCA) was conducted with a data matrix 25,084 SNPs for the 245 wheat landraces. The individual landraces were plotted on their scores on principal components 1 and 2 and identified with symbols for province of origin to examine any relationships. The relationship of RF to the first two principal components was also investigated by regression analysis.

Genome-wide association mapping was conducted according to the two-model procedure reported by Pasam et al. (2017) to determine significant marker trait associations. Firstly, a LMM with PCA and kinship matrix as correction for population structure was implemented to identify the significantly associated SNPs based on *P*-values. Both PCA and GWAS with LMM were performed using the R Package GAPIT (Lipka et al. 2012). Secondly, the BayesR method (Moser et al. 2015) was also used to compute marker trait associations and

identify SNPs contributing to a significant effect on the trait. Marker-trait associations were initially established using a relaxed significance threshold of $P \geq 0.001$ in the LMM and for BayesR, the 0.2% of SNPs with the largest effects were considered significant (Pasam et al. 2017). SNPs that were significant across the two approaches were selected for further investigation. Markers around these SNPs with a significant *P*-value <0.001 in the LMM approach and that were in LD with the significant BayesR SNPs, were reported as flanking SNPs. The LD between the markers was used to confirm that QTL mapped on the same chromosomes were segregating independently in the population.

Subsequently, KASP primers were obtained for 16 of the SNPs that correspond to the flanking marker positions of the eight QTL, either from the PolyMarker website (http://www.polymarker.info/designed_primers), the CerealsDB website (https://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/), or designed manually using NetPrimer (https://www.premierbiosoft.com/netprimer/). Additionally, KASP markers were obtained from literature for eight SNPs that have been reported previously, namely *Kukri_rep_c117487_334* (2B) and *Kukri_rep_c105352_281* (6D) (Linsell et al. 2014) and *BS00002660* (2B), *BS00023068* (2B), *Uat6* (7B), *Uat7* (7B), *Uat8* (7B) and *Uat14* (6D) (AWBMMP, University of Adelaide, http://www.markers.net.au/). All 24 SNP loci were assayed on the wheat cultivars Bobwhite, Gregory, Wylie, resistant landrace parents (AUS28369, AUS28372, AUS28451, AUS28470, AUS28645 and AUS28677) and BC_1F_4 selection lines. Flanking SNPs that showed significant *P*-values in LMM were used to determine the QTL interval. From these 24 KASP markers, 13 were polymorphic and 11 markers were monomorphic in the selection lines and parents. A list of KASP markers and their corresponding loci information is provided in Accessory Table 1.

Results

288 **Population structure of ILW**

289 Principal components analysis with SNPs showed no prominent relationships among the
290 landraces. The amount of genetic variation explained by the first and second principal
291 components was 27 and 13%, respectively. No clear clustering by resistance level or
292 geographic origin was observed.

293

294 **Association Mapping**

295 Based on the LMM analysis, a total of 55 SNPs showed associations above the significance
296 threshold ($P<0.001$) (Fig 2a) and in the BayesR analysis, 24 SNPs were considered
297 significant (Fig 2b). The two-model procedure (Pasam et al. 2017) identified 11 SNPs that
298 were significant across both approaches (Table 1). In addition, 27 flanking markers were
299 linked to these SNPs (Table 1). Seven QTL for *P. thornei* resistance and one for *P. thornei*
300 susceptibility were identified based on genetic mapping positions and LD values (Fig. 3). The
301 susceptibility QTL was located on chromosome 1B (*QRlnt.usq-1B.1*), three of the resistance
302 QTL were located on chromosome 2B (*QRlnt.usq-2B.1*, *QRlnt.usq-2B.2* and *QRlnt.usq-2B.3*)
303 and one each on chromosomes 3B (*QRlnt.usq-3B.1*), 5B (*QRlnt.usq-5B.1*), 6D (*QRlnt.usq-*
304 *6D.1*) and 7B (*QRlnt.usq-7B.1*). These QTL were then compared with eight known markers
305 for *P. thornei* resistance (Linsell et al. 2014; Schmidt et al. 2005; Toktay et al. 2006; Zwart et
306 al. 2010). Two *P. thornei*-resistance QTL, located on chromosomes 2B (*QRlnt.usq-2B.1*) and
307 6D (*QRlnt.usq-6D.1*), and the *P. thornei*-susceptibility QTL located on chromosome 1B
308 (*QRlnt.usq-1B.1*), corresponded to previously published QTL. The five remaining *P. thornei*-
309 resistance QTL, *QRlnt.usq-2B.2*, *QRlnt.usq-2B.3*, *QRlnt.usq-3B.1*, *QRlnt.usq-5B.1* and
310 *QRlnt.usq-7B.1*, have not been previously reported and were considered novel. There were

two significant positive epistatic interactions among these QTL, with *QRlnt.usq-2B.2* and *QRlnt.usq-3B.1* both epistatic with *QRlnt.usq-5B.1* (data not shown).

Presence of QTL among ILW accessions

The number of *P. thornei* resistance QTL detected per accession in this collection ranged from one to six. Notably, average reproduction factor of the accessions decreased exponentially as the number of *P. thornei* resistance QTL per accession increased, indicating that the *P. thornei* resistance in this collection was polygenic with dose-dependent gene action (Fig. 4).

Geographic distribution of QTL

The eight QTL identified in this collection varied in their frequency of distribution among the provinces of Iran (Table 2). *QRlnt.usq-6D.1* was the most common *P. thornei*-resistance QTL, occurring in 88% of accessions and in every province. *QRlnt.usq-2B.1*, *QRlnt.usq-2B.2*, *QRlnt.usq-2B.3* and *QRlnt.usq-3B.1* were also found in all provinces and in 59% to 87% of accessions. *QRlnt.usq-7B.1* was less common, occurring in 41% of accessions, while *QRlnt.usq-5B.1* was the rarest QTL, only occurring in 14% of accessions across five provinces. Consequently, the *QRlnt.usq-2B.2* and *QRlnt.usq-3B.1* epistatic interactions with *QRlnt.usq-5B.1* were also limited to the same five provinces with only 32 (13%) and 33 (13%) accessions carrying them, respectively. A total of 32 accessions (13%) carried both pairs of epistatic QTL. The QTL that conferred susceptibility, *QRlnt.usq-1B.1*, was found in all provinces and in 34% of accessions.

QTL validation in ILW-derived BC_1F_4 populations

Four ILW-derived BC_1F_4 populations were phenotyped in Experiment 1 and three in Experiment 2. Subsequently, the seven populations were evaluated for the presence of the QTL identified in this and other studies (Table 3). All seven populations had unique QTL combinations with six of the seven carrying QTL for *P. thornei* resistance and the AUS28451/Gregory//Wylie population carrying only *QRlnt.usq-1B.1*, which conferred *P. thornei* susceptibility. Individually, *QRlnt.usq-6D.*1 and *QRlnt.usq-1B.1* were the most frequently recovered QTL, being found in four and three of the seven populations, respectively. *QRlnt.usq-2B.1*, *QRlnt.usq-2B.2* and *QRlnt.usq-7B.1* were each recovered in two populations and *QRlnt.usq-2B.3*, *QRlnt.usq-3B.1* and *QRlnt.usq-5B.1* were only recovered in one population each. Cumulatively, the three QTL located on chromosome 2B were recovered in five of the seven populations, indicating the importance of this region when breeding for *P. thornei* resistance. No BC_1F_4 lines carrying the pairs of QTL (*QRlnt.usq-2B.2*/*QRlnt.usq-5B.1* or *QRlnt.usq-3B.1*/*QRlnt.usq-5B.1*) that produced the positive epistatic interactions for *P. thornei* resistance were recovered.

Development of advanced breeding lines

Twenty-two *P. thornei* resistant BC_1F_5 advanced breeding lines were selected from six of the BC_1F_4 populations (Table 3). After two years of evaluation in replicated trials, 12 of those lines recovered *P. thornei* resistance levels similar to, or exceeding, the best levels commercially available (Fig. 5).

Discussion

This GWAS established that seven QTL associated with *P. thornei* resistance and one associated with *P. thornei* susceptibility were present in this set of 245 ILW. This is the first

report for five of the resistance QTL, namely *QRlnt.usq-2B.2*, *QRlnt.usq-2B.3*, *QRlnt.usq-3B.1*, *QRlnt.usq-5B.1* and *QRlnt.usq-7B.1*. The susceptibility QTL, *QRlnt.usq-1B.1*, and two of the resistance QTL, *QRlnt.usq-2B.1* and *QRlnt.usq-6D.1*, were located near SNPs that have been reported in other landrace and/or synthetic hexaploid-derived populations (Rahman et al. 2019; Schmidt et al. 2005; Toktay et al. 2006; Zwart et al. 2005; 2010). Notably, three of the seven resistance QTL identified in this study were located on chromosome 2B. This is consistent with the findings of Linsell et al. (2014) where three of eight *P. thornei*-resistance QTL identified in the Sokoll x Krichauff population were located on 2B and the conclusion of Toktay et al. (2006) that the presence of *P. thornei*-resistance QTL on 2B was relatively common in the genetic studies they reported. The novel 2B QTL reported here and those reported in landrace-derived populations (Schmidt et al. 2005) and the Sokoll-derived population (Linsell et al. 2014; Rahman et al. 2019) are not associated with *P. neglectus* resistance, however, the synthetic hexaploid-derived 2B QTL that is analogous with *QRlnt.usq-2B.1* has been associated with both *P. thornei* and *P. neglectus* resistance (Zwart et al. 2010). Interestingly, phenotypic characterisation of a subset of the ILW accessions studied here has shown that some accessions were resistant or moderately so to both *P. thornei* and *P. neglectus* (J. Sheedy unpublished data).

A marker-trait association analogous with *QRlnt.usq-1B.1*, the only QTL associated with *P. thornei* susceptibility in this study, was reported in the now obsolete Australian commercial wheat cultivar Janz (Schmidt et al. 2005). At its commercial peak, Janz was widely grown across Australia and consequently was commonly used as a parent in breeding programs (Parker et al. 2002). It is likely that the 1B *P. thornei*-susceptibility locus is widely distributed through Australian wheat germplasm and that using MAS to exclude this trait from breeding populations, would be an efficient process that would complement the incorporation of *P. thornei* resistance into a breeding program. Chromosomes 3B, 5B and 7B, the locations of the

383 remaining novel *P. thornei*-resistance QTL, may also carry several *P. thornei* resistances with
384 additional QTL identified on 3B (Toktay et al. 2006) and 7B (Thompson et al. 2015) from
385 landrace-derived populations and a minor QTL on 5B from CIMMYT breeding lines
386 (Dababat et al. 2016). It is not surprising that so many QTL associated with *P. thornei*
387 resistance have been identified on the B-genome given that the evaluation of a collection of
388 progenitor and related species to wheat found that 100% of *Aegilops speltoides* accessions
389 were moderately resistant or better to *P. thornei* (Sheedy et al. 2012), where *A. speltoides* is
390 an S-genome diploid that can substitute for chromosomes on the B-genome and is a close
391 relative of the unknown B-genome donor species of wheat (Marcussen et al. 2014). In
392 contrast, the same study found 86% and 91% of accessions of the A-genome diploid species
393 *Triticum urartu* (donor species of wheat A-genome [Marcussen et al. 2014]) and *T.*
394 *monococcum*, respectively, were moderately resistant to *P. thornei* (Sheedy et al. 2012), but
395 in this study and other published reports, major QTL for *P. thornei* resistance on the A-
396 genome were notably absent.

397 The QTL identified in this research varied in their frequency of occurrence among the ILW
398 accessions, but only *QRlnt.usq-5B.1* appeared to have a limited geographic distribution.
399 *QRlnt.usq-3B.1* and *QRlnt.usq-6D.1* were ubiquitous, occurring in all 10 province groups and
400 87% and 88% of accessions, respectively. The *P. thornei* resistance associated with
401 *QRlnt.usq-6D.1* has often been reported in populations derived from landraces, synthetic
402 hexaploids and breeding lines (Linsell et al. 2014; Schmidt et al. 2005; Thompson et al. 2015;
403 Toktay et al. 2006; Zwart et al. 2005; 2006). Quantitative trait loci associated with *P. thornei*
404 resistance located on 3B have been reported in populations derived from the landrace
405 accessions AUS13124 (syn. Morocco 426), AUS4926 (syn. Iraq 43) (Schmidt et al. 2005)
406 and AUS4930 (syn. Iraq 48) (Toktay et al. 2006), CIMMYT breeding lines (Dababat et al.
407 2016) and Indian wheat genotypes (Kumar et al. 2021). However, subsequent higher marker

density analysis of the AUS13124 and AUS4926-derived populations concluded that for AUS13124, *P. thornei* resistance QTL were located on 2B and 7B and for AUS4926 on 6D and 7B (Thompson et al. 2015). The 3B QTL reported by Dababat et al. (2016) and Kumar et al. (2021) were not considered the major *P. thornei* resistance QTL in those studies leaving only the AUS4930-derived population having a primary *P. thornei* resistance QTL on 3B. It is surprising, given the frequency of occurrence in the ILW collection, that primary *P. thornei* resistance QTL located on 3B have not been reported more often. The introduction of this available additional genetic diversity into wheat breeding programs would be helpful to manage *P. thornei* in wheat-based farming systems.

Resistance alleles for *QRlnt.usq-2B.1*, *QRlnt.usq-2B.2*, *QRlnt.usq-2B.3* and *QRlnt.usq-6D.1* were common in this ILW collection, occurring in all 10 province groups and in 59% to 88% of accessions. The susceptibility QTL *QRlnt.usq-1B.1* and the resistance QTL *QRlnt.usq-7B.1* were recovered less frequently, in 34% and 41% of accessions, respectively, but still had a wide geographic distribution being found in 10 and eight province groups, respectively. Conversely, the only QTL to have a limited geographic distribution was the resistance QTL *QRlnt.usq-5B.1*, which was the rarest of the QTL and occurred in only 14% of accessions. This suggests that the geographic distribution of an individual *P. thornei* resistance/susceptibility QTL may not be limited until its frequency of occurrence in a population is as little as one in six accessions. Thirty-four of 35 accessions that carried *QRlnt.usq-5B.1*, were from four contiguous western-Iranian provinces, Kordestan, Kermanshah, Ilam and Hamadan.

The maximum number of *P. thornei* resistance QTL found in any single accession was six, indicating the polygenic nature of the resistance in this collection. This is consistent with reports of WANA landrace wheats carrying two to six *P. thornei* resistance genes (Thompson and Seymour 2011) and synthetic hexaploid wheats carrying three to six genes (Thompson et

al. 2012). In this study, regression analysis of the reproduction factor of accessions and the number of *P. thornei* resistance QTL per accession established that *P. thornei* reproduction decreased exponentially as the number of resistance QTL per accession increased. This supports the conclusion that *P. thornei* resistance was dose-dependent, as has been reported for WANA landraces (Thompson and Seymour 2011) and synthetic hexaploid wheats (Thompson 2008; Zwart et al. 2004). The evidence from this study and others is that *P. thornei* resistance in wheat is polygenic and dose-dependent.

Association mapping is an effective process that combines the germplasm discovery and marker identification phases without the need to produce biparental populations. This process can produce many putative markers that should be validated, ideally, against a genetically diverse panel of genotypes (Toth et al. 2019) or with other suitable populations. In this study, we validated the eight putative QTL against seven ILW-derived breeding populations and found that the markers effectively detected all eight QTL in the breeding populations. The populations derived from AUS28372, AUS28369, AUS28470 and AUS28645 produced, on average, the lowest final *P. thornei* population densities and were enriched with at least two *P. thornei*-resistance QTL, but did not carry the *QRlnt.usq-1B.1 P. thornei*-susceptibility locus. One population was enriched with only *QRlnt.usq-1B.1* and produced the highest *P. thornei* population densities, while the remaining two populations were enriched with both resistance and susceptibility QTL and produced intermediate *P. thornei* population densities. This supports the conclusion that the exclusion of *QRlnt.usq-1B.1* from breeding populations will reduce *P. thornei* population densities and maximise the benefit of any resistance QTL present in the population.

The BC_1F_4 populations used in this study were derived from *P. thornei*-tolerant BC_1F_3 populations that had previously been selected phenotypically for resistance to *P. thornei* in the BC_1F_1 and BC_1F_2 generations. We screened a sufficient number of individuals per family

458 in each generation to enrich subsequent generations with the target traits. In the BC_1F_4
459 generation, resistant selections were recovered from each segregating population, with a
460 higher proportion of resistant selections recovered from the populations carrying two to three
461 *P. thornei*-resistance QTL. Selecting for *P. thornei* resistance or tolerance phenotypically in
462 each of the early generations successfully identified individuals with superior resistance and
463 tolerance in better-adapted backgrounds than the donor parent, however, the process was very
464 labour intensive and consequently expensive. Since resistance to *P. thornei* in wheat is
465 polygenic and dose dependent, MAS offers the opportunity to improve the efficiency of this
466 process. Using MAS to select in the F_2 generation has been reported to reduce by 80% the
467 necessary population size to recover six target alleles (Bonnett et al. 2005), and that large
468 responses to MAS in the BC_1F_1 generation were also observed in derived populations
469 (Kuchel et al. 2007). These studies have determined that maximum genetic gain within
470 breeding populations, at the lowest cost, was achieved by the use of markers closely-linked to
471 target genes to enrich the early generations of segregating populations, rather than to fix
472 target loci, with homozygous individuals selected in later generations (Bonnett et al. 2005;
473 Kuchel et al. 2007). This process would likely be well-suited to the development of *P.*
474 *thornei*-resistant wheat cultivars that combine several resistance loci and produce an overall
475 higher level of phenotypic resistance. Further refining the QTL identified in this research
476 through a fine mapping process would greatly assist the production of closely-linked markers
477 and would also provide insight into the mechanisms of *P. thornei* resistance.

478 This is the first report of a GWAS being used to identify novel QTL associated with
479 resistance to root-lesion nematodes from ILW. Analysing existing phenotypic data of a
480 germplasm collection in a GWAS framework proved an effective tool to identify putative *P.*
481 *thornei* resistance QTL without the need to develop biparental populations. Validation of the
482 QTL in active breeding populations rather than developing additional biparental populations

also reduced the time taken and cost of delivering novel QTL combinations in four advanced breeding lines suitable for use by plant breeding programs. ILW are a valuable but largely untapped source of genetic diversity for resistance to RLN. The five novel QTL for *P. thornei* resistance offer the opportunity both to increase diversity in the genetic management of *P. thornei* and to develop new gene combinations to increase the overall level of resistance available to plant breeders.

Acknowledgements

The authors would like to thank the Grains Research and Development Corporation (GRDC) (Project Numbers: USQ00019, DAV00127) and the Queensland Department of Agriculture and Fisheries through the Broadacre Cropping Initiative (BACI) partnership for co-funding this research with the University of Southern Queensland (USQ). We thank Jing Lin, Mei Wang and Dr Rebecca Zwart (USQ) for technical assistance. The authors declare no conflicts of interest. Reference in this document to any specific commercial product, process, or service, or the use of any trade, firm or corporation name is for the information and convenience of the reader, and does not constitute endorsement, recommendation, or favouring by the authors or their affiliates.

References

Alipour H, Bihamta MR, Mohammadi V, Peyghambari SA, Bai G, Zhang G (2017) Genotyping-by-sequencing (GBS) revealed molecular genetic diversity of Iranian wheat landraces and cultivars. Frontiers in Plant Science 8:1293.

505 Barrett JC (2009) Haploview: visualisation and analysis of SNP genotype data. Cold Spring
506 Harbour Protocols 2009; doi:10.1101/pdb.ip71

507 Bernard GC, Egnin M, Bonsi C (2017) The Impact of Plant-Parasitic Nematodes on
508 Agriculture and Methods of Control. In Nematology Concepts, Diagnosis and Control. Ed
509 MM Shah. (IntechOpen Limited: London, UK.) https://doi.org/10.5772/66851

510 Bonnett DG, Rebetzke GJ, Spielmeyer W (2005) Strategies for efficient implementation of
511 molecular markers in wheat breeding. Mol Breed 15:75-85.

512 Browning BL, Zhou Y, Browning SR (2018). A one-penny imputed genome from next
513 generation reference panels. American Journal of Human Genetics 103:338-348.

514 Butler DG, Cullis BR, Gilmour AR, Gogel BJ, Thompson R (2017) ASReml-R reference
515 manual version 4. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

516 Castillo P, Jimenez-Diaz RM, Gomez-Barcina A, Vovlas N (1995) Parasitism of the root-
517 lesion nematode *Pratylenchus thornei* on chickpea. Plant Pathology 44:728-733.

518 Castillo P, Vovlas N (2007) *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology,
519 Pathogenicity and Management. In Nematology Monographs and Perspectives Volume 6
520 Series 9. Eds DJ Hunt and RN Perry. (Brill: Leiden, the Netherlands.)

521 Curtis BC (2002) Wheat in the world. In 'Bread Wheat; Improvement and Production' FAO
522 Plant Production and Protection Series No. 30. Eds. Curtis BC, Rajaram S, Gómez
523 Macpherson H. Food and Agriculture Organisation of the United Nations Rome 2002.

524 Dababat AA, Ferney G-BH, Erginbas-Orakci G, Dreisigacker S, Imren M, Toktay H,
525 Eleckcioglu HI, Mekete T, Nicol JM, Ansari O, Ogbonnaya F (2016) Association analysis of
526 resistance to cereal cyst nematodes (*Heterodera avenae*) and root lesion nematodes
527 (*Pratylenchus neglectus* and *P. thornei*) in CIMMYT advanced spring wheat lines for semi-
528 arid conditions. Breed Sci 66:692-702.

529 Fatemah F, Reza F-N, Reza AA, Vahe M, Frahnaz JA, Zeynab B (2012) Determination of
530 reaction of some wheat lines/cultivars to root-lesion nematodes (*Pratylenchus thornei* and *P.*
531 *neglectus*) under controlled conditions in southwest Iran. International Journal of Nematology
532 22:73-80.

533 Food and Agriculture Organisation of the United Nations Cereal Supply and Demand Brief
534 (2021) http://www.fao.org/worldfoodsituation/csdb/en/. Accessed 19 April 2021.

535 Fortuner R (1977) *Pratylenchus thornei*. Commonwealth Institute of Helminthology
536 Descriptions of Plant-parasitic Nematodes Set 7, No. 93.

537 Greco N, Di Vito M, Saxena MC and Reddy MV (1988) Investigation on the root lesion
538 nematode *Pratylenchus thornei*, in Syria. Nematologia Mediterranea 16: 101-105

539 Keil T, Laubach E, Sharma S, Jung C (2009) Screening for resistance in the primary and
540 secondary gene pool of barley against the root-lesion nematode *Pratylenchus neglectus*. Plant
541 Breeding 128:436-442.

542 Koenning SR, Overstreet C, Noling JW, Donald PA, Becker JO, Fortnum BA (1999) Survey
543 of crop losses in response to phytoparasitic nematodes in the United States for 1994. J
544 Nematol 31(4S): 587-618.

545 Kuchel H, Fox R, Reinheimer J, Mosionek L, Willey N, Bariana H, Jefferies S (2007) The
546 successful application of a marker-assisted wheat breeding strategy. Mol Breed 20:295-308.

547 Kumar D, Sharma S, Sharma R, Pundir S, Kumar Singh V, Chaturvedi D, Singh B, Kumar S,
548 Sharma S (2021) Genome-wide association study in hexaploid wheat identifies novel
549 genomic regions associated with resistance to root lesion nematode (*Pratylenchus thornei*).
550 Scientific Reports 11:3572.

551 Linsell KJ, Rahman MS, Taylor JD, Davey RS, Gogel BJ, Wallwork H, Forrest KL, Hayden
552 MJ, Taylor SP, Oldach KH (2014) QTL for resistance to root lesion nematode (*Pratylenchus*
553 *thornei*) from a synthetic hexaploid wheat source. Theor Appl Genet 127:1409-1421.

554 Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang Z
555 (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397-
556 2399.

557 Lucas H (2012) The Wheat Initiative – an international research initiative for wheat
558 improvement. Second Global Conference on Agricultural Research for Development. Punta
559 del Este, Uruguay, 29 October – 1 November 2012.

560 Marcussen T, Sandve SR, Heier L, Spannagl M, Pfeifier M, The International Wheat Genome
561 Consortium, Jakobsen KS, Wulff BBH, Steuernagl B, Mayer KFX, Olsen O-A (2014)
562 Ancient hybridisations among the ancestral genomes of bread wheat. Science 345.
563 http://dx.doi.org/10.1126/science.1250092

564 May DB, Johnson WA, Zuck PC, Chen CC, Dyer AT (2016) Assessment and management of
565 root lesion nematodes in Montana wheat production. Plant Disease 100:2069-2079.

566 Mishra SM, Gupta P (1988) Sweet pea - a new host of *Pratylenchus thornei* in India. Indian
567 Journal of Nematology 18:357

568 Mokrini F, Waeyenberger L, Viaene N, Abbad Andaloussi F, Moens M (2016) Diversity of
569 root-lesion nematodes (*Pratylenchus* spp.) associated with wheat (*Triticum aestivum* and *T.*
570 *durum*) in Morocco. Nematology 18:781-801.

571 Moser G, Lee SH, Hayes BJ, Goddard ME, Wray NR, Visscher PM (2015) Simultaneous
572 discovery, estimation and prediction analysis of complex traits using a Bayesian mixture
573 model. PLoS Genetics 11:e1004969. https://doi.org/10.1371/journal.pgen.1004969

574 Mulki MA, Jighly A, Ye G, Emebiri LC, Moody D, Ansari O, Ogbonnaya FC (2013)
575 Association mapping for soilborne pathogen resistance in synthetic hexaploid wheat. Mol
576 Breed 31:299-311.

577 Nicol JM, Ortiz-Monasterio I (2004) Effects of the root-lesion nematode, *Pratylenchus*
578 *thornei*, on wheat yields in Mexico. Nematology 6:485-493.

579 Oakey H, Verbyla AP, Cullis BR, Wei X, Pitchford WS (2007) Joint modelling of additive
580 and non-additive (genetic line) effects in multi-environment trials. Theor Appl Genet
581 114:1319-1332.

582 Parker GD, Fox PN, Langridge P, Chalmers K, Whan B, Ganter PF (2002) Genetic diversity
583 within Australian wheat breeding programs based on molecular and pedigree data. Euphytica
584 124:293-306.

585 Pasam RK, Bansal U, Daetwyler HD, Forrest KL, Wong D, Petkowski J, Willey N,
586 Randhawa M, Chhetri M, Miah H, Tibbits J, Bariana H, Hayden MJ (2017) Detection and
587 validation of genomic regions associated with resistance to rust diseases in a worldwide
588 hexaploid wheat landrace collection using BayesR and mixed linear model approaches. Theor
589 Appl Genet 130:777-793.

590 Quraishi UM, Pont C, Ain Q-u, Flores R, Burlot L, Alaux M, Quesneville H, Salse J (2017)
591 Combined genomic and genetic data integration of major agronomical traits in bread wheat
592 (*Triticum aestivum* L.). Front Plant Sci 8: 1843. https://doi.org/10.3389/fpls.2017.01843

593 R Core Team (2017). R: A language and environment for statistical computing. R Foundation
594 for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

595 Rahman MS, Linsell KJ, Taylor JD, Hayden MJ, Collins NC, Oldach KH (2019) Fine
596 mapping of root lesion nematode (*Pratylenchus thornei*) resistance loci on chromosomes 6D
597 and 2B of wheat. Theor Appl Genet 133:635–652.

598 Saghai-Maroof M, Biyashev R, Yang G, Zhanq Q, Allard R (1984) Extraordinary
599 polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and
600 population dynamics. Proc Natl Acad Sci USA 91:5466-5470.

601 Scherer A, Christensen GB (2016) Concepts and relevance of genome-wide association
602 studies. Science Progress 99:59-67.

603 Schmidt AL, McIntyre CL, Thompson JP, Seymour NP, Liu CJ (2005) Quantitative trait loci
604 for root lesion nematode (*Pratylenchus thornei*) resistance in Middle-Eastern landraces and
605 their potential for introgression into Australian bread wheat. Australian Journal of
606 Agricultural Research 56:1059-1068.

607 Sheedy JG, Thompson JP (2009) Resistance to the root-lesion nematode *Pratylenchus thornei*
608 of Iranian landrace wheat. Australas Plant Path 38:478-489

609 Sheedy JG, Thompson JP, Kelly A (2012) Diploid and tetraploid progenitors of wheat are
610 valuable sources of resistance to the root lesion nematode *Pratylenchus thornei*. Euphytica
611 186:377-391.

612 Shewry PR, Hey SJ (2015) The contribution of wheat to human diet and health. Food and
613 Energy Security 4:178-202

614 Subramaniyan S, Sivakumar CV (1991) '*Pratylenchus* species.' Current Research, University
615 of Agricultural Sciences, Bangalore

616 Thompson CH, Beckman GG (1959) Soils and land use in the Toowoomba area, Darling
617 Downs, Queensland. Soils and land use series No. 28. CSIRO Melbourne.

618 Thompson JP (1990) Treatments to eliminate root-lesion nematode (*Pratylenchus thornei*
619 Sher and Allen) from a vertisol. Nematologica 36:123-127

620 Thompson JP, Brennan PS, Clewett TG, Sheedy JG and Seymour NP (1999) Progress in
621 breeding wheat for tolerance and resistance to root-lesion nematode (*Pratylenchus thornei*).
622 Australas Plant Pathol 28:45-52

623 Thompson JP, Clewett TG, O'Reilly MM (2014) Temperature response of root-lesion
624 nematode (*Pratylenchus thornei*) reproduction on wheat cultivars has implications for
625 resistance screening and wheat production. Ann Appl Biol 167:1-10

626 Thompson JP, Owen KJ, Stirling GR, Bell MJ (2008) Root-lesion nematodes (*Pratylenchus*
627 *thornei* and *P. neglectus*): a review of recent progress in managing a significant pest of grain
628 crops in northern Australia. Australas Plant Pathol 37:235-242

629 Thompson JP, Seymour NP (2011) Inheritance of resistance to root-lesion nematode
630 *Pratylenchus thornei* in wheat landraces and cultivars from the WANA region. Crop Pasture
631 Sci 62:82-93.

632 Thompson JP, Sheedy JG, Robinson NA (2020) Resistance of wheat genotypes to root-lesion
633 nematode (*Pratylenchus thornei*) can be used to predict final nematode population densities,
634 crop greenness, and grain yield in the field. Phytopathology 110: 505-516.

635 Thompson J, Sheedy J, Robinson N, Clewett TG (2021) Tolerance of wheat (*Triticum*
636 *aestivum*) genotypes to root-lesion nematode (*Pratylenchus thornei*) in the subtropical grain
637 region of eastern Australia. Euphytica 217:48. https://doi.org/10.1007/s10681-020-02761-0

638 Thompson J, Sheedy J, Robinson N, Khoo K, Chalmers K, Mather D (2015) Utilisation of the
639 Watkins collection of wheat landraces for root-lesion nematode resistance. Ninth
640 International Wheat Conference, Sydney, 20-25 September. p. 149.

641 Thompson JP, Sheedy JG, Seymour NP, Clewett TG, Mason LR, Sheppard JA, Banks PM
642 (2001) Advances in breeding wheat for tolerance and resistance to *Pratylenchus thornei* and

643 *P. neglectus* for the northern region. In 'Proceedings of the 10th Assembly of the Wheat
644 Breeding Society of Australia Inc.'. 16-21 September 2001, Mildura, Australia. pp. 123-127.

645 Thompson JP, Zwart RS, Butler D (2012) Inheritance of resistance to root-lesion nematodes
646 (*Pratylenchus thornei* and *P. neglectus*) in five doubled-haploid populations of wheat.
647 Euphytica 188:209-219.

648 Toktay H, McIntyre CL, Nicol JM, Ozkan H, Elekcioglu HI (2006) Identification of common
649 root-lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in wheat. Genome 49:1319-
650 1323.

651 Toth J, Pandurangan S, Burt A, Fetch JM, Kumar S (2019) Marker-assisted breeding of
652 hexaploid spring wheat in the Canadian prairies. Can J Plant Sci 99:111-127.

653 Townshend JL, Anderson RV (1976) *Pratylenchus neglectus*. Commonwealth Institute of
654 Helminthology Descriptions of Plant-parasitic Nematodes Set 6, No. 82.

655 Van Raden PM (2008) Efficient methods to compute genomic predictions. J Dairy Sci
656 91:4414-4423.

657 Vanstone VA, Hollaway GJ, Stirling GR (2008) Managing nematode pests in the southern
658 and western regions of the Australian cereal industry: continuing progress in a challenging
659 environment. Australas Plant Pathol 37:220-234.

660 Viccars L, Spindler L, Haak I, Wildermuth GB, Thompson JP, Banks PM, Appels R,
661 Lagudah ES (1999) Genetic markers for resistance to crown rot and root lesion nematode in
662 wheat. In 'Proceedings of the 9th assembly of the Wheat Breeding Society of Australia inc.'.
663 September 1999, Toowoomba, Australia. pp. 118-122.

664 Vikram P, Franco J, Burgueño J, Li H, Sehgal D, Saint-Pierre C, Ortiz C, Kumar Singh V,
665 Sneller C, Sharma A, Tattaris M, Guzman C, Pena J, Paola Sansaloni C, Amador J, Serna C,
666 Thiyagarajan K, Fuentes Davila G, Reynolds M, Sonder K, Govindan V, Ellis M, Bhavani S,

667 Jalal Kamali MR, Roosatei M, Singh S, Basandrai D, Singh Bains N, Basandrai A, Payne T,
668 Crossa J, Singh S (2020) Strategic use of Iranian bread wheat landrace accessions for genetic
669 improvement: Core set formulation and validation. Plant Breed 2020;00:1–13.
670 https://doi.org/10.1111/pbr.12885

671 Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, Yang J (2017) 10
672 years of GWAS discovery: Biology, function and translation. The American Journal of
673 Human Genetics 101:5-22.

674 Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG,
675 Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plieske J,
676 International Wheat Genome Sequencing Consortium, Lillemo M, Mather D, Appels R,
677 Dolferus R, Brown-Guedira G, Korol A, Akhunova AR, Feuillet C, Salse J, Morgante M,
678 Pozniak C, Luo M-C, Dvorak J, Morell M, Dubcovsky J, Ganal M, Tuberosa R, Lawley C,
679 Mikoulitch I, Cavanagh C, Edwards KJ, Hayden M, Akhunov E (2014) Characterisation of
680 polyploid wheat genomic diversity using a high-density 90,000 single nucleotide
681 polymorphism array. Plant Biotechnology Journal 12:787-796.

682 Whitehead AG, Hemming JR (1965) A comparison of some quantitative methods of
683 extracting small vermiform nematodes from soil. Ann Appl Biol 55:25-38.

684 Yang J, Zaitlen NA, Goddard ME, Visscher PM, Price AL (2014) Advantages and pitfalls in
685 the application of mixed-model association methods. Nat Genet 46:100-106.

686 Yu Q (1997) First report of *Pratylenchus thornei* from spring wheat in southern Ontario. Can
687 J Plant Pathol 19:289-292.

688 Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals.
689 Weed Res 14:415-421.

690 Zwart RS, Thompson JP, Godwin ID (2004) Genetic analysis of resistance to root-lesion
691 nematode (*Pratylenchus thornei*) in wheat. Plant Breed 123:209-212.

692 Zwart RS, Thompson JP, Godwin ID (2005) Identification of quantitative trait loci for
693 resistance to two species of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in
694 wheat. Aust J of Agric Res 56:345-352.

695 Zwart RS, Thompson JP, Sheedy JG, Nelson JC (2006) Mapping quantitative trait loci for
696 resistance to *Pratylenchus thornei* from synthetic hexaploid wheat in the International
697 Triticeae Mapping Initiative (ITMI) population. Aust J Agric Res57:525-530.

698 Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, Bariana HS
699 (2010) QTL mapping of multiple foliar disease and root-lesion nematode resistances in
700 wheat. Mol Breed 26:107-124.

701

702

703

List of Tables

713 Table 1. Twenty-seven flanking markers linked to 11 SNPs, were delineated into eight QTL for reproduction factor (P_f/P_i) of *Pratylenchus*
714 *thornei*, based on significant p-values.

715 *indicates novel QTL identified in this study.

SNP	Genetic Position (cM)[1]	Chromosome	Physical position[2]	QTL Name	MAF[3]	Log_{10}(P-value)	SNPs selected through BayesR + LMM analyses	KASP Marker
Tdurum_contig8081_2331	134.68	1B	415230355		0.480	2.871		
GENE-0235_131	134.47	1B	.		0.444	3.294		
BobWhite_c43322_203	136.43	1B	384300477		0.397	3.872		
Ra_c18323_183	136.43	1B	388757998		0.389	3.573		
IACX184	136.43	1B	388383252		0.389	3.573		
wsnp_Ex_c22006_31180883	136.43	1B	451076976	*QRlnt.usq-1B.1*	0.492	4.092		77218_1B
Kukri_rep_c116003_106	136.43	1B	385157636		0.421	3.434	Kukri_rep_c116003_106	
BS00073094_51	136.90	1B	421670804		0.413	2.949		10642_1B
Excalibur_c39191_82	138.32	1B	390707788		0.397	2.746		
CAP7_c302_518	138.40	1B	388010989		0.389	3.573		
IACX8446	68.00	2B	.	*QRlnt.usq-2B.1*	0.107	3.827	IACX8446	36300_2B
Tdurum_contig54649_915	75.00	2B	16871375		0.282	3.847	Tdurum_contig54649_915	72375_2B
GENE-2192_463	172.00	2B	.	*QRlnt.usq-2B.2**	0.401	3.084	GENE-2192_463	32838_2B
wsnp_CAP11_c5554_2580044	174.00	2B	534842647		0.377	3.141	wsnp_CAP11_c5554_2580044	75885_2B
Excalibur_c5438_274	252.00	2B	774958099	*QRlnt.usq-2B.3**	0.317	4.910	Excalibur_c5438_274	27473_2B
Excalibur_rep_c109577_698	253.00	2B	775368259		0.234	3.744		30421_2B
BS00075108_51	189.00	3B	66870072	*QRlnt.usq-3B.1**	0.111	7.486	BS00075108_51	10783_3B
wsnp_Ex_c11246_18191331	189.00	3B	67942455		0.147	5.680	wsnp_Ex_c11246_18191331	76275_3B
Excalibur_c50887_231	105.00	5B	377980058		0.218	3.095		27185_5B
Tdurum_contig84745_267	108.00	5B	378816535	*QRlnt.usq-5B.1**	0.218	3.095		73671_5B
Excalibur_c30346_54	108.19	5B	388393869		0.147	3.079		
CAP11_c919_204	109.49	5B	387173377		0.143	3.093		
Jagger_c505_232	113.21	5B	403855635		0.052	3.931	Jagger_c505_232	

Excalibur_rep_c84264_67	32.82	6D	.	*QRlnt.usq-6D.1*	0.119	3.150	Excalibur_rep_c84264_67	31346_6D
Kukri_rep_c68823_696	39.83	6D	5178439		0.115	2.858		49821_6D
BS00041397_51	186.97	7B	.	*QRlnt.usq-7B.1**	0.107	3.955	BS00041397_51	8724_7B
Kukri_c10172_396	199.68	7B	739384773		0.456	3.042		40270_7B

716 [1] Based on the genetic reference map of Quraishi et al. (2017).

717 [2] Based on the International Wheat Genome Sequencing Consortium reference sequence of bread wheat, IWGSC RefSeq v1.0.

718 https://wheat.pw.usda.gov/GG3/node/674

719 [3] Minor allele frequency

720

Table 2. Average *Pratylenchus thornei* reproduction factor (RF; P_f/P_i) and number and percentage of accessions carrying QTL identified in this study for each Iranian province.

Province	**Average**		**Number and percentage of accessions carrying QTL**															
	RF	**Total**	***QRlnt.usq-1B.1***		***QRlnt.usq-2B.1***		***QRlnt.usq-2B.2***		***QRlnt.usq-2B.3***		***QRlnt.usq-3B.1***		***QRlnt.usq-5B.1***		***QRlnt.usq-6D.1***		***QRlnt.usq-7B.1***	
			No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
MQZ[1]	1.58	4	3	75%	3	75%	1	25%	3	75%	4	100%	0	0%	4	100%	0	0%
YK[2]	2.80	4	3	75%	4	100%	1	25%	2	50%	4	100%	0	0%	4	100%	0	0%
Esfahan	3.47	11	9	82%	8	73%	2	18%	10	91%	11	100%	1	9%	10	91%	7	64%
Khorasan[3]	3.59	10	9	90%	8	80%	1	10%	9	90%	10	100%	0	0%	9	90%	1	10%
Hamadan	3.65	19	9	47%	15	79%	6	32%	10	53%	19	100%	1	5%	19	100%	4	21%
East Azerbaijan	3.84	26	16	62%	22	85%	8	31%	23	88%	26	100%	0	0%	22	85%	5	19%
Kordestan	4.38	33	13	39%	27	82%	17	52%	25	76%	28	85%	2	6%	28	85%	10	30%
Ilam	4.95	35	4	11%	20	57%	28	80%	20	57%	31	89%	9	26%	32	91%	19	54%
West Azerbaijan	5.41	29	2	7%	16	55%	19	66%	29	100%	29	100%	0	0%	27	93%	17	59%
Kermanshah	7.09	74	15	20%	35	47%	61	82%	34	46%	52	70%	22	30%	61	82%	37	50%
TOTAL		**245**	**83**	**34%**	**158**	**64%**	**144**	**59%**	**165**	**67%**	**214**	**87%**	**35**	**14%**	**216**	**88%**	**100**	**41%**

[1] Combined accessions from neighbouring Markazi (2), Qazvin (1) and Zanjan (1) provinces

[2] Combined accessions from neighbouring Yazd (2) and Kerman (2) provinces

[3] Combined accessions from neighbouring North Khorasan (1) and Razavi Khorasan (9) provinces

728 Table 3. Average final *Pratylenchus thornei* population density (P_f) for each BC_1F_4 population, enrichment for *Pratylenchus thornei* resistance
729 (R) and susceptibility (S) quantitative trait loci (QTL) and their associated KASP markers and advanced breeding lines (ABL) produced.

Province	QTL enriched in population	Average P_f $\log_e(x)$	BTM[1]	Accessions per family	KASP Markers	No. of ABL selected from the population for yield evaluation
Experiment 1						
AUS28372/2*Gregory	QRlnt.usq-3B.1 (R)	8.81	6,701	17	76275_3B	2
	QRlnt.usq-6D.1 (R)				49086_6D	
AUS28369/2*Wylie	QRlnt.usq-2B.1 (R)	9.42	12,333	17	36300_2B	8
	QRlnt.usq-6D.1 (R)				31346_6D; 49086_6D	
	QRlnt.usq-7B.1 (R)				8724_7B; Uat8_7B	
AUS28645/2*Gregory	QRlnt.usq-2B.3 (R)	9.90	19,930	17	27473_2B	3
	QRlnt.usq-6D.1 (R)				31346_6D; 49086_6D	
AUS28677/2*Wylie	QRlnt.usq-1B.1 (S)	10.36	31,571	17	10642_1B[1]; 77218_1B[2]	3
	QRlnt.usq-2B.1 (R)				36300_2B	
	QRlnt.usq-5B.1 (R)				73671_5B	
	QRlnt.usq-6D.1 (R)				Uat14_6D	
Experiment 2						
AUS28470/2*Gregory	QRlnt.usq-2B.2 (R)	9.46	12,836	16	32838_2B; 75885_2B	4
	QRlnt.usq-7B.1 (R)				8724_7B	
AUS28451/2*Gregory	QRlnt.usq-1B.1 (S)	10.31	30.031	15	10642_1B	2
	QRlnt.usq-2B.2 (R)				32838_2B; 75885_2B	
AUS28451/Gregory//Wylie	QRlnt.usq-1B.1 (S)	10.65	42,193	16	10642_1B	0

730 [1] Back-transformed mean

731 [2] Partial enrichment for this QTL in this population.

732

List of Figures

757 AUS28369, AUS28372 and AUS28451 are Iranian landrace wheats rated as moderately
758 resistant (MR) to *P. thornei*. EGA Gregory and EGA Wylie are Australian wheat cultivars
759 rated as moderately susceptible–susceptible (MSS) to *P. thornei*.
760

761 

762 Figure 1. Iranian provinces from which the landrace wheat accessions evaluated in this study

763 originated.

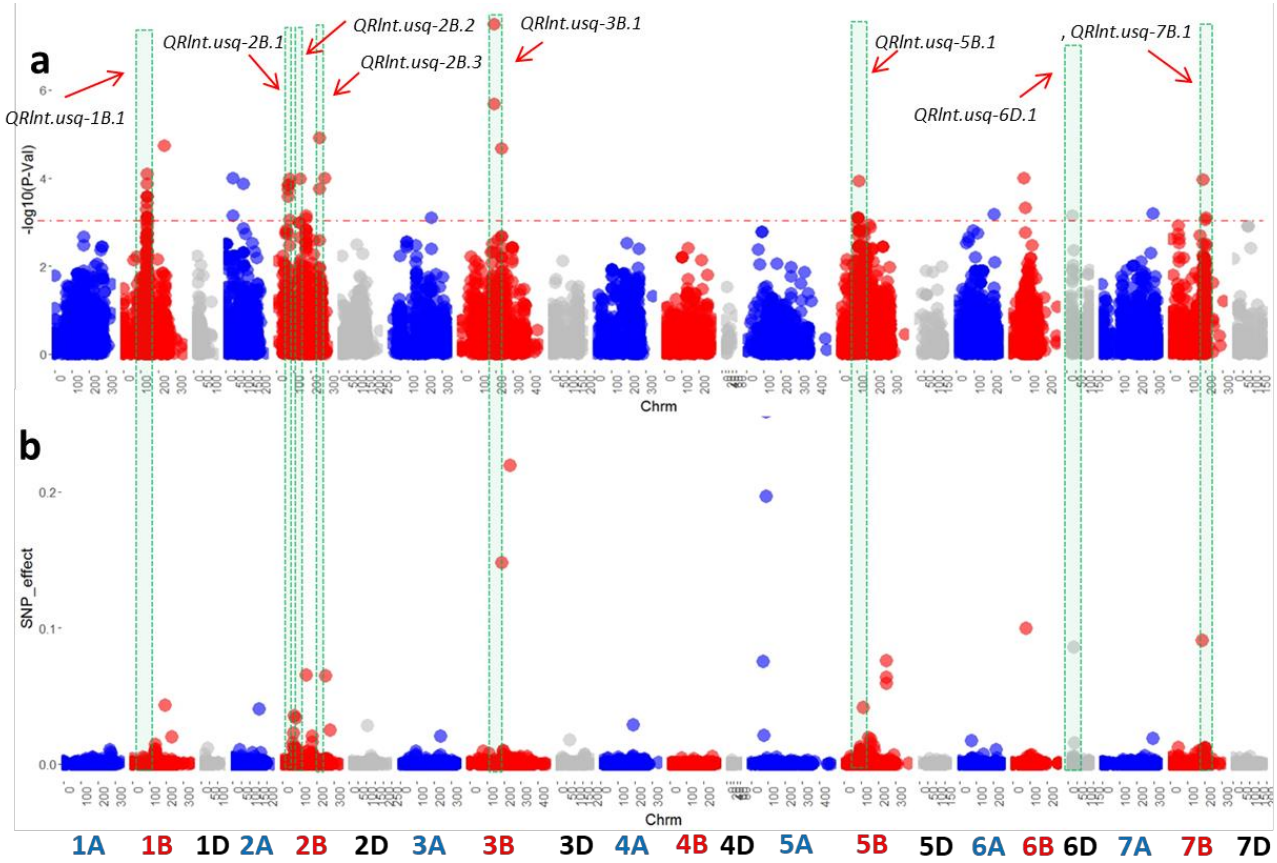


Figure 2. Comparison of GWAS results using two approaches for root-lesion nematode (*P. thornei*) resistance based on reproduction factor: a) Linear mixed model with principal components analysis and kinship matrix as correction for population structure; Y-axis shows $-\log_{10}$(P value) and red dotted line represents the threshold of significance, and b) a Bayesian approach (BayesR); Y-axis shows the effect of each SNP in BayesR analysis. X-axis shows SNPs ordered on their genetic position along the 21 chromosomes based on the consensus map of Quraishi et al. (2017). The SNPs that were significant in both approaches were identified and used for further investigation. 11 SNPs were significant across both approaches and they correspond to eight QTL. The QTL are named after their chromosome location and chromosome order. QTL: *QRlnt.usq-1B.1*, *QRlnt.usq-2B.1*, *QRlnt.usq-2B.2*, *QRlnt.usq-2B.3*, *QRlnt.usq-3B.1*, *QRlnt.usq-5B.1*, *QRlnt.usq-6D.1*, *QRlnt.usq-7B.1*.

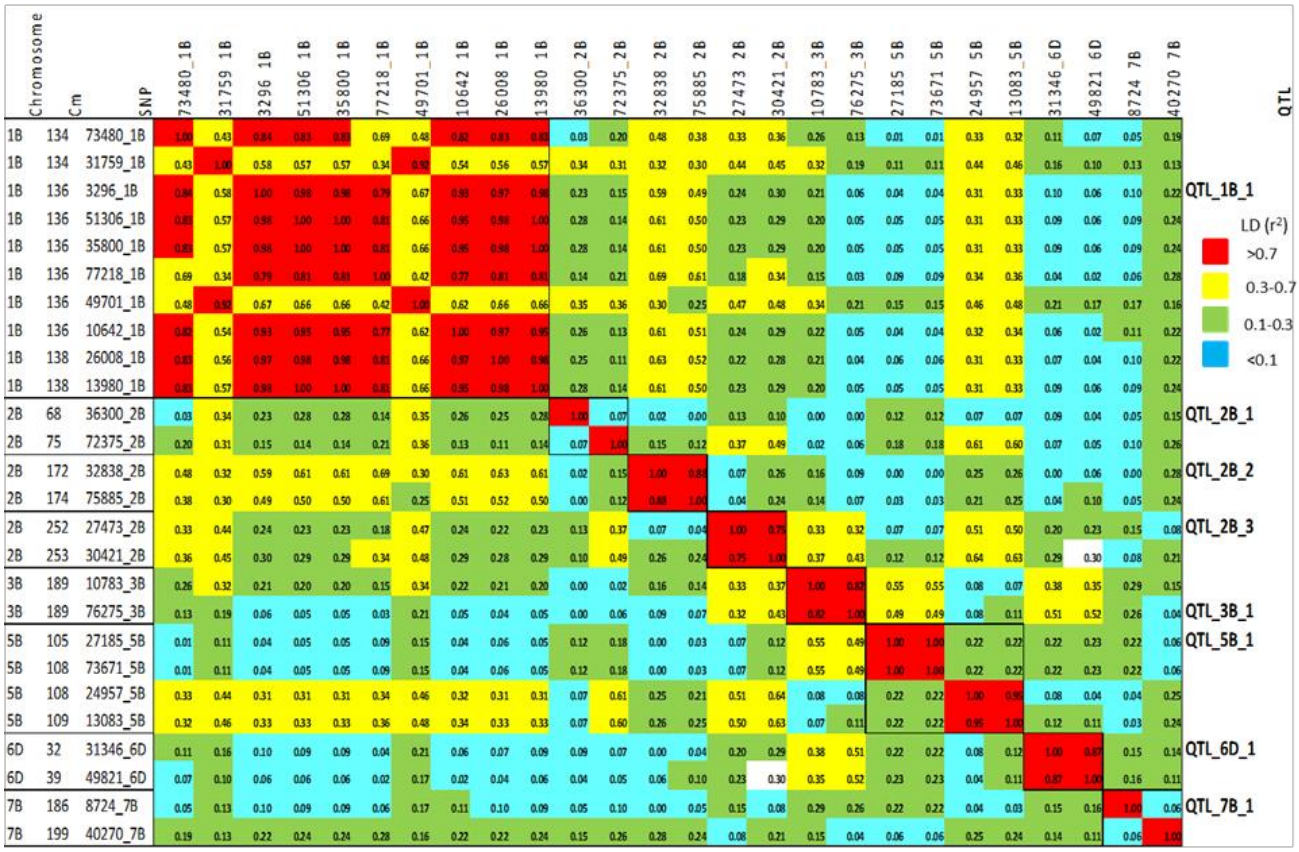
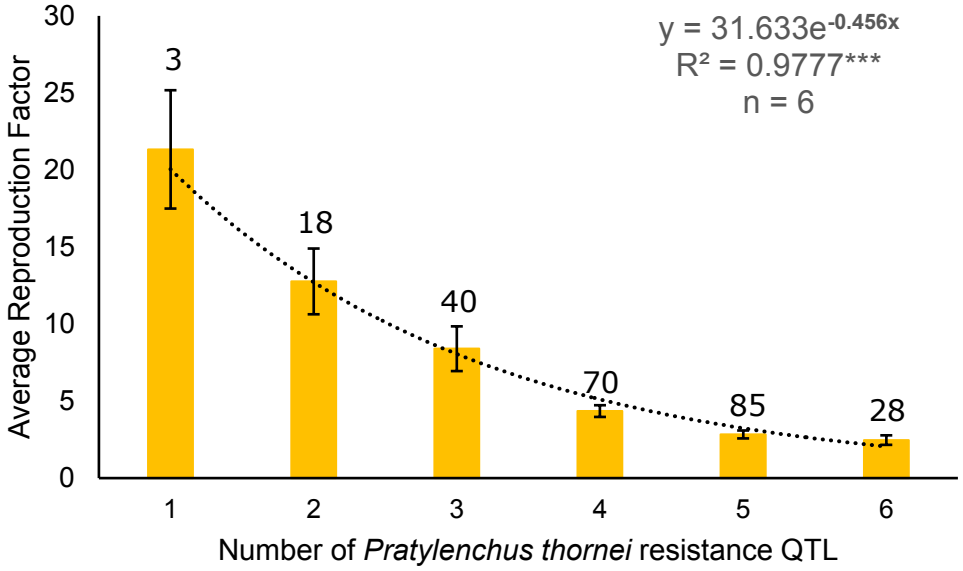


Figure 3. Linkage Disequilibrium matrix of the significant SNPs and their flanking SNPs in the region. Regions highlighted in a box correspond to each QTL.



781

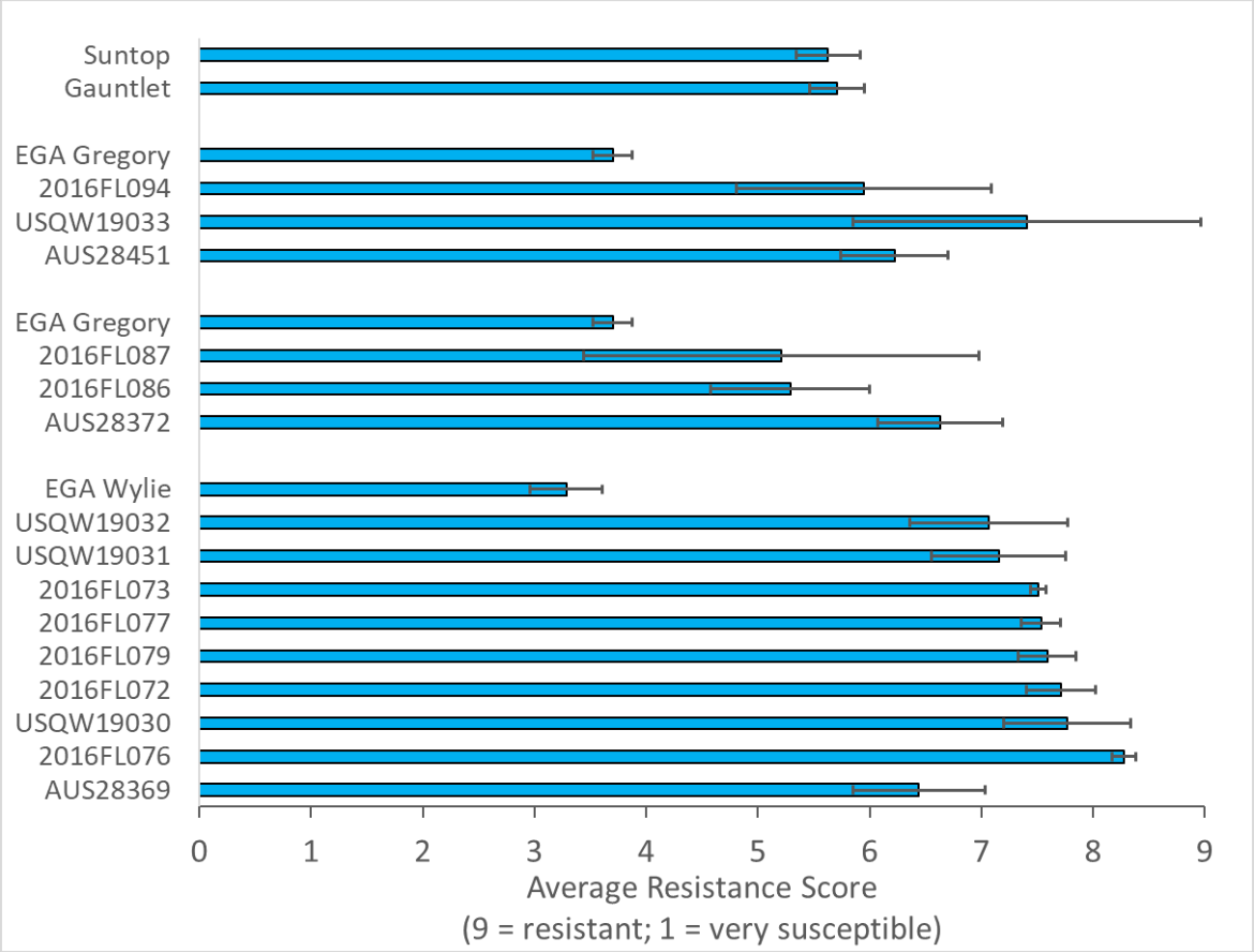
782 Figure 4. Average reproduction factor of Iranian landrace wheat accessions decreased

783 exponentially as the number of *Pratylenchus thornei* resistance QTL per accession increased,

784 indicating that the *P. thornei* resistance in this collection was polygenic and dose-dependent.

785 Numbers above the standard error bars indicate the relevant number of accessions.

786



787

788 Figure 6. Average resistance scores and standard errors of advanced breeding lines selected
789 from BC_1F_4 populations compared with their parents and commercial standards. Suntop and
790 Gauntlet are Australian wheat cultivars rated as moderately resistant–moderately susceptible
791 (MRMS) to *P. thornei* and represent the best level of resistance commercially available.
792 AUS28369, AUS28372 and AUS28451 are Iranian landrace wheats rated as moderately
793 resistant (MR) to *P. thornei*. EGA Gregory and EGA Wylie are Australian wheat cultivars
794 rated as moderately susceptible–susceptible (MSS) to *P. thornei*.

3.1 Implications of Chapter 3

Iranian landrace wheats are an important source of genetic diversity and traits useful to modern wheat improvement programs. Although *P. thornei*-resistant genotypes had been identified by Sheedy and Thompson (2009), there are no reports on the genetics of the resistance. The GWAS proved an effective procedure to reanalyse existing phenotypic data to improve our knowledge of the genetics of *P. thornei* resistance in the ILW collection. Five novel *P. thornei* resistance QTL were identified as well as three previously reported QTL (two associated with *P. thornei* resistance and one with *P. thornei* susceptibility) were confirmed. Twelve ABLs that recovered *P. thornei* resistance levels similar to, or exceeding their donor ILW parent were developed. The *P. thornei* resistance in the ILW collection was polygenic, controlled by up to six QTL per genotype, and dose-dependent. This knowledge provides the opportunity to develop additional ABLs that carry novel QTL combinations and deliver a higher level of resistance than is commercially available. The KASP markers that were developed will aid in that process, however, fine mapping of these QTL would greatly improve the efficiency of selection of genotypes with superior resistance. It would also be valuable to evaluate this ILW collection, or at least the *P. thornei*-resistant genotypes, for their resistance to *P. neglectus*. Genotypes that combine resistance to *P. thornei* and *P. neglectus* are relatively rare but would be valuable genetic resources to develop commercial cultivars to manage mixed RLN populations in Australian farming systems.

CHAPTER 4: PAPER 2 – DISCOVERY OF RESISTANCE TO *Pratylenchus neglectus* AMONG *P. thornei*-RESISTANT IRANIAN LANDRACE WHEATS AND THE INTROGRESSION OF BOTH RESISTANCES INTO ADVANCED BREEDING LINES

Sheedy JG, Lin J, Thompson JP (2022) Discovery of resistance to *Pratylenchus neglectus* among *P. thornei*-resistant Iranian landrace wheats and the introgression of both resistances into advanced breeding lines. *Plant Pathology* 71: 2017-2028. https://doi.org/10.1111/ppa.13616

In this study a collection of 91 *P. thornei*-resistant ILWs was evaluated for their resistance to *P. neglectus* in four glasshouse experiments in order to 1) identify genotypes with resistance to both RLN, 2) determine if any genotypes carried *Rlnn1* and/or *QRlnn.lrc-2B* and 3) develop ILW-derived advanced breeding lines (ABL) with resistance to both RLN. Seven *P. neglectus*-resistant genotypes were identified, with five that had potentially novel resistance. Subsequently, six breeding lines that were resistant to both RLN were developed by crossing six ILW with Australian cultivars and selecting for resistance in each generation. Both the ILWs and ABLs will be valuable genetic resources for wheat breeders to develop cultivars with dual resistance to better manage mixed RLN populations with novel *P. neglectus* resistance that potentially is not linked with yellow flour colour.

Supplementary information referenced in this chapter is documented in Appendix B.

Received: 3 June 2022 | Accepted: 4 July 2022

DOI: 10.1111/ppa.13616

Plant Pathology WILEY

ORIGINAL ARTICLE

Discovery of resistance to *Pratylenchus neglectus* among *P. thornei*-resistant Iranian landrace wheats and the introgression of both resistances into advanced breeding lines

Jason G. Sheedy | Jing Lin | John P. Thompson

University of Southern Queensland, Centre for Crop Health, Toowoomba, Australia

Correspondence
Jason G. Sheedy, University of Southern Queensland, Centre for Crop Health, Toowoomba, QLD, Australia.
Email: jason.sheedy@usq.edu.au

Funding information
Department of Agriculture and Fisheries, Queensland Government, Grant/Award Number: Broadacre Cropping Initiative; Department of Agriculture and Fisheries; Grains Research and Development Corporation, Grant/Award Number: DAQ00171 and USQ00019; University of Southern Queensland

Abstract

Root-lesion nematodes (RLNs) *Pratylenchus thornei* and *P. neglectus* are globally important pathogens of cereal and pulse crops. These RLNs can occur together in farming systems and must be managed concurrently to minimize substantial yield losses in intolerant crop cultivars. Australian wheat cultivars with resistance to *P. neglectus*, have either the *Rlnn1* resistance gene, which provides a high level of resistance but is linked with yellow flour colour that reduces cultivar marketability for bread production, or *QRlnn.lrc-2B*, which provides moderate resistance. We evaluated a collection of 91 *P. thornei*-resistant Iranian landrace wheats (ILWs) for their resistance to *P. neglectus* in four glasshouse experiments to (a) identify genotypes with resistance to both RLNs, (b) determine if any genotypes carried *Rlnn1* and/or *QRlnn.lrc-2B* and (c) develop ILW-derived advanced breeding lines (ABLs) with resistance to both RLNs. A factor analytic linear mixed model (FA-1) that explained 70% of the genetic variation, where the genetic correlations between the experiments ranged from 0.54 to 0.77, was used for the combined analysis of all experiments. Seven *P. neglectus*-resistant genotypes were identified, with five that had potentially novel resistance. Subsequently, six breeding lines that were resistant to both RLNs were developed by crossing six ILWs with Australian cultivars and selecting for resistance in each generation. Both the ILWs and ABLs will be valuable genetic resources for wheat breeders to develop cultivars with dual resistance, enabling better management of mixed RLN populations with novel *P. neglectus* resistance that potentially is not linked with yellow flour colour.

KEYWORDS
factor analytic linear mixed model, nematode resistance, plant breeding, *Pratylenchus neglectus*, *Pratylenchus thornei*, *Triticum aestivum*

1 | INTRODUCTION

The root-lesion nematodes (RLNs) *Pratylenchus thornei* and *P. neglectus* are significant pathogens of cereal and pulse crops. Wheat (*Triticum aestivum*) is an economically important host, in which *P. thornei* and *P. neglectus* have been reported to reduce the grain yields of intolerant cultivars by up to 60% (Thompson et al., 2021) and 20% (Taylor et al., 1999), respectively. Both species have a global

distribution (Castillo & Volvas, 2007) and are commonly reported to cohabit farmers' fields (Smiley et al., 2008; Thompson et al., 2010). RLNs are best managed through the incorporation into commercial crop cultivars of both genetic tolerance, that is the ability of a plant to minimize yield loss when grown in nematode-infested soil, and genetic resistance, that is the ability of a plant to inhibit nematode reproduction. Many quantitative trait loci (QTLs) for *P. thornei* resistance have been reported in wheat (Kumar et al., 2021; Schmidt et al., 2005; Zwart et al., 2010). Several studies have reported QTLs associated with *P. neglectus* resistance (Dababat et al., 2016; Mulki et al., 2013; Zwart et al., 2010); however, the QTLs often explained only a low proportion of genetic variation or they required validation in a genetically diverse panel or appropriate breeding populations before being suitable for use in marker-assisted selection (MAS).

The only catalogued wheat gene (McIntosh et al., 2020) conferring resistance to *P. neglectus* is *Rlnn1*, which is located on chromosome 7A (Williams et al., 2002). It has been widely used in Australian wheat germplasm and effectively controls *P. neglectus* populations (Vanstone et al., 1998). *Rlnn1* can be readily detected using the Kompetitive allele-specific PCR (KASP) markers *uat128* and *uat129* (Australian Wheat and Barley Molecular Marker project [AWBMMP], University of Adelaide, http://www.markers.net.au/); however, this gene is strongly linked with yellow flour colour (Jayatilake et al., 2013). Generally, cultivars with white flour are selected in wheat breeding programmes because yellow pigments are considered a detrimental quality factor for bread making (Zhang & Dubcovsky, 2008). The *phytoene synthase 1* (*Psy1*) gene contributes to yellow flour colour variation in wheat, with the *Psy-A1t* ("very yellow") allele strongly influencing yellow flour colour in Australian germplasm (Crawford et al., 2011). Jayatilake et al. (2013) hypothesized that the linkage between *Psy-A1t* and *Rlnn1* appeared unlikely to be broken because genotypes with *Rlnn1* resistance carry a chromosome rearrangement on 7AL that suppresses genetic recombination in that region.

The QTL *QRlnn.lrc-2B* is a synthetic-derived QTL associated with *P. neglectus* resistance and is located on chromosome 2B near the *P. thornei*-resistance QTL, *QRlnt.lrc-2B* (Zwart et al., 2010). It is not currently known if these two resistances are closely linked to separate genes or a single gene; however, wheat genotypes that carried *QRlnt.lrc-2B*, as detected by the *uat20* KASP marker (AWBMMP, University of Adelaide, http://www.markers.net.au/), were generally moderately resistant to both *P. thornei* and *P. neglectus* (Sheedy et al., 2017), suggesting that *uat20* was also reasonably diagnostic for *QRlnn.lrc-2B*.

Although several synthetic hexaploid wheat genotypes are resistant to both *P. thornei* and *P. neglectus* (Ogbonnaya et al., 2008; Thompson, 2008), the development of synthetic-derived breeding lines is not without its challenges. The inheritance of undesirable characteristics from the resistant parent, sometimes called linkage drag, is a common feature that can require several rounds of crossing and selection to properly manage (Rosyara et al., 2019). Landrace wheats offer an alternative genetic resource, that, while not without agronomic flaws, are a substantially improved starting point when developing germplasm suited to modern commercial production.

Iranian landrace wheats (ILWs) have proven to be a genetically diverse source of traits that have been beneficial for wheat improvement, including tolerances to abiotic stresses, disease resistance and end-use quality traits (Vikram et al., 2020). Notably, a collection of 274 ILWs were characterized for their resistance to *P. thornei* with 46% proving to be at least moderately resistant (Sheedy & Thompson, 2009). The objectives of this study were to (a) identify genotypes with resistance to both *P. thornei* and *P. neglectus*, (b) determine if any genotypes carried *Rlnn1* and/or *QRlnn.lrc-2B* and (c) develop advanced breeding lines (ABLs) with resistance to both *P. thornei* and *P. neglectus* through crosses between RLN-resistant ILWs and Australian commercial cultivars.

2 | MATERIALS AND METHODS

2.1 | Germplasm phenotyped for *P. neglectus* resistance

A collection of 91 wheat landraces, previously identified as resistant or moderately resistant to *P. thornei* (Sheedy & Thompson, 2009) and originating from 13 Iranian provinces (Figure 1), was characterized for *P. neglectus* resistance in four separate glasshouse experiments (Table S1). Twenty-two wheat standards ranging from resistant to susceptible to *P. neglectus* were included in each experiment for comparison. These standards included three wheat cultivars that carry *Rlnn1* (Excalibur, Wyalkatchem, Yenda), five synthetic hexaploid wheats (CPI133842, CPI133859, CPI133872, TAMD870167/AUS18913 and Yallaroi/AUS24152) and six CPI133872-derived doubled-haploid lines known to be moderately resistant to both *P. thornei* and *P. neglectus* and that carry *QRlnn.lrc-2B*/*QRlnt.lrc-2B* (Thompson, 2008; Zwart et al., 2010). The eight remaining standards ranged in resistance to *P. neglectus* from moderately susceptible (EGA Wylie, GBA Ruby, GBA Sapphire) to susceptible (Brookton, Cunningham, Janz, Machete, Petrie; Table S2).

The ILW genotypes were visually assessed for grain colour before and after soaking three seeds per genotype in 5% NaOH for 15 min at room temperature (20–25°C) (Baker, 1981). The 5% NaOH treatment intensified the kernel colour so that genetically red kernels turned deep red and genetically white kernels tuned a light cream colour, facilitating colour differentiation.

2.2 | Phenotyping procedure for *P. neglectus* resistance

All four glasshouse experiments characterizing the ILW collection followed the procedure described by Sheedy and Thompson (2009). Briefly, each experiment comprised 113 wheat genotypes (91 ILW, 22 standards) replicated three times in a randomized block design. Each genotype was grown in a 0.54 L pot suitable for bottom-watering

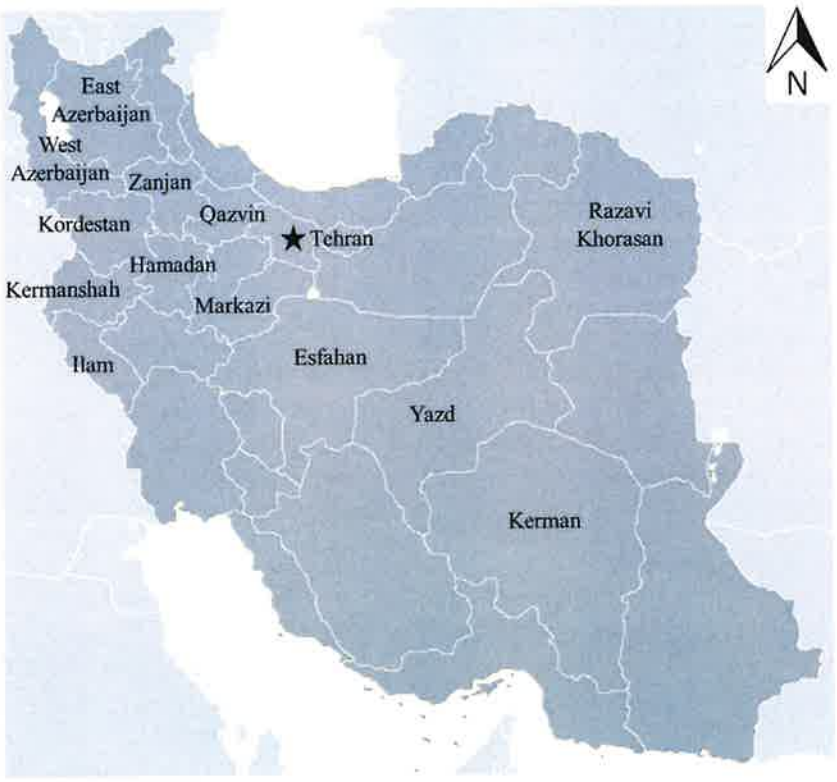


FIGURE 1 Iranian provinces from which the landrace wheat genotypes evaluated in this study originated. The number of genotypes evaluated per province, in parentheses after the province name, were as follows: East Azerbaijan (7); Esfahan (5); Hamadan (9); Ilam (14); Kerman (1); Kermanshah (29); Kordestan (13); Markazi (2); Qazvin (1); Razavi Khorasan (5); West Azerbaijan (3); Yazd (1); Zanjan (1).

containing 0.33 kg (oven-dry equivalent) of a vertosolic soil of the Irving clay soil association, pasteurized using aerated steam at 80°C for 45 min and fertilized with 1 g Osmocote Landscape Plus Micronutrients (21.2:1.9:5.7 NPK) slow-release fertilizer (Scotts Australia Pty Ltd). All experiments were grown on benches fitted with a bottom-watering system regulated by a float valve set to a water tension of 2 cm. Each pot was inoculated with 3300 *P. neglectus* at planting and plants were grown for 16 weeks. Soil and air temperatures were maintained at 20–25°C by the use of under-bench heating, evaporative coolers and shade cloth as required. Final *P. neglectus* population densities were determined by extraction from a 150 g subsample of homogenized soil and roots that had been processed so that soil aggregate diameter and root length were <1 cm. The extractions were performed using the Whitehead tray method at 22°C for 48 h (Whitehead & Hemming, 1965). Nematodes were collected on a 20 μm aperture sieve (Glenammer Engineering) and stored in 30 ml vials at 3°C until they were counted once using a 1 ml gridded nematode counting slide (Chalex LLC) under a compound microscope (40×; Olympus Corp.). Gravimetric soil moisture content was determined by drying a 100 g subsample of the soil and roots in a forced draught oven at 105°C for 48 h. Final nematode population density per kg of oven-dry soil and roots was calculated for each pot.

2.3 | Genotyping for *P. neglectus* resistance

Single plants of all 113 wheat genotypes (91 ILW, 22 standards) were grown under glasshouse conditions similar to those described for the phenotyping procedures but without the addition of *P. neglectus*. Four weeks after planting, up to five 2 cm-long sections of fresh leaf were harvested from each plant, placed in 1.5 ml microcentrifuge tubes and stored at −80°C. The leaf material was freeze dried using an Alpha 2–4 LDplus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH) and DNA extracted following the protocol of the Wizard Genomic DNA purification kit (Promega). The KASP markers *uat128* and *uat129* were used to detect *Rlnn1*, and *uat20* was used to detect *QRlnt.lrc.2B/QRlnn.lrc.2B*. Primers were synthesized by Macrogen Inc. and KASP markers were amplified following the protocols of the AWBMMP, University of Adelaide, (http://www.markers.net.au/) using a CFX384 real-time PCR machine (Bio-Rad). Data analysis was performed using Klustercaller software (LGC Genomics) to identify marker alleles. An ILW genotype was deemed to have the *Rlnn1* resistance gene if it was homozygous for both *uat128* and *uat129* marker alleles associated with resistance, and *QRlnt.lrc.2B/QRlnn.lrc.2B* if it was homozygous for the *uat20* allele associated with resistance.

2.4 | Development of advanced breeding lines

Specific cross combinations were made between six ILW genotypes (AUS28369, AUS28372, AUS28451, AUS28470, AUS28645 and AUS28677) and the *P. thornei*-tolerant Australian commercial cultivars EGA Gregory, EGA Wylie and Leichhardt and the *P. thornei*-resistant ABLs QT8343 and QT8447 to produce 14 F_1 populations. From these F_1 populations, 14 back-cross (BC_1F_1) populations were

developed using the same recurrent parents, and eight top-cross (TC_1F_1) populations were developed using the *P. thornei*-tolerant and moderately resistant wheat cv. Suntop. Lines from a subset of these populations were selected for resistance to *P. thornei*, or resistance to *P. neglectus*, or to both *P. thornei* and *P. neglectus* using mixed inoculum, in each generation until BC_1F_4. When selecting for *P. thornei* resistance, the methodology was similar to that described for the ILW collection except that plants were inoculated with 3300 *P. thornei* per pot. When selecting for resistance to both *P. thornei* and *P. neglectus*, each plant was inoculated with 1650 of each nematode species to provide a total of 3300 RLNs per pot. The use of mixed RLN inoculum facilitates the selection of wheat genotypes with resistance to both *P. thornei* and *P. neglectus* (authors' unpublished data). In the BC_1F_5 generation, 22 ILW-derived ABLs with resistance to *P. thornei*, or to *P. neglectus* or to both *P. thornei* and *P. neglectus* were selected, given accession numbers, and evaluated as fixed lines for subsequent replicated phenotyping.

2.5 | Phenotyping of ABLs for RLN resistance

The ILW-derived ABLs were characterized for their resistance to *P. thornei* and *P. neglectus* in separate replicated experiments using methodology similar to that previously described. All ABLs were evaluated twice for *P. thornei* resistance and compared with standard genotypes that ranged from resistant to susceptible (Table S3). For *P. neglectus*, all ABLs were evaluated once and the six genotypes with the best combination of resistance to *P. thornei* and *P. neglectus* were again evaluated for their *P. neglectus* resistance. In both *P. neglectus* resistance experiments, the ABLs were compared with standard genotypes that ranged from resistant to susceptible (Table S4).

2.6 | Statistical analysis of the Iranian landrace collection

For the ILW collection, final *P. neglectus* population densities were initially analysed for each of the four individual experiments to understand spatial trends and then combined into a multi-environment trial analysis based on a linear mixed model (LMM) where each experiment was considered a separate environment. A $\log_e(x)$ transformation, where x = nematodes per kg of soil and roots, was applied to the data to ensure homoscedastic variance over the range of fitted values. The overall mean for each experiment, crop type and any spatial trend within the trial were fitted as fixed effects while replicate effect and effects due to pot position for each experiment were fitted as random. To account for potential spatial variation across each experimental layout, the variance–covariance of the residuals were assumed to follow a separable AR1 by AR1 (AR1 = autoregressive structure of order 1) correlation structure in row and column directions and different residual variances were estimated for each experiment (Gilmour et al., 1997). Genotype effects within each experiment were fitted as random.

Initially, a simple model was considered for the combined analysis of all experiments assuming different genetic variance for each experiment and independent genetic effects between experiments. Then, a factor analytic (FA) model (Smith et al., 2001, 2015) was used, which allowed for a different genetic variance for each experiment and heterogeneous covariance (and hence correlation) between each pair of experiments. To determine the effective number of factors (order) in the FA model, multiple models were fitted by successively adding factors. To select the best model, a likelihood ratio test was applied to determine if the successively added factors significantly improved the model and minimized the Akaike information criterion (AIC).

The estimated FA loadings from the selected FA model were rotated using a principal component (PC) solution. Hence, the first PC axis (PA1) accounted for the maximum proportion of the genetic covariance of the data. The second PC axis (PA2) explained the next greatest proportion and so on for subsequent PAs while all PAs are orthogonal (Cullis et al., 2010). Furthermore, a genetic correlation matrix between pairs of experiments was produced.

Estimates of variance parameters were generated using restricted maximum likelihood (REML) estimation. The fixed effects in the model were estimated through best linear unbiased estimates (BLUEs) while for the random effects, empirical best linear unbiased predictions (E-BLUPs) were used (Cullis et al., 2010). The FA model produced predictions of genotypes in each experiment and an overall prediction for each genotype. Predictions for wheat genotype effects were rescaled by the addition of the estimate for the mean of each experiment, and also for the overall mean, in units of $\log_e$ (*P. neglectus* per kg of soil and roots) and then back-transformed by exponentiation to produce final *P. neglectus* population densities per kg of soil and roots. Pairwise comparisons were made to calculate the probability that overall genotype means were significantly different from those of selected standard genotypes. The FA model was fitted using the ASReml-R package (Butler et al., 2017) in the R software environment.

2.7 | Statistical analysis of the ABLs

For each experiment characterizing ABLs, final *P. neglectus* or *P. thornei* population densities were transformed by $\log_e(x+1)$, where x = nematodes per kg of soil and roots. These transformed values were analysed using a LMM that included the experiment mean and genotype as fixed-effect terms. Replicate, to account for nongenetic variation from experimental design blocks, was modelled as a random effect, as was the AR1 by AR1 structure. To detect linear trends or random effects across rows and columns, these terms were added to the model individually and tested for significant reductions in the model deviance using chi-squared principles. If an effect term was significant, it was added to the model and then rescaled BLUEs were calculated from the final model of each experiment and used to rank genotypes. Once ranked, the genotypes were divided into nine equal subranges and allocated a score on a 1–9 scale (Thompson et al., 2020). Average

scores and standard errors across experiments were calculated for each genotype and were converted to an alpha classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/). The LMM was performed using Genstat for Windows 19th Edition (VSN International).

3 | RESULTS

3.1 | *P. neglectus* resistance of the ILW collection

The pairwise genetic correlations among the four experiments ranged from 0.55 to 0.77, indicating that they were suitable for combined analysis. Initial evaluation of the LMM produced an AIC score of 1476. Sequentially adding factors to the model showed that the first factor (FA-1) accounted for 70% of the variance with an AIC score of 1411 and a significantly decreased log likelihood compared with the initial model. The second factor (FA-2) increased the variance explained to 74.5% but also produced a higher AIC score (1417) and did not significantly decrease the log likelihood compared with the FA-1 model. Because the second factor did not significantly improve the model, the FA-1 model was selected (Table 1). The wheat genotypes formed a continuous distribution ranging from resistant to very susceptible when they were ranked according to their E-BLUPs (Table 2). The majority of the ILW genotypes ranged from moderately susceptible (MS) to very susceptible (VS). However, two ILW genotypes (AUS28372 and AUS28369) were classified as resistant (R) and five (AUS28645, AUS28430, AUS28434, AUS28677 and AUS28399) were classified as moderately resistant-moderately susceptible (MRMS), a resistance level that would still effectively manage *P. neglectus* population densities in a cropping system (authors' unpublished data). In all, seven of 91 (8%) ILW genotypes in this collection were at least moderately resistant to *P. neglectus*. Given that this collection was a *P. thornei*-resistant subset of a larger collection of 274 genotypes, genotypes with at least moderate resistance to both *P. thornei* and *P. neglectus* comprised 2.6% of the collection. The most resistant genotype, AUS28372, produced *P. neglectus* population densities that were 89% less than the most susceptible genotype (AUS28321). On average, the resistant ILW genotypes ($n = 2$) and the MRMS ILW genotypes ($n = 5$) produced *P. neglectus* population densities that were respectively 88% and 73% less than that of the most susceptible genotype.

All seven of these genotypes with at least moderate resistance to *P. neglectus* originated from north-western Iran. Both of the resistant ILW genotypes (AUS28372 and AUS28369) originated from the West Azerbaijan province, three of the MRMS genotypes (AUS28645, AUS28430 and AUS28677) were from Kermanshah province and the remaining two MRMS genotypes (AUS28434 and AUS28399) were from Hamadan province. Despite this, there was no apparent geographic trend associated with the distribution of *P. neglectus* resistance in this collection, with similar means for *P. neglectus* population densities for genotypes collected from each province.

Visual grain colour assessment before soaking in the 5% NaOH solution concurred with the post-soaking assessment in 94% of samples. In all discordant samples, the grain was assessed as white before soaking and red after. The two resistant ILW genotypes were red-grained, while the five MRMS ILW genotypes were white-grained. Three of the six ILW genotypes used as donor parents in the development of ABLs were red-grained (AUS28369, AUS28372 and AUS28470) with the remaining three white-grained (AUS28451, AUS28645 and AUS28677). Across the collection, the red-grained genotypes ranged from R to susceptible–very susceptible (SVS) and the white-grained genotypes ranged from MRMS to VS with similar mean E-BLUPs of 11.94 and 12.03 respectively, indicating that grain colour was not associated with resistance (Table 2).

3.2 | Genotyping for known *P. neglectus* resistance loci

The two *P. neglectus*-resistant ILW genotypes (AUS28372 and AUS28369) carried *Rlnn1*. The five MRMS ILW genotypes carried neither *Rlnn1* nor *QRlnt.lrc-2B* (Table 2) and therefore, were putatively novel types of *P. neglectus* resistance. Two ILW genotypes (AUS28308 and AUS28323) carried *QRlnt.lrc-2B* but both were SVS to *P. neglectus*. As expected, *Rlnn1* was detected in Excalibur, Wyalkatchem and Yenda and *QRlnt.lrc-2B* was detected in the five wheat synthetic hexaploids and the six synthetic-derived doubled-haploid lines. All five *Rlnn1* genotypes were rated moderately resistant (MR) or better and had average *P. neglectus* population

TABLE 1 Restricted maximum likelihood (REML) estimates of experiment means, variance components, rotated loadings and the percentage variance accounted for (VAF) of four experiments analysed together using a factor analytic linear mixed model (FALMM)

Trial name	Mean *Pratylenchus neglectus*/kg of soil + roots (x)		Variance components		Rotated loadings	VAF (%)
	$Log_e(x)$	BTM	Genetic	Error		
Exp01	11.77	189,125	0.442	0.269	0.665	100.0
Exp02	10.10	42,006	0.178	0.812	0.298	49.8
Exp03	8.01	11,592	0.725	1.817	0.659	59.8
Exp04	11.16	119,974	0.221	0.754	0.362	59.4
FA-1 model						70.0

Abbreviation: BTM, back transformed mean.

TABLE 2 *Pratylenchus neglectus* population densities, resistance classifications and grain colour of a collection of Iranian landrace wheats, where *P. neglectus* population densities are empirical best linear unbiased predictions (E-BLUPS) from a combined analysis of four experiments

Genotype	Grain colour	*P. neglectus*/kg soil + root			Reduction (%)[a]	Classification[b]	Resistance gene/QTL[c]
		$\log_e(x)$	$p <$ Petrie[d]	BTM[e]			
AUS28372	Red	10.55	<0.001	38,147	89	R	*Rlnn1*
Yenda	White	10.61	<0.001	40,440	88	R	*Rlnn1*
AUS28369	Red	10.61	<0.001	40,581	88	R	*Rlnn1*
Excalibur	White	10.76	<0.001	47,154	86	R	*Rlnn1*
CPI133872_Janz DH074	Red	10.84	<0.001	51,087	85	RMR	*QRlnt.lrc-2B*
CPI133872_Janz DH083	Red	10.87	<0.001	52,367	84	RMR	*QRlnt.lrc-2B*
CPI133872	Red	10.87	<0.001	52,413	84	RMR	*QRlnt.lrc-2B*
CPI133872_Janz DH043	Red	10.93	<0.001	55,998	83	RMR	*QRlnt.lrc-2B*
CPI133872_Janz DH024	Red	11.09	<0.001	65,668	80	MR	*QRlnt.lrc-2B*
Wyalkatchem	White	11.11	<0.001	66,526	80	MR	*Rlnn1*
TAMD870167/ AUS18913	Red	11.13	<0.001	68,479	80	MR	*QRlnt.lrc-2B*
CPI133859	Red	11.15	<0.001	69,333	79	MR	*QRlnt.lrc-2B*
CPI133872_Janz DH001	Red	11.24	<0.001	75,820	77	MR	*QRlnt.lrc-2B*
CPI133842	Red	11.29	<0.001	79,713	76	MRMS	*QRlnt.lrc-2B*
AUS28645	White	11.33	<0.001	83,046	75	MRMS	
AUS28430	White	11.39	<0.001	88,175	74	MRMS	
AUS28434	White	11.45	0.002	94,244	72	MRMS	
AUS28677	White	11.47	0.002	95,862	71	MRMS	
AUS28399	White	11.50	0.003	98,568	71	MRMS	
AUS28302	White	11.55	0.004	103,744	69	MS	
AUS28294	White	11.55	0.011	103,990	69	MS	
AUS28723	White	11.59	0.006	107,540	68	MS	
GBA Ruby	White	11.63	0.012	112,742	66	MS	
AUS36669	Red	11.68	0.059	118,191	65	MS	
GBA Sapphire	White	11.68	0.017	118,737	65	MS	
AUS28326	White	11.70	0.015	120,616	64	MS	
AUS28703	White	11.71	0.016	121,399	64	MS	
AUS28452	White	11.71	0.016	122,135	64	MS	
AUS28401	White	11.72	0.017	122,897	63	MS	
AUS28295	White	11.72	0.018	123,077	63	MS	
Yallaroi/AUS24152	Red	11.72	0.017	123,466	63	MS	*QRlnt.lrc-2B*
CPI133872_Janz DH010	Red	11.73	0.018	124,093	63	MS	*QRlnt.lrc-2B*
AUS28304	Red	11.75	0.023	127,133	62	MS	
AUS28309	White	11.76	0.024	127,713	62	MS	
AUS28649	White	11.78	0.028	130,947	61	MSS	
EGA Wylie	White	11.80	0.039	133,408	60	MSS	
AUS28714W	White	11.82	0.037	136,067	60	MSS	

TABLE 2 (Continued)

Genotype	Grain colour	*P. neglectus*/kg soil + root			Reduction (%)[a]	Classification[b]	Resistance gene/QTL[c]
		$\log_e(x)$	$p <$ Petrie[d]	BTM[e]			
AUS28706	Red	11.83	0.038	136,743	59	MSS	
AUS28681	White	11.85	0.047	140,510	58	MSS	
AUS28718	White	11.86	0.047	141,048	58	MSS	
AUS28424	White	11.87	0.050	142,475	58	MSS	
AUS28333	White	11.87	0.051	142,789	58	MSS	
AUS28415	Red	11.87	0.051	142,809	57	MSS	
AUS28668	White	11.88	0.053	143,735	57	MSS	
AUS28284	White	11.88	0.055	144,504	57	MSS	
AUS28329	White	11.89	0.057	145,260	57	MSS	
AUS28338	Red	11.89	0.059	145,958	57	MSS	
AUS28451	White	11.90	0.061	146,675	56	MSS	
AUS28727	White	11.90	0.062	146,921	56	MSS	
AUS28426	White	11.90	0.063	147,497	56	MSS	
AUS28398	White	11.91	0.098	148,844	56	MSS	
AUS28728	White	11.92	0.069	149,791	55	MSS	
Brookton	White	11.96	0.087	156,070	54	MSS	
AUS28457	White	11.96	0.089	156,235	53	MSS	
AUS28400	White	11.97	0.128	158,444	53	MSS	
AUS28290	White	11.98	0.101	159,709	52	MSS	
AUS28301	White	11.98	0.102	160,129	52	MSS	
AUS28413	Red	11.99	0.108	161,830	52	MSS	
AUS28667	White	12.00	0.110	162,444	52	MSS	
AUS28443	White	12.00	0.113	163,399	51	S	
AUS28714R	Red	12.01	0.125	165,046	51	S	
AUS28342	Red	12.03	0.130	167,511	50	S	
AUS28630	White	12.05	0.142	170,506	49	S	
AUS28391	White	12.06	0.148	172,303	49	S	
AUS28322	White	12.06	0.162	172,565	49	S	
AUS28632	White	12.06	0.150	172,577	49	S	
AUS28712	Red	12.08	0.179	176,432	47	S	
AUS28462	White	12.08	0.170	177,140	47	S	
AUS28690	White	12.11	0.191	181,710	46	S	
AUS28305	White	12.12	0.202	183,969	45	S	
Cunningham	White	12.12	0.233	184,075	45	S	
AUS28389	White	12.14	0.220	187,508	44	S	
AUS28631	White	12.14	0.220	187,544	44	S	
AUS28442	Red	12.16	0.233	190,141	43	S	
AUS28687	Red	12.16	0.233	190,155	43	S	
AUS28311	White	12.16	0.235	190,425	43	S	
AUS28433	White	12.16	0.239	191,389	43	S	
AUS28693	White	12.16	0.242	191,741	43	S	
AUS28470	Red	12.17	0.243	192,153	43	S	
AUS28402	White	12.17	0.249	192,743	43	S	
AUS28334	Red	12.17	0.252	193,789	42	S	

(Continues)

TABLE 2 (Continued)

Genotype	Grain colour	*P. neglectus*/kg soil + root			Reduction (%)[a]	Classification[b]	Resistance gene/QTL[c]
		$\log_e(x)$	$p <$ Petrie[d]	BTM[e]			
AUS28635	White	12.18	0.261	195,597	42	S	
Janz	White	12.18	0.273	195,699	42	S	
AUS28417	White	12.20	0.290	199,225	41	S	
Machete	White	12.20	0.309	199,650	41	S	
AUS28638	White	12.23	0.310	204,619	39	S	
AUS28644	White	12.23	0.307	204,650	39	S	
AUS28686	Red	12.23	0.322	205,231	39	S	
AUS28315	Red	12.23	0.315	205,594	39	S	
AUS28308	White	12.26	0.356	211,539	37	SVS	*QRlnt.Irc-2B*
AUS28423	White	12.27	0.369	214,127	36	SVS	
AUS28392	Red	12.28	0.366	214,694	36	SVS	
AUS28657	White	12.28	0.370	215,559	36	SVS	
AUS28307	White	12.28	0.373	216,061	36	SVS	
AUS28291	Red	12.29	0.380	217,239	35	SVS	
AUS28407	White	12.29	0.385	218,123	35	SVS	
AUS28699	White	12.30	0.391	219,202	35	SVS	
AUS28666	White	12.33	0.428	225,844	33	SVS	
AUS28387	Red	12.34	0.443	228,668	32	SVS	
AUS28384	White	12.34	0.446	229,128	32	SVS	
AUS28671	White	12.35	0.460	231,857	31	SVS	
AUS28642	White	12.35	0.460	231,868	31	SVS	
AUS28336	Red	12.36	0.470	233,575	30	SVS	
Petrie	White	12.39	na	239,265	29	SVS	
AUS28408	White	12.40	0.515	242,074	28	SVS	
AUS28689	White	12.40	0.515	242,089	28	SVS	
AUS28332	White	12.41	0.530	244,967	27	SVS	
AUS28323	White	12.46	0.589	256,877	24	SVS	*QRlnt.Irc-2B*
AUS28700	White	12.49	0.630	265,497	21	VS	
AUS28375	White	12.51	0.648	269,912	20	VS	
AUS28366	White	12.51	0.650	272,051	19	VS	
AUS28701	White	12.56	0.713	285,725	15	VS	
AUS28321	White	12.72	0.860	335,985	na	VS	

[a]Percentage reduction in final *P. neglectus* population densities compared with the most susceptible genotype (AUS28321).

[b]Genotype classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/) using the method of Thompson et al. (2020).

[c]Resistance gene/quantitative trait locus (QTL) status determined by the presence of markers *uat128* and *uat129* for *Rlnn1* and *uat20* for *QRlnt.Irc-2B* (Australian Wheat and Barley Molecular Marker project, University of Adelaide, http://www.markers.net.au/).

[d]Probability that genotypes produced significantly lower final *P. neglectus* population densities than the susceptible standard wheat cv. Petrie.

[e]Back-transformed means.

densities that were 86% less than the most susceptible genotype. Nine of 11 synthetic and synthetic-derived genotypes were rated MRMS or better. The remaining two genotypes (Yallaroi/AUS24152, CPI133872_Janz DH010) were MS. On average, the synthetic and synthetic-derived genotypes that carried *QRlnt.Irc-2B* had *P. neglectus* population densities that were 78% less than the most susceptible genotype.

3.3 | Development of ILW-derived advanced breeding lines

Thirteen of the 22 ILW-derived ABLs were at least MRMS to *P. thornei*, seven were at least MRMS to *P. neglectus* and six of these had combined resistance to both *P. thornei* and *P. neglectus* (Table S5). Of the seven *P. neglectus*-resistant genotypes, one (2016FL085)

was AUS28677-derived and probably carries novel *P. neglectus* resistance. The remaining six genotypes were AUS28369-derived (*Rlnn1*-type) and on average had better combined resistance to both *P. thornei* and *P. neglectus* than current commercial cultivars (Table 3).

4 | DISCUSSION

ILWs are a genetically diverse resource that have the potential to contribute resistances and tolerances to biotic and abiotic stresses, and to improve the quality of end-use products of wheat cultivars produced in modern breeding programmes. We identified seven ILW genotypes that are resistant to both *P. thornei* and *P. neglectus*. Five of these genotypes do not carry the known *P. neglectus* resistance loci *Rlnn1* or *QRlnt.lrc-2B* and are probable novel sources of resistance. Subsequently, we developed six ABLs that combined effective levels of ILW-derived resistance to the RLNs *P. neglectus* and *P. thornei*. These genotypes have the parentage AUS28369/2*EGA Wylie where AUS28369 is an ILW that carries *Rlnn1* and is rated as resistant–moderately resistant (RMR) to *P. neglectus* and MR to *P. thornei*, and EGA Wylie is an Australian wheat cultivar that is MSS but moderately tolerant to *P. thornei* (Thompson et al., 2021). All six ABLs have resistance to both nematode species that is phenotypically superior to commercial cultivars and are agronomically similar to their recurrent parent EGA Wylie.

The five ILWs with putatively novel *P. neglectus* resistance produced final *P. neglectus* population densities that were, on average, 73% less than the most susceptible genotype. This was a lower percentage reduction than genotypes that carried the *Rlnn1* resistance gene (86%) and the synthetic and synthetic-derived genotypes that carried *QRlnt.lrc-2B* (78%), but would still effectively manage *P. neglectus* population densities in cropping systems. They also offer the prospect of *P. neglectus* resistance that is not linked with the yellow flour colour defect associated with *Rlnn1*.

Notably, two ILW genotypes carried *QRlnt.lrc-2B* but were phenotypically SVS to *P. neglectus*. The presence of *QRlnt.lrc-2B* did reliably predict resistance to *P. neglectus* in the synthetic and synthetic-derived genotypes but was not effective in discriminating *P. neglectus* resistant and susceptible phenotypes in the ILW collection. This suggests that the resistances to *P. thornei* and *P. neglectus* on chromosome 2B have separate genetic controls, and although closely linked in synthetic wheats (Zwart et al., 2010), recombination has occurred in that region to produce ILW genotypes resistant to *P. thornei* but not to *P. neglectus*.

Of the 91 ILW genotypes evaluated, 8% were at least moderately resistant to *P. neglectus*. Considering they were a component of a larger collection that was evaluated for *P. thornei* resistance (Sheedy &

TABLE 3 Average *Pratylenchus thornei* and *P. neglectus* resistance scores and standard errors of Iranian landrace wheat-derived advanced breeding lines (ABLs) compared with their parents and Australian commercial wheat cultivars

	P. thornei resistance				*P. neglectus* resistance			
Genotype	Score[a]	SE[b]	Rating[c]	n[d]	Score	SE	Rating	n
2016FL076	8.27	0.11	R	2	7.89	0.51	RMR	2
2016FL072	7.71	0.31	RMR	2	6.93	0.27	MR	2
USQW19030	7.77	0.57	RMR	2	6.46	0.35	MR	2
AUS28369	6.44	0.59	MR	4	7.25	0.60	RMR	7
USQW19031	7.16	0.60	RMR	2	5.77	0.01	MRMS	2
USQW19032	7.07	0.71	RMR	2	5.77	1.08	MRMS	2
2016FL073	7.51	0.07	RMR	2	5.05	0.63	MRMS	2
Yenda[e]	3.73	0.48	MSS	6	6.68	0.14	MR	62
Suntop[f]	5.63	0.29	MRMS	19	2.87	0.45	S	5
Gauntlet[f]	5.72	0.23	MRMS	26	2.70	0.38	S	5
EGA Wylie	3.28	0.32	MSS	23	3.47	0.17	MSS	56
Sunprime[g]	1.63	0.63	SVS	3	2.86	0.86	S	2
Strzelecki[g]	1.93	0.18	SVS	48	2.22	0.39	S	12

Note: The ABLs have the parentage AUS28369/2*EGA Wylie where AUS28369 is an Iranian landrace wheat and EGA Wylie is an Australian wheat cultivar.

[a]Average resistance score using the method of Thompson et al. (2020). Scores use a 1 to 9 scale where 1 = very susceptible and 9 = resistant.

[b]Standard error of the average resistance score.

[c]Genotype classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/).

[d]Number of experiments used to determine *P. thornei* and *P. neglectus* resistance ratings.

[e]Australian wheat cultivar with the best level of *P. neglectus* resistance commercially available.

[f]Australian wheat cultivars with the best level of *P. thornei* resistance commercially available.

[g]*P. thornei*- and *P. neglectus*-susceptible Australian commercial wheat cultivars.

Thompson, 2009), only 2.6% (7 of 274) of the total genotypes were at least moderately resistant to both *P. thornei* and *P. neglectus*. This frequency is substantially lower than the 41% (32 of 78) of ILW genotypes reported by Thompson et al. (2016) but is similar to the frequency of 2.4% (4 of 169) of synthetic hexaploid genotypes reported to be at least moderately resistant to both *P. thornei* and *P. neglectus* (Ogbonnaya et al., 2008). The findings of this study, and others, indicate that resistance to one nematode species does not necessarily confer resistance to another nematode species, even if those species are closely related (Ogbonnaya et al., 2008). Consequently, genotypes that are resistant to multiple nematode species are relatively rare and of substantial value for crop improvement efforts. Three ILW genotypes (AUS28369, AUS28370 and AUS28430) were characterized as at least moderately resistant to both *P. thornei* and *P. neglectus* in this study and by Thompson et al. (2016), with the remaining common genotypes characterized as MS to SVS to *P. neglectus* in this study.

Sheedy and Thompson (2009) hypothesized that *P. neglectus* resistance, in addition to the *P. thornei* resistance they reported, may be found in the ILW collection due to those RLN species being the most commonly recovered from Iranian wheat fields (Pourjam et al., 1999). Given that genotypes with resistance to both *P. thornei* and *P. neglectus* were identified in this collection, the findings of this research do support the hypothesis that a proportion of genotypes that evolve in an environment where diseases are endemic will probably carry genetic resistance to these diseases. This association is important when considering that on-farm nematode populations are often composed of several RLN species (Mokrini et al., 2018a; Thompson et al., 2010). Therefore, to manage mixed RLN populations effectively, it is necessary to develop wheat genotypes with genetic resistance to the prevailing combinations of RLN species.

Previously, only synthetic-derived wheat genotypes had been reported with resistance to both *P. thornei* and *P. neglectus* (Ogbonnaya et al., 2008; Thompson, 2008) and an Iraqi wheat landrace (AUS4930 syn. Iraq 48)-derived ABL produced by the International Maize and Wheat Improvement Center (CIMMYT) with resistance to both *P. thornei* and *P. penetrans* (Mokrini et al., 2018b). Recently, a core set of 305 ILW genotypes that captures 93% of the rare alleles of the entire ILW collection (about 6800 genotypes) was compiled (Vikram et al., 2020). Only three of these ILW genotypes were assessed for their resistance to *P. neglectus* in this study and 13 for their resistance to *P. thornei* by Sheedy and Thompson (2009). It is likely that genotypes with resistance to one or more of the eight *Pratylenchus* spp. that have been reported from cereal-producing regions of Iran (Mokrini et al., 2018a) would be present in Vikram's core set. These genotypes would be of value for breeding programmes to develop wheat cultivars that could manage the various combinations of *Pratylenchus* spp. encountered by farmers around the world.

Starting the breeding process with a single donor genotype that contributes resistances to multiple diseases would facilitate the rapid development of commercial wheat cultivars with resistance to diseases, when compared with the sequential incorporation of resistances to several diseases. Several synthetic wheat-derived ABLs that are resistant to both *P. thornei* and *P. neglectus* have been developed and delivered to Australian plant breeders (Sheedy et al., 2017) so that the identified resistances could be incorporated into commercial wheat cultivars. The ILW genotypes identified, and the ABLs developed in this research, offer plant breeders genetically diverse alternatives to the RLN resistances currently available, while maintaining the advantage of breeding with a single genotype resistant to both *P. thornei* and *P. neglectus*.

AUTHOR CONTRIBUTIONS

J.G.S. conceived and supervised the experiments, developed the breeding populations, conducted statistical analyses and wrote the manuscript. J.P.T. initially acquired the landrace collection, conceived the experiments and contributed to scientific planning. J.L. contributed to the phenotyping experiments, development of breeding populations and genetic marker screening. All authors contributed to the final manuscript and approved its publication.

ACKNOWLEDGEMENTS

The authors thank the University of Southern Queensland (USQ) staff Mei Wang, Hannah Rostad, and Michelle Thompson for technical assistance in the overall conduct of the experiments, Neil Robinson for assistance in the development of breeding populations, and Dr Rebecca Zwart for assistance with the KASP marker assays. This research was co-funded by the Grains Research and Development Corporation (GRDC) and the Queensland Department of Agriculture and Fisheries Broadacre Cropping Initiative (BACI) in partnership with USQ through projects DAQ00171 and USQ00019. Open access publishing facilitated by University of Southern Queensland, as part of the Wiley - University of Southern Queensland agreement via the Council of Australian University Librarians. Open access publishing facilitated by University of Southern Queensland, as part of the Wiley - University of Southern Queensland agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

The authors declare no conflict of interest. Reference in this document to any specific commercial product, process or service, or the use of any trade, firm or corporation name is for the information and convenience of the reader, and does not constitute endorsement, recommendation or favouring by the authors or their affiliates.

DATA AVAILABILITY STATEMENT

The genotypes evaluated in this research are available upon request from the Australian Grains Genebank, USDA National Small Grains Collection or International Maize and Wheat Improvement Center (CIMMYT). The data remain the intellectual property of the funding partners.

ORCID

Jason G. Sheedy https://orcid.org/0000-0001-6569-5016
Jing Lin https://orcid.org/0000-0001-8852-5335
John P. Thompson https://orcid.org/0000-0002-2097-5695

REFERENCES

Baker, R.J. (1981) Inheritance of seed coat colour in eight spring wheat cultivars. *Canadian Journal of Plant Science*, 61, 719–721.

Butler, D.G., Cullis, B.R., Gilmour, A.R., Gogel, B.J. & Thompson, R. (2017) *ASReml-R reference manual version 4*. Hemel Hempstead, UK: VSN International Ltd.

Castillo, P. & Vovlas, N. (2007) Pratylenchus *(Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management*. In: *Nematology monographs and perspectives, volume 6 series 9*. Eds DJ Hunt and RN Perry. Leiden, Netherlands: Brill.

Crawford, A.C., Stefanova, K., Lambe, W., McLean, R., Wilson, R., Barclay, I. et al. (2011) Functional relationships of phytoene synthase 1 alleles on chromosome 7A controlling flour colour variation in selected Australian wheat genotypes. *Theoretical and Applied Genetics*, *123*, 95–108.

Cullis, B.R., Smith, A.B., Beeck, C.P. & Cowling, W.A. (2010) Analysis of yield and oil from a series of canola breeding trials. Part II. Exploring variety by environment interaction using factor analysis. *Genome*, *53*, 1002–1016.

Dababat, A.A., Ferney, G.-B.H., Erginbas-Orakci, G., Dreisigacker, S., Imren, M., Toktay, H. et al. (2016) Association analysis of resistance to cereal cyst nematodes (*Heterodera avenae*) and root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) in CIMMYT advanced spring wheat lines for semi-arid conditions. *Breeding Science*, *66*, 692–702.

Gilmour, A.R., Cullis, R.R. & Verbyla, A.P. (1997) Accounting for natural and extraneous variation in the analysis of field experiments. *Journal of Agricultural, Biological and Environmental Statistics*, *2*, 269–293.

Jayatilake, D.V., Tucker, E.J., Bariana, H., Kuchel, H., Edwards, J., McKay, A.C. et al. (2013) Genetic mapping and marker development for resistance of wheat against the root lesion nematode *Pratylenchus neglectus*. *BMC Plant Biology*, *13*, 230.

Kumar, D., Sharma, S., Sharma, R., Pundir, S., Kumar Singh, V., Chaturvedi, D. et al. (2021) Genome-wide association study in hexaploid wheat identifies novel genomic regions associated with resistance to root lesion nematode (*Pratylenchus thornei*). *Scientific Reports*, *11*, 3572.

McIntosh, R.A., Dubcovsky, J., Rogers, W.J., Xia, X.C. & Raupp, W.J. (2020) Catalogue of gene symbols for wheat. *Annual Wheat Newsletter*, *66*, 109–128. Available at: https://wheat.pw.usda.gov/GG3/sites/default/files/Catalogue%20of%20Gene%20Symbols%20for%20Wheat%20-%20supplement2020.pdf [Accessed 19 July 2022].

Mokrini, F., Viaene, N., Waeyenberge, L., Dababat, A.A. & Moens, M. (2018a) Root-lesion nematodes in cereal fields: importance, distribution, identification, and management strategies. *Journal of Plant Diseases and Protection*, *126*, 1–11.

Mokrini, F., Viaene, N., Waeyenberge, L., Dababat, A.A. & Moens, M. (2018b) Investigation of resistance to *Pratylenchus penetrans* and *P. thornei* in international wheat lines and its durability when inoculated together with the cereal cyst nematode *Heterodera avenae*, using qPCR for nematode quantification. *European Journal of Plant Pathology*, *151*, 875–889.

Mulki, M.A., Jighly, A., Ye, G., Emebiri, L.C., Moody, D., Ansari, O. et al. (2013) Association mapping for soilborne pathogen resistance in synthetic hexaploid wheat. *Molecular Breeding*, *31*, 299–311.

Ogbonnaya, F.C., Imtiaz, M., Bariana, H.S., McLean, M., Shankar, M.M., Hollaway, G.J. et al. (2008) Mining synthetic hexaploids for multiple disease resistance to improve bread wheat. *Australian Journal of Agricultural Research*, *59*, 421–431.

Pourjam, E., Kheiri, A., Geraert, E. & Alizadeh, A. (1999) Variations in Iranian populations of *Pratylenchus neglectus* and *P. thornei* (Nematoda: Pratylenchidae). *Iranian Journal of Plant Pathology*, *35*, 23–27.

Rosyara, U., Kishii, M., Payne, T., Sansaloni, C.P., Singh, R.P., Braun, H.-J. et al. (2019) Genetic contribution of synthetic hexaploid wheat to CIMMYT's spring bread wheat breeding germplasm. *Scientific Reports*, *9*, 12355.

Schmidt, A.L., McIntyre, C.L., Thompson, J.P., Seymour, N.P. & Liu, C.J. (2005) Quantitative trait loci for root lesion nematode (*Pratylenchus thornei*) resistance in middle-eastern landraces and their potential for introgression into Australian bread wheat. *Australian Journal of Agricultural Research*, *56*, 1059–1068.

Sheedy JG, Robinson NA, Lin J, Reen RA, Clewett TG & Thompson JP (2017) Prebreeding to produce wheat cultivars that combine root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) resistance and tolerance. In: *Australian wheat breeders assembly 2017*. August 2017, Sydney, Australia, pp. 23–25.

Sheedy, J.G. & Thompson, J.P. (2009) Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. *Australasian Plant Pathology*, *38*, 478–489.

Smiley, R.W., Sheedy, J.G. & Easley, S.A. (2008) Vertical distribution of *Pratylenchus* spp. in silt loam soil and Pacific northwest dryland crops. *Plant Disease*, *92*, 1662–1668.

Smith, A.B., Cullis, B.R. & Thompson, R. (2001) Analysing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. *Biometrics*, *57*, 1138–1147.

Smith, A.B., Ganesalingam, A., Kuchel, H. & Cullis, B.R. (2015) Factor analytic mixed models for the provision of grower information from national crop variety testing programs. *Theoretical and Applied Genetics*, *128*, 55–72.

Taylor, S.P., Vanstone, V.A., Ware, A.H., McKay, A.C., Szot, D. & Russ, M.H. (1999) Measuring yield loss in cereals caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) with and without nematicide. *Australian Journal of Agricultural Research*, *50*, 617–622.

Thompson, A.L., Smiley, R.W., Paulitz, T.C. & Garland-Campbell, K. (2016) Identification of resistance to *Pratylenchus neglectus* and *Pratylenchus thornei* in Iranian landrace accessions of wheat. *Crop Science*, *56*, 654–672.

Thompson, J.P. (2008) Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. *Australian Journal of Agricultural Research*, *59*, 432–446.

Thompson, J.P., Clewett, T.G., Sheedy, J.G., Reen, R.A., O'Reilly, M.M. & Bell, K.L. (2010) Occurrence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in the northern grain region of Australia. *Australasian Plant Pathology*, *39*, 254–264.

Thompson, J.P., Sheedy, J.G. & Robinson, N.A. (2020) Resistance of wheat genotypes to root-lesion nematode (*Pratylenchus thornei*) can be used to predict final nematode population densities, crop greenness, and grain yield in the field. *Phytopathology*, *110*, 505–516.

Thompson, J.P., Sheedy, J.G., Robinson, N.A. & Clewett, T.G. (2021) Tolerance of wheat (*Triticum aestivum*) genotypes to root-lesion nematode (*Pratylenchus thornei*) in the subtropical grain region of eastern Australia. *Euphytica*, *217*, 48.

Vanstone, V.A., Rathjen, A.J., Ware, A.H. & Wheeler, R.D. (1998) Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. *Australian Journal of Experimental Agriculture*, *38*, 181–188.

Vikram, P., Franco, J., Burgueño, J., Li, H., Sehgal, D., Saint-Pierre, C. et al. (2020) Strategic use of Iranian bread wheat landrace accessions for genetic improvement: core set formulation and validation. *Plant Breeding*, *140*, 87–99.

Whitehead, A.G. & Hemming, J.R. (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, *55*, 25–38.

Williams, K.J., Taylor, S.P., Bogacki, P., Pallotta, M., Bariana, H.S. & Wallwork, H. (2002) Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. *Theoretical and Applied Genetics*, *104*, 874–879.

Zhang, W. & Dubcovsky, J. (2008) Association between allelic variation at the *phytoene synthase 1* gene and yellow pigment content in the wheat grain. *Theoretical and Applied Genetics*, *116*, 635–645.

Zwart, R.S., Thompson, J.P., Milgate, A.W., Bansal, U.K., Williamson, P.M., Raman, H. et al. (2010) QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Molecular Breeding*, *26*, 107–124.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sheedy, J. G., Lin, J. & Thompson, J. P. (2022). Discovery of resistance to *Pratylenchus neglectus* among *P. thornei*-resistant Iranian landrace wheats and the introgression of both resistances into advanced breeding lines. *Plant Pathology*, *00*, 1–12. https://doi.org/10.1111/ppa.13616

4.1 Implications of Chapter 4

Wheat genotypes that are resistant to multiple pathogens, particularly phytoparasitic nematodes, are relatively rare and valuable to wheat improvement programs. *Pratylenchus thornei* and *P. neglectus* commonly occur together in farmers' fields, therefore the development of commercial wheat cultivars that are resistant to both would be beneficial. Resistance to *P. thornei* was identified among ILW genotypes (Sheedy and Thompson 2009), and I have reported the identification of novel *P. thornei* resistance QTL identified among ILW genotypes in Chapter 3. However, those genotypes had not been characterised for their resistance to *P. neglectus* to determine if genotypes with resistance to both RLN were present in the ILW collection. In Chapter 4, seven ILW genotypes that were resistant to both *P. thornei* and *P. neglectus* were identified. Five of those genotypes did not carry the previously identified *Rlnn1* gene or *QRlnn.lrc-2B* QTL and were likely novel sources of *P. neglectus* resistance. Subsequently, six ILW-derived ABLs that were resistant to both RLN, and suitable for use in wheat improvement programs, were developed. It would be valuable to develop mapping populations derived from the ILW genotypes that carry the novel *P. neglectus* resistances and to undertake QTL analysis of them. This would improve our knowledge of the genetics of the resistances and would produce markers that could be used for marker-assisted selection by plant breeders. An alternative approach would be to characterise Vikram's ILW core set (Vikram et al. 2020) for resistance to *P. neglectus* and analyse the data using a GWAS framework. This approach would identify *P. neglectus*-resistant genotypes and markers that could be used to select for those resistances without requiring the time and expense of developing custom breeding populations. Either approach would facilitate the identification of novel *P. neglectus* resistance genes that are not linked with the yellow flour colour defect associated with *Rlnn1*.

CHAPTER 5: PAPER 3 – THE FIRST TRANSFER OF RESISTANCE TO THE ROOT-LESION NEMATODE (*Pratylenchus thornei*) FROM DIPLOID EINKORN (*Triticum monococcum*) TO HEXAPLOID WHEAT (*T. aestivum*)

The first transfer of resistance to the root-lesion nematode (*Pratylenchus thornei*) from diploid Einkorn (*Triticum monococcum*) to hexaploid wheat (*T. aestivum*). Jason G Sheedy, Jing Lin, Mandy Christopher and John P Thompson. This chapter was prepared according to the instructions to authors given by the journal *Crop Science*.

In this study four recombinant inbred line (RIL) wheat populations (RILPs) were produced that were derived from RLN-resistant einkorn (*T. monococcum*; diploid A^mA^m-genome) accessions. These populations were then evaluated for resistance in the BC_1F_2, BC_1F_6, BC_3F_5 and/or BC_3F_6 generations and the effective numbers of resistance genes were determined using segregation and quantitative genetic analyses. *Pratylenchus thornei*-resistant genotypes that likely carry novel A-genome resistances were identified in all populations. Both segregation and quantitative genetics analyses indicated 1 or 2 genes controlled resistance in all populations and that RLN population densities were reduced by up to 99% when compared with the most susceptible RIL. These RLN-resistant RILs will provide plant breeders with novel genetic resources to develop genotypes with resistance superior to that currently available.

Supplementary information referenced in this chapter is documented in Appendix C.

Transfer of Pt resistance from einkorn to wheat

Core ideas (3-5 impact statements, 95 char max for each)

1. *Pratylenchus thornei* resistance was successfully transferred from einkorn to wheat.
2. Einkorn-derived resistance to *Pratylenchus thornei* was controlled by 1–2 additive genes.
3. Einkorn-derived RILs will deliver novel A-genome *P. thornei* resistance for wheat improvement.

The first transfer of resistance against root-lesion nematode (*Pratylenchus thornei*) from diploid einkorn (*Triticum monococcum*) to hexaploid wheat (*T. aestivum*)

Jason G Sheedy[1,3], Jing Lin[1], Mandy Christopher[2] and John P Thompson[1]

[1] University of Southern Queensland, Centre for Crop Health, Toowoomba, QLD, Australia

[2] Queensland Department of Agriculture and Fisheries, Toowoomba, QLD, Australia

[3] Corresponding Author. Email: jason.sheedy@usq.edu.au Telephone: +61 7 46311185

Abbreviations: ABL, advanced breeding line; AR1, autoregressive structure of order 1; BLUP, best linear unbiased prediction; GWAS, Genome-wide association studies; h^2, heritability LMM, linear mixed model; MR, moderately resistant; MRMS, moderately resistant – moderately susceptible; MS, moderately susceptible; MSS, moderately susceptible – susceptible; QTL, quantitative trait loci; R, resistant; REML, restricted maximum likelihood; RIL, recombinant inbred line; RILP, recombinant inbred line population; RLN, root-lesion nematode (*Pratylenchus thornei*); RMR, resistant – moderately resistant; S, susceptible; SSD, single seed descent; SVS, susceptible – very susceptible; VS, very susceptible.

ABSTRACT

The root-lesion nematode (RLN; *Pratylenchus thornei*) is a serious constraint of global wheat production and can reduce grain yield by up to 60%. Breeding resistance into wheat (*Triticum aestivum*; hexaploid BBA^uA^uDD-genomes) genotypes has proven an effective method to minimise these losses. We produced four recombinant inbred line (RIL) wheat populations (RILPs) derived from RLN-resistant einkorn (*T. monococcum*; diploid A^mA^m-genome)

accessions and evaluated them for resistance in the BC_1F_2, BC_1F_6, BC_3F_5 and/or BC_3F_6 generations and estimated the effective number of resistance genes using segregation and quantitative genetic analyses. Three BC_1F_2 RILPs (Tma1GRIL, Tmm1GRIL, Tmm1JRIL) had 16 to 35% of RILs at least moderately resistant, and two of these (Tma1GRIL and Tmm1JRIL) had 2 to 21% resistant when re-evaluated in the BC_1F_6 generation. A fourth RILP (Tma3GRIL) was assessed in the BC_3F_5 and BC_3F_6 generations where combined data analysis showed that 15% of RILs were resistant. Both segregation and quantitative genetics analyses indicated 1 or 2 genes controlled resistance in all populations and that RLN population densities were reduced by up to 99% when compared with the most susceptible RIL. This is the first report of RLN resistance being transferred from einkorn to wheat. Previously, resistance to RLN has been predominantly reported in the B- and D-genomes of wheat. Since einkorn was the only resistant genotype in the parentage of these RILPs, they are likely to carry novel A-genome resistances. These RLN-resistant RILs will provide plant breeders with novel genetic resources to develop genotypes with resistance superior to that currently available.

INTRODUCTION

Wheat (*Triticum aestivum*; hexaploid BBA^uA^uDD-genomes) is an internationally significant crop with ~790 million tonnes (Mt) forecast to be produced in 2022 with ~192 Mt (24%), worth nearly 50 billion USD (wheat priced at 262 USD/t), expected to be traded (FAO 2021, 2022). Demand for wheat in 2022 is anticipated to exceed production, thereby reducing global reserves (FAO 2021). This trend is expected to continue given the linkage between increasing world population and the increased demand for wheat for both human and livestock consumption (FAO 2021). To meet this increasing demand, it will be necessary to increase wheat production commensurately. Three avenues to achieve this are by increasing the production area, increasing

crop genetic yield potential and reducing the gap between actual and attainable yields (Hatfield & Beres, 2019; van Wart et al., 2013).

Opportunities to increase the crop production area are regionally dependent, with only South America, Asia and Africa having increased their agricultural production area since 2002 (Grassini et al. 2013). However, total global agricultural production area, after peaking at 4.88 billon hectares in 2001, has steadily fallen to 4.75 billion hectares by 2019 (FAOSTAT, 2022. A recent study found that projected global food demand will increase by up to 56% during the period 2010 to 2050 (van Dijk et al., 2021). To meet this demand, crop production will need to increase by around 1.4% per year. The International Maize and Wheat Improvement Center (CIMMYT) have reported genetic gains in wheat yields, when compared with local check genotypes, of 1.17% and 0.73% in high and low rainfall environments respectively (Gerard et al., 2020). Nine of the top ten wheat-producing countries have been reported to have a gap between actual (Y_a) and attainable (Y_{at}) yields, referred to as the yield gap (Y_g) and calculated as $Y_g = (Y_{at} - Y_a)/Y_{at}$, with the yield gap of those countries averaging 0.15 and ranging from 0.04 (India) to 0.24 (Australia, Canada) (Hatfield & Beres, 2019). The higher yield gaps, like those reported for Australia and Canada, can be attributed to large variations in the climatic conditions during the growing season, whereas, low yield gaps occur where wheat producing regions enjoy moderate temperatures and above-normal precipitation during the growing season (Hatfield & Beres, 2019). Since crop production area has not increased since 2001 and genetic yield gain alone is unlikely to meet the projected global food demand, a reduction in yield variability, which leads to the yield gap, will be important to meet future food demand. Yield variability is often explained in terms of plant nutrition, water availability and climate (Hatfield & Beres, 2019) but pests and diseases are also important contributing factors (Gerard et al., 2020), of

which nematodes are estimated to cause USD 157 billion per year in damage to crops (Mendoza-de Gives, 2022).

The root-lesion nematode (RLN), *Pratylenchus thornei*, has a cosmopolitan distribution (Castillo & Volvas, 2007) and can reduce wheat yields by up to 60% (Thompson et al., 2021). The use of crop genotypes that are tolerant, that is, they have the ability to maintain their yield in the presence of damaging *P. thornei* population densities, can reduce these yield losses (Thompson et al. 2021). The incorporation of resistance, that is, the ability to restrict nematode multiplication in the plant's roots, can further reduce yield loss (Thompson et al., 2001; 2020; 2021) and provides the additional benefit of reducing *P. thornei* population densities residual in the soil to attack subsequent crops in the cropping system. Wheat genotypes that combine tolerance and resistance to *P. thornei* are relatively rare and require a targeted breeding approach to produce commercial-quality varieties that combine those traits (Thompson et al., 2008).

The identification of wheat genotypes that are resistant to *P. thornei* and the quantitative trait loci (QTL) associated with some of those resistances has been well documented. Primarily, *P. thornei* resistance has been identified in wheat landraces (Sheedy & Thompson, 2009; Thompson et al., 2009), synthetic hexaploid wheats (Ogbonnaya et al., 2008; Thompson, 2008) and in wheat progenitors and closely related species (Sheedy et al., 2012; Thompson & Haak, 1997). The QTL that were associated with, and explained the greatest amount of phenotypic variation for, *P. thornei* resistance have commonly been located on the B- and D-genomes of both wheat landraces (Schmidt et al., 2005; Toktay et al., 2006; Thompson et al., 2015) and synthetic hexaploid wheats (Rahman et al., 2020; Zwart et al., 2010). Similar findings were reported from genome-wide association studies (GWAS) of wheat breeding lines from CIMMYT (Dababat et al., 2016) and wheat genotypes from India (Kumar et al., 2021).

While a limited number of QTL associated with *P. thornei* resistance have been reported on the A-genome (Dababat et al., 2016; Linsell et al., 2014; Schmidt et al., 2005), those QTL have generally explained a low proportion of the total phenotypic variation, or have not been consistently significant in repeat experiments, and have not been reported to be the subject of fine mapping, like those on the B- and D-genomes (Rahman et al., 2020; Zwart et al., 2010), to produce markers suitable for use by wheat breeding programs. It is surprising that A-genome resistance to *P. thornei* is relatively rare in hexaploid wheat given that resistance to *P. thornei* is common among accessions of *Triticum urartu* (the A^u-genome diploid progenitor of wheat) and einkorn (*T. monococcum*; a closely related A^m-genome diploid species that is homeologus with the A-genome of wheat) (Sheedy et al., 2012).

Understanding the genetic basis of plant resistance is helpful for plant breeding purposes and can provide valuable guidance in developing crossing and selection programs, particularly in the absence of reliable marker-trait associations. Evaluation of five synthetic hexaploid wheat-derived populations showed that their *P. thornei* resistance was polygenic, controlled by three to six genes, and additive (Thompson et al. 2012). Similarly, another study has shown *P. thornei* resistance to be additive and that the minimum number of effective resistance genes ranged from two to six in populations derived from wheat landraces from the West Asia North Africa (WANA) region (Thompson and Seymour 2011). The same study indicated that the *P. thornei* resistance of GS50a, a resistant selection from the obsolete Australian wheat cv. Gatcher (Thompson et al., 1999), was controlled by two genes (Thompson and Seymour 2011). The only report of phytoparasitic nematode resistance being transferred from *T. monococcum* to wheat indicated that cereal cyst nematode (*Heterodera avenae*) resistance was controlled by two genes for which QTL were identified on chromosomes 1AS and 2AS (Singh et al. 2010). However, a

single gene, *Rlnn1*, has been reported to confer an effective level of resistance to *P. neglectus* in wheat (Williams et al. 2002). While it is reasonable to conclude that *P. thornei* resistance in wheat is likely polygenic, there are examples where resistance to *Pratylenchus* may be controlled by only one or two genes. This may be particularly relevant where the resistance donor is not a hexaploid wheat but rather a related diploid species. There are no reports of einkorn-derived introgression lines resistant to *Pratylenchus* and therefore there has been no genetic analysis to establish if the *P. thornei* resistance reported in einkorn is also polygenic with an additive gene action.

The objectives of this research were to 1) produce recombinant inbred line (RIL) wheat populations (RILPs) after wide-crossing of *P. thornei*-resistant einkorn accessions with adapted wheat genotypes, 2) phenotypically characterise the RILPs in the BC_1F_2, BC_1F_6, BC_3F_5 and/or BC_3F_6 generations for their resistance to *P. thornei* and 3) study the genetic basis of the resistance by estimating the effective number of resistance genes in each population using segregation and quantitative genetic analyses.

MATERIALS AND METHODS

Development of recombinant inbred line populations

Four RILPs were developed to transfer *P. thornei* resistance from einkorn to wheat (Table 1). Two einkorn accessions, namely AUS27012 (*T. monococcum* ssp. *monococcum*) and AUS27045 (*T. monococcum* ssp. *aegilopoides*), which had previously been identified as resistant to *P. thornei* (Sheedy et al., 2012), were used as the male parent in crosses with wheat cv. Chinese Spring (susceptible-very susceptible [SVS] to *P. thornei*) to produce F_1 seed at the respective rates of 31% and 14% of pollinated florets of each einkorn accession. The F_1 seed germinated

normally and produced amphiploid plants that all resembled wheat but were male sterile and partially female fertile. The F_1 plants were crossed with the Australian wheat cvs. Gregory (moderately susceptible-susceptible [MSS] to *P. thornei*) or Janz (susceptible [S] to *P. thornei*) to produce BC_1F_1 seed that formed the basis of the Tma1GRIL (Chinese Spring/AUS27045//Gregory), Tmm1GRIL (Chinese Spring/AUS27012//Gregory) and Tmm1JRIL (Chinese Spring/AUS27012//Janz) RILPs. BC_1F_1 seed set was relatively low in these crosses with 13%, 16% and 14% of pollinated florets respectively for the Tma1GRIL, Tmm1GRIL and Tmm1JRIL RILPs. A proportion of the Chinese Spring/AUS27045//Gregory BC_1F_1 seed was backcrossed twice more to Gregory to produce BC_3F_1 seed that formed the basis of the Tma3GRIL (Chinese Spring/AUS27045//3*Gregory) RILP. The BC_3F_1 seed set of Tma3GRIL was 14% of pollinated florets.

One to three BC_1F_1 seed of the Tma1GRIL, Tmm1GRIL and Tmm1JRIL RILPs were grown under glasshouse conditions and produced 145 to 300 BC_1F_2 seeds with self-pollination rates of 39%, 11% and 36% respectively for the three RILPs. The BC_1F_2 seeds of each population were subsequently grown in separate glasshouse experiments where they were characterized for their resistance to *P. thornei* and the BC_1F_3 seed was harvested from those plants that produced seed. The Tma1GRIL and Tmm1JRIL RILPs were developed through single seed descent (SSD) until the BC_1F_6 generation where they were considered fixed $BC_1F_{3:6}$ RILs and characterized for their resistance to *P. thornei* in separate replicated experiments. The SSD of the Tmm1GRIL RILP was discontinued at the BC_1F_3 generation due to its very low fertility and seed production. Twenty-eight BC_3F_1 seed of the Tma3GRIL RILP were grown in a field increase and produced 2,176 BC_3F_2 seeds. Three hundred and three BC_3F_2 seeds were progressed via SSD until the BC_3F_5 generation where they were considered fixed $BC_3F_{2:5}$ RILs and characterized for their

resistance to *P. thornei* in replicated experiments in the BC_3F_5 and BC_3F_6 generations. During the population development phase, the single seed descent efficiency (E_{ssd}) for each generation was calculated as the number of generation F_x plants that produced generation F_{x+1} seed divided by the number of F_x seeds used to establish the F_x plants.

Phenotyping of recombinant inbred line populations for *Pratylenchus thornei* resistance

The four RILPs were characterized for their resistance to *P. thornei* in seven separate glasshouse experiments. Each experiment comprised one RILP, the parental genotypes, BC_xF_1 lines where available, a set of five to 10 standard genotypes that ranged from resistant (R) to SVS and an inoculated/unplanted treatment (Accessory Table 1). Each experiment characterizing a BC_1F_2 RILP was arranged using a randomized complete block (RCB) design with four blocks where each block contained an equal number of segregating RILs and a complete set of parents and standard genotypes and treatments. The experiments characterizing the BC_1F_6, BC_3F_5 and BC_3F_6 RILPs were also arranged using RCB designs with three replicates of the RILs, parents and standard genotypes and treatments. For all experiments, single plants of each genotype or treatment were grown in 0.55 L pots (7 cm x 7 cm across x 16 cm high) designed for bottom-watering (T70SSUD; Garden City Plastics) on benches fitted with a bottom-watering system regulated by a float valve set to a water tension of 2 cm. Each pot held 0.33 kg (oven-dry equivalent) of a vertosolic soil of the Irving clay soil association that had been pasteurized at 80°C for 45 min using forced-draught steam (modified from Thompson 1990) and fertilized with 1 g of Osmocote® Landscape Plus Micronutrients (21.2:1.9:5.7 NPK) slow-release fertilizer (Scotts Australia Pty Ltd.). Each pot was inoculated with 3,300 *P. thornei* at planting and plants were grown for 16 weeks. Soil and air temperatures were maintained between 20–25°C by the use of under-bench heating or on-bench electric heat mats, glasshouse heating and cooling

systems (evaporative coolers and/or reverse-cycle air conditioners) and shade cloth as required. At harvest, the plant and soil were removed from each pot, with the lower 50% of the soil and roots removed for subsequent processing. The plant, remaining soil and roots and an amount of pasteurized soil equivalent to the removed sample volume of soil and roots were repotted and returned to the same glasshouse benches. The plants continued growing until maturity, when the seed was harvested. The soil and root samples were processed so that soil aggregate diameter and root length were <1 cm. Final *P. thornei* population densities were determined by extraction from a 150 g subsample of homogenized soil and roots using the Whitehead tray method at 22°C for 48 h (Whitehead and Hemming 1965). Nematodes were collected on a 20 μm aperture sieve (Glenammer Engineering) and stored in 30 mL vials at 3°C until they were counted once using a 1 mL gridded nematode counting slide (Chalex LLC) under a compound microscope (40x) (Olympus Corp.). Gravimetric soil moisture content was determined by drying a 100 g subsample of the soil and roots in a forced draught oven at 105°C for 48 h. Final nematode population density per kg of oven-dry soil and roots was calculated for each pot.

Statistical analyses of *Pratylenchus thornei* phenotyping experiments

Final *P. thornei* population densities were analyzed for each of the seven experiments and a combined analysis of the two Tma3GRIL data sets using linear mixed models (LMM) where estimates of variance parameters were generated using residual maximum likelihood (REML) estimation (Patterson and Thompson, 1971). Prior to analysis, a $\log_e(x)$ transformation, where x = nematodes per kg of soil and roots, was applied to ensure the data followed a normal distribution (Berry, 1987). The variance-covariance of the residuals were assumed to follow a separable AR1 by AR1 (AR1 = autoregressive structure of order 1) correlation structure in row and column directions to account for any spatial variation across each experimental layout

(Gilmour et al., 1997). Each LMM included the experiment mean as a fixed effect and genotype, replicate and the AR1 by AR1 structure as random effects. To detect linear trends or random effects across rows and columns, these terms were individually added to the model and tested for significant reductions in the model deviance using Chi-square principles. Terms with significant effects were added to the model. The random effects in the model were estimated through best linear unbiased predictions (BLUPs) (Cullis et al., 2010). Predictions for wheat genotype effects were rescaled by the addition of the estimate for the mean of each experiment in units of $\log_e$ (*P. thornei* per kg of soil and roots) and then back-transformed by exponentiation to produce final *P. thornei* population densities per kg of soil and roots. Least significant differences and a generalized measure of heritability (h^2) were calculated for each experiment using the formula $h^2 = 1 - PEV \div (2\sigma^2_g)$ where PEV = average pairwise prediction error variance of test line effects (calculated as the square of the average standard error of differences [AvSED] of the test line effects) and σ^2_g = genetic variance (Cullis et al., 2006). Predicted mid-parent values for BC_1F_1 or BC_3F_1 generations were calculated for each population. Genotypes were ranked according to the rescaled BLUPs and then divided into nine equal subranges and assigned an alpha classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/) (Thompson et al., 2020). Those alpha classifications were R, resistant – moderately resistant (RMR), moderately resistant (MR), moderately resistant – moderately susceptible (MRMS), moderately susceptible (MS), MSS, susceptible (S), SVS and very susceptible (VS). Statistical analyses were performed using Genstat for Windows 21st Edition (VSN International 2020).

231 **Estimation of effective gene number controlling *Pratylenchus thornei* resistance**

232 We estimated the number of genes controlling the *P. thornei* resistance in the RILPs using both
233 qualitative (Mendelian) and quantitative genetic approaches. Mendelian BC_1F_2 segregation ratios
234 were calculated assuming self-pollination for six genetic models, namely, 1-gene (5:2:1) and 2-
235 gene (25:20:14:4:1) additive models, 1-gene dominance model with dominant susceptibility
236 (7:1), 1-gene dominance model with dominant resistance (5:3), 2-gene dominance model with
237 dominant susceptibility (49:14:1) and a 2-gene dominance model with dominant resistance
238 (25:29:5). To compare the genotypes from each BC_1F_2 RILP with the Mendelian segregation
239 ratios, the genotypes were grouped according to their alpha classification to form two, three or
240 five categories depending on the genetic model (Accessory Table 2). To determine if observed
241 segregation ratios were similar to the predicted ratios, we calculated the predicted number of
242 genotypes for each category by proportioning the total number of genotypes according to the
243 predicted segregation ratios. The Chi-squared (χ^2) value for each category was calculated using
244 the formula: (Observed number of genotypes - predicted number of genotypes)^2 ÷ predicted
245 number of genotypes. The sum of the χ^2 values for all categories within a genetic model was
246 compared with the appropriate threshold for statistical difference ($P = 0.05$) χ^2 value for (number
247 of categories in the genetic model - 1) degrees of freedom (Mead et al., 1993). Where the χ^2
248 value exceeded the threshold for statistical difference, we considered that the observed ratio did
249 not conform to the predicted ratio and rejected the genetic model.

250 Two quantitative methods for estimating effective resistance gene number were applied to the
251 RILP in each of the seven experiments. Firstly, we applied the Castle-Wright estimator of gene
252 number using the formula $k = (PR)^2 \div 8\sigma^2_g$ where k = minimum number of effective genes, PR =
253 parental phenotypic range and σ^2_g = genetic variance (Wu et al., 2007). This approach assumes

that (a) all genes contribute equally to the phenotypic effect; (b) an additive genetic model exists and that no linkage, epistasis or dominance effects contribute to the phenotypic range; and (c) the target genes were contributed by one parental genotype and consequently the parental range adequately represents the full phenotypic range of the trait (Snape et al., 1984; Herrera-Foessel et al., 2008).

Secondly, we applied the modified Castle-Wright formula used by Singh et al. (1995) where $k = (GR \times h^2)^2 \div F_g\sigma^2_g$ where k = minimum number of effective genes, GR = the phenotypic range of the RIL means, h^2 = heritability, F_g = an inbreeding coefficient of 8 for BC_xF_2 lines (Wu et al., 2007), 4.57 for BC_xF_5 lines (Herrera-Foessel et al., 2008) or 4.27 for BC_xF_6 lines (Singh et al., 1995) and σ^2_g = genetic variance. This approach relies on the aforementioned assumptions (a) and (b), but does not assume that the target genes come from a single parent. It does assume (c) the two extreme opposite RILs contain all the increasing and decreasing alleles, respectively, segregating between the parents and that they adequately represent the full phenotypic range of the trait (Snape et al., 1984; Herrera-Foessel et al., 2008).

RESULTS

Recombinant inbred line population development

The four einkorn-derived RILPs displayed variable establishment and fertility during the SSD process. The three populations (Tma1GRIL, Tmm1GRIL, Tmm1JRIL) with only one backcross with an adapted wheat cultivar (Gregory or Janz) had relatively low plant establishment (range: 66% – 92%) and fertility (range: 56% – 84%) during the BC_1F_2 to BC_1F_4 generations resulting in low single seed descent efficiencies ranging from 0.47 to 0.58 (Table 2). In the BC_1F_5 generation, both plant establishment (range: 92% – 98%) and fertility (range: 85% – 96%) stabilized and consequently E_{ssd} improved to 0.84 to 0.88. Tma3GRIL, which had three

backcrosses to Gregory, had higher E_{ssd} in the BC_3F_2 (0.87) and BC_3F_3 (0.84) generations and normal E_{ssd} in the BC_3F_4 (0.96) generation (Table 2).

Pratylenchus thornei resistance of the BC_1F_2 populations

The Tma1GRIL (Fig. 1a), Tmm1GRIL (Fig. 1b) and Tmm1JRIL (Fig. 1c) BC_1F_2 populations all had continuous and normally distributed final *P. thornei* population densities. The heritabilities were moderate for all populations, ranging from 0.42 to 0.52. The Tma1GRIL population ranged from R to VS with 76 lines (35%) at least moderately resistant, eight of which produced lower final *P. thornei* population densities than AUS27045 (Fig 1a). The most resistant RIL reduced *P. thornei* population densities by 99% when compared with the most susceptible RIL.

The Tmm1GRIL population also ranged from R to VS (Fig. 1b). Twenty-one lines (16%) were at least moderately resistant, with four lines producing lower final *P. thornei* populations than AUS27012. The most resistant RIL reduced *P. thornei* population densities by 77%. Notably, the proportion of resistant lines derived from the cultivated form of *T. monococcum* (AUS27012) in this population was less than half that of the wild form (AUS27045) in the Tma1GRIL population.

The Tmm1JRIL BC_1F_2 lines ranged from RMR to VS with 25 lines (16%) at least moderately resistant, however, none produced lower final *P. thornei* populations than AUS27012 (Fig. 1c). The most resistant RIL reduced *P. thornei* population densities by 89%. The proportion of resistant lines derived from AUS27012 was similar to that observed in the Tmm1GRIL population and again was less than half that of the *T. monococcum* wild form (AUS27045) in the Tma1GRIL population.

The effective number of resistance genes in the BC_1F_2 populations

Our Mendelian segregation analysis of the *P. thornei* resistance data from the three RILPs evaluated in the BC_1F_2 generation indicated that one to two resistance genes were present in each population (Table 3). Tma1GRIL, derived from the wild form of einkorn (AUS27045; *T. monococcum* ssp. *aegilopoides*), had segregation ratios that conformed to both the 1-gene additive (5:2:1) and 1-gene dominance (5:3) models with resistance genetically dominant in the dominance model. The two populations derived from the cultivated form of einkorn (AUS27012; *T. monococcum* ssp. *monococcum*) conformed to the 1-gene (7:1) and 2-gene (49:14:1) dominance models where susceptibility was genetically dominant.

Our quantitative estimation of gene number supported the Mendelian segregation analysis with all populations effectively carrying one resistance gene (Table 4). The Castle-Wright approach provided estimates in the range 0.5 to 1.1 genes and the modified Castle-Wright formula provided lower estimates in the range 0.2 to 0.8 genes.

Pratylenchus thornei resistance of the recombinant inbred line populations

The final *P. thornei* population densities of the Tmm1JRIL (Fig 2a) and Tma1GRIL (Fig 2b) populations were continuous and normally distributed in their BC_1F_6 generations, as they were in their BC_1F_2 generations. The Tmm1JRIL population had moderate heritability ($h^2 = 0.54$) with individual RILs ranging from MR to VS (Accessory Table 3). Tmm1JRIL047 was the only MR genotype of the 107 evaluated and produced *P. thornei* population densities 88% lower than the most susceptible RIL. The Tma1GRIL population had a lower, but still moderate, heritability ($h^2 = 0.37$) with individual RILs ranging from R to VS (Accessory Table 4). Fourteen (9%) of the

320 163 genotypes were at least moderately resistant to *P. thornei* with the most resistant RIL
321 (Tma1GRIL042) reducing *P. thornei* population densities by 78%.

322 From the combined analysis of the BC_3F_5 and BC_3F_6 generations of the Tma3GRIL
323 population, 11 (5%) of the 226 genotypes were found to be at least moderately resistant to *P.*
324 *thornei* (Accessory Table 5) with a high heritability ($h^2 = 0.66$). Similar to the other RILPs, the
325 final *P. thornei* population densities were continuous and normally distributed (Fig 2c). The most
326 resistant RIL (Tma3GRIL142) reduced *P. thornei* population densities by 93% when compared
327 with the most susceptible RIL.

328 **The effective number of resistance genes in the recombinant inbred line populations**

329 Quantitative assessment of the Tma1GRIL BC_1F_6 population indicated that it effectively
330 carried one *P. thornei* resistance gene. The Castle-Wright and modified Castle-Wright
331 approaches estimated the gene number as 0.4 and 0.2 respectively (Table 4). The Tmm1JRIL
332 BC_1F_6 population effectively carried one to two *P. thornei* resistance genes with estimates using
333 the Castle-Wright and modified Castle-Wright approaches of 1.9 and 0.6 respectively (Table 4).
334 Our genetic analysis of the combined Tma3GRIL BC_3F_5 and BC_3F_6 data sets showed that the
335 Tma3GRIL population effectively carried two *P. thornei* resistance genes with estimates using
336 the Castle-Wright and modified Castle-Wright approaches of 2.0 and 2.1 respectively (Table 4).

337 # DISCUSSION

338 Crop wild relatives are a valuable genetic resource for plant improvement programs. The use
339 of plant species related to wheat in breeding programs has increased genetic diversity within the
340 crop and has delivered novel traits that have improved resistance to disease, tolerance to abiotic
341 stress and the quality of end-use products (Brozynska et al., 2016; Dempewolf et al., 2017).

342 Through a program of interspecific hybridization, we developed four einkorn-derived RILPs that
343 were characterized for their resistance to *P. thornei* in the BC_1F_2, BC_1F_6, BC_3F_5 and/or BC_3F_6
344 generations. During this process, we identified 26 RILs (in the BC_1F_6 and BC_3F_6 generations)
345 that likely carry novel A-genome resistance to *P. thornei*. This is the first report of *P. thornei*
346 resistance being transferred from einkorn to wheat. Our segregation analysis of the BC_1F_2
347 generation and the application of quantitative genetic formulae for estimation of number of
348 resistance genes in the BC_1F_2, BC_1F_6, and BC_3F_6 generations indicated that one to two genes
349 controlled the *P. thornei* resistance in all four RILPs.

350 The *P. thornei* resistant RILs developed during this research recovered resistance levels
351 comparable with their einkorn parent. The most resistant RIL reduced *P. thornei* population
352 densities by 78% to 93% when compared with the most susceptible RIL of each population. The
353 Tmm1JRIL population, derived from cultivated einkorn (AUS27012), produced the lowest
354 proportion of resistant RILs (1%). In contrast, the Tma1GRIL population derived from wild
355 einkorn (AUS27045) produced the highest proportion of resistant RILs (9%). The wild einkorn
356 (AUS27045)-derived Tma3GRIL population produced an intermediate proportion of resistant
357 RILs (5%). Furthermore, the additional backcrossing during the Tma3GRIL population
358 development resulted in RILs that were agronomically similar to the adapted parent Gregory and
359 agronomically superior to the RILs with less backcrossing.

360 The identification of closely linked marker trait associations with the *P. thornei* resistance
361 identified in these populations and the development of effective markers to detect the
362 quantitative trait loci (QTL) will be necessary to maximize their value to plant breeders. The
363 novel *P. thornei* resistances identified in these RILPs will be valuable to increase the genetic
364 diversity of resistances available to breeders and offer the opportunity to develop new gene

365 combinations. Several synthetic hexaploid wheat genotypes have been reported to carry *P*.
366 *thornei* resistance on their B- and D-genomes (Zwart et al., 2010), which has led to the
367 development of advanced breeding lines (ABLs) that carry both resistances combined with
368 improved agronomic characteristics (Sheedy et al., 2017). A targeted intercrossing program
369 among the synthetic-derived ABLs and the *P*. *thornei* resistant RILs that we have identified in
370 this research will facilitate the development of ABLs that combine *P*. *thornei* resistances on each
371 of the A-, B-, and D-genomes of wheat that will likely deliver a higher level of *P*. *thornei*
372 resistance than is currently available.

373 Previous genetic studies have concluded that *P*. *thornei* resistance was polygenic in synthetic
374 hexaploid wheats (Thompson et al., 2012) and Middle Eastern landraces (Thompson & Seymour
375 2011), controlled by three to six genes. The *P*. *thornei* resistance carried by the wheat genotypes
376 GS50a and El Neilain (ISR455.3) was oligogenic, with two genes effectively controlling their
377 resistance (Thompson & Seymour 2011). Similarly, we have concluded that one to two genes
378 controlled the einkorn-derived *P*. *thornei* resistance. The resistances of the synthetic hexaploid
379 wheats, Middle Eastern landraces (including El Neilain) and GS50a were all determined to have
380 an additive gene action (Thompson & Seymour 2011; Thompson et al., 2012). Our segregation
381 analyses of the BC_1F_2 RILPs indicated that additive gene action or genetically dominant
382 resistance were plausible models in the wild einkorn-derived Tma1GRIL population and that
383 genetically dominant susceptibility was likely in the cultivated einkorn-derived Tmm1GRIL and
384 Tmm1JRIL populations. Quantitative genetic analyses of the BC_1F_2 RILPs estimated similar
385 numbers of effective resistance genes as the segregation analyses, however, the mean *P*. *thornei*
386 population density of each RILP was similar to the predicted mid-parent value of each RILP,
387 suggesting an additive gene action (Snape et al., 1984).

Subsequent evaluation of the BC_1F_6 generation of the Tmm1JRIL population using quantitative genetic analysis indicated that the mean *P. thornei* population density of the RILs (11.28) was statistically similar to both the predicted mid-parent value (11.26) and the observed mean of the BC_1F_1 treatment (11.09). This finding indicates that the one to two genes estimated to control the *P. thornei* resistance in the Tmm1JRIL population had an additive gene action. The evaluation of the BC_xF_6 generations of the Tma1GRIL and Tma3GRIL populations produced similar outcomes where the mean *P. thornei* population densities of the RILPs were statistically similar to both the predicted mid-parent values and the observed means of the BC_xF_1 treatments of each population. This finding supports the conclusion of additive gene action from the segregation analysis of the BC_1F_2 generation of the Tma1GRIL population. The Tma3GRIL population was not evaluated in its BC_3F_2 generation but it shares the resistant parental genotype (AUS27045) with the Tma1GRIL population and demonstrated a similar additive gene action.

A high strategic priority for practical cereal improvement worldwide is to enrich the cultivated gene pools by incorporating favorable alleles, genes or gene complexes from wild relatives (Feuillet et al. 2007; Rosyara et al., 2019). Traditionally, wheat breeders have preferred to use germplasm that is well adapted to the domestic environment. When unadapted parents must be used to provide the desired type and level of genetic variation, the order of preference is i) landraces and synthetic hexaploid wheats (primary gene pool), ii) closely related species (secondary gene pool) and iii) more distantly related species or genera in the tertiary gene pool (Harlan & de Wet 1971; Cox 1991; Ogbonnaya et al., 2008). einkorn, although from the secondary gene pool, has proven to be a valuable source of desirable traits and genetic diversity for wheat improvement (Adhikari et al., 2022) and this research has introgressed novel resistances to *P. thornei* into wheat lines that will be valuable to plant breeders.

411 However, developing these einkorn-derived RILPs was not without its challenges.
412 Interspecific hybridization, or wide-crossing, produces well documented defects in the hybrids
413 including reduced fertility (Johnson & Dhaliwal 1976; Ma & Hughes 1993) and the inheritance
414 of undesirable characteristics from the resistant parent, also known as linkage drag or genetic
415 load (Rosyara et al., 2019). In this study, we developed three BC_1 RILPs to maximize our
416 chances of transferring the *P. thornei* resistance of einkorn to wheat and one BC_3 RILP to reduce
417 the amount of linkage drag while still recovering resistant genotypes. Our BC_1 RILPs all had
418 relatively low fertility and variable seed vigor in the BC_1F_2 to BC_1F_4 generations with E_{ssd} values
419 ranging from 0.47 to 0.58. The fertility was restored to near normal levels for wheat in the BC_1F_5
420 generation of the two RILPs that were progressed to that stage (E_{ssd} range: 0.84 – 0.88). Many of
421 the 15 *P. thornei* resistant genotypes identified from these two RILPs were generally slower
422 maturing and taller than their adapted parents. The BC_3 RILP had near normal fertility in the
423 BC_3F_2 and BC_3F_3 generations (E_{ssd} range: 0.84 – 0.88) with normal fertility recovered in the
424 BC_3F_4 generation (E_{ssd} = 0.96). The 11 *P. thornei* resistant genotypes identified in the
425 Tma3GRIL population had similar height and maturity to its adapted parent Gregory. The
426 resistant genotypes produced statistically similar *P. thornei* population densities to their resistant
427 einkorn parent AUS27045, indicating that there had not been the dilution of resistance reported
428 for some disease resistances transferred from a species of a lower level of ploidy to one of a
429 higher level (Cox 1991; Gill et al. 1986; Kerber and Green 1980; Potgieter et al. 1991).

430 The development of these einkorn-derived RILPs and the selection of 26 *P. thornei* resistant
431 RILs has broadened the useable genetic base of resistance available to breeding programs by
432 transferring resistance from the secondary gene pool to the primary gene pool. Exploitation by
433 plant breeders of this largely untapped pool of resistance to *P. thornei* will deliver additional

genetic resources that increase diversity within their breeding programs. Combined with the knowledge that the resistance is controlled by one to two genes and operates with an additive gene action, this germplasm will facilitate the efficient development of genotypes with novel *P. thornei*-resistance combinations that can better manage *P. thornei* population densities in commercial farming systems.

ACKNOWLEDGMENTS

The authors would like to thank University of Southern Queensland (UniSQ) staff Neil Robinson, Mei Wang, Roslyn Reen, Hannah Rostad, Michelle Thompson, Sriram Padmanaban and Martin Fiske for technical assistance in the overall conduct of the experiments and germplasm population development. This research was co-funded by the Grains Research and Development Corporation (GRDC) and the Queensland Department of Agriculture and Fisheries Broadacre Cropping Initiative (BACI) in partnership with UniSQ through projects DAQ00171 and USQ00019.

AUTHOR CONTRIBUTIONS

Jason Sheedy: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. Jing Lin: Investigation, Writing – review & editing. Mandy Christopher: Investigation, Supervision, Writing – review & editing. John Thompson: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. Reference in this document to any specific commercial product, process, or service, or the use of any trade, firm or corporation name is for the information and convenience of the reader, and does not constitute endorsement, recommendation, or favoring by the authors or their affiliates.

ORCID

JG Sheedy: 0000-0001-6569-5016

J Lin: 0000-0001-8852-5335

M Christopher: 0000-0001-6108-3514

JP Thompson: 0000-0002-2097-5695

SUPPLEMENTAL MATERIAL

Accessory Table 1 (AT01): *Pratylenchus thornei* resistance ratings of parents, standard genotypes and treatments used to characterize einkorn-derived recombinant inbred line populations.

Accessory Table 2 (AT02): Alpha classifications of resistance to *Pratylecnchus thornei* of BC_1F_2 genotypes used to form categories to compare the segregation ratios of recombinant inbred line populations with predicted Mendelian segregation ratios for 1-gene and 2-gene additive and dominance genetic models.

Accessory Table 3 (AT03): *Pratylenchus thornei* population densities (best linear unbiased predictions) and resistance classifications of the Tmm1JRIL recombinant inbred line population.

Accessory Table 4 (AT04): *Pratylenchus thornei* population densities (best linear unbiased predictions) and resistance classifications of the Tma1GRIL recombinant inbred line population.

Accessory Table 5 (AT05): *Pratylenchus thornei* population densities (best linear unbiased predictions) and resistance classifications of the Tma3GRIL recombinant inbred line population after combined analysis of the BC_3F_5 and BC_3F_6 generations.

REFERENCES

Adhikari, L., Raupp, J., Wu, S., Wilson, D., Evers, B., Koo, D. H., Singh, N., Friebe, B., & Poland, J. (2022). Genetic characterization and curation of diploid A-genome wheat species. *Plant Physiol*. *188*: 2101–2114. https://doi.org/10.1093/plphys/kiac006

Berry, D. A. (1987). Logarithmic transformations in ANOVA. *Biometrics 43*: 439-456. https://doi.org/10.2307/2531826

Brozynska, M., Furtado, A., & Henry, R. J. (2016). Genomics of crop wild relatives: expanding the gene pool for crop improvement. *Plant Biotechnol. J. 14*: 1070–1085. https://doi.org/10.1111/pbi.12454

Castillo, P., & Vovlas, N. (2007). *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. In Nematology Monographs and Perspectives Volume 6 Series 9. Eds DJ Hunt and RN Perry. (Brill: Leiden, the Netherlands.) ISBN: 978-90-04-15564-0. https://brill.com/view/title/13449

Cox, T. S. (1991). The contribution of introduced germplasm to the development of U.S. wheat cultivars. In, Use of Plant Introductions in Cultivar Development, Part 1, Crop Science Society of America Special Publication No. 17, USA. pp. 25-47. https://doi.org/10.2135/cssaspecpub17.c3

Cullis, B. R., Smith, A. B., & Coombes, N. E. (2006). On the design of early generation variety trials with correlated data. *J. Agric. Biol. Environ. Stat. 11*: 381-393. https://doi.org/10.1198/108571106X154443

Cullis, B. R., Smith, A. B., Beeck, C. P., & Cowling, W. A. (2010). Analysis of yield and oil from a series of canola breeding trials. Part II. Exploring variety by environment interaction using factor analysis. *Genome 53*:1002-1016. https://doi.org/10.1139/G10-080

Dababat, A. A., Ferney, G-B. H., Erginbas-Orakci, G., Dreisigacker, S., Imren, M., Toktay, H., Elekcioglu, H. I., Mekete, T., Nicol, J. M., Ansari, O., & Ogbonnaya, F. (2016). Association analysis of resistance to cereal cyst nematodes (*Heterodera avenae*) and root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) in CIMMYT advanced spring wheat lines for semi-arid conditions. *Breed Sci 66*:692-702. https://doi.org./10.1270/jsbbs.15158

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., & Guarino, L. (2017). Past and future use of wild relatives in crop breeding. *Crop Sci. 57*:1–13. https://doi.org/10.2135/cropsci2016.10.0885

FAO. 2021. Food Outlook – Biannual report on global food markets. Food Outlook, November 2021. Rome. https://doi.org/10.4060/cb7491en

FAO. 2022. Crop Prospects and Food Situation – Quarterly Global Report No. 1, March 2022. Rome. https://doi.org/10.4060/cb8893en

FAOSTAT. 2022. Agricultural land – Area. https://www.fao.org/faostat

Feuillet, C., Langridge, P., & Waugh, R. (2007). Cereal breeding takes a walk on the wild side. *Trends in Genetics 24*:24-32. https://doi.org/10.1016/j.tig.2007.11.001

Gerard, G. S., Crespo-Herrera, L. A., Crossa J., Mondal, S., Velu, G., Juliana, P., Huerta-Espino, J., Vargas, M., Rhandawa, M. S., Bhavani, S., Braun, H., & Singh, R. P. (2020). Grain yield genetic gains and changes in physiological related traits for CIMMYT's High Rainfall Wheat Screening Nursery tested across international environments. *Field Crops Res 249*: 107742. https://doi.org/10.1016/j.fcr.2020.107742

Gill, B. S., Multani, D. S., & Dhaliwal, H. S. (1986). Transfer of Isoproturon resistance from *Triticum monococcum* to *T. durum*. *Crop Improv. 13*:200-203.

Gilmour, A. R., Cullis, R. R., & Verbyla, A. P. (1997). Accounting for natural and extraneous variation in the analysis of field experiments. *J. Agric. Biol. Environ. Stat. 2*:269-293. https://doi.org/10.2307/1400446

Grassini, P., Eskridge, K. M., & Cassman, K. G. (2013). Distinguishing between yield advances and yield plateaus in historical crop production trends. *Nat Commun 4*: 2918. https://doi.org/10.1038/ncomms3918

Harlan, J. R., & de Wet, J. M. J. (1971). Toward a rational classification of cultivated plants. *Taxon 20*:509-517. https://doi.org/10.2307/1218252

Hatfield, J. L., & Beres, B. L. (2019). Yield gaps in wheat: Path to enhancing productivity. *Front. Plant Sci. 10*:1603. https://doi.org/10.3389/fpls.2019.01603

Herrera-Foessel, S. A., Singh, R. P., Huerta-Espino, J., Crossa, J., Djurle, A., & Yuen, J. (2008). Genetic analysis of slow-rusting resistance to leaf rust in durum wheat. *Crop Science 48*: 2132-2140. https://doi.org/10.2135/cropsci2007.11.0606

Johnson, B. L., & Dhaliwal, H. S. (1976). Reproductive isolation of *Triticum boeoticum* and *Triticum urartu* and the origin of the tetraploid wheats. *Am. J. Bot. 63*:1088-1094. https://doi.org/10.2307/2441653

Kerber, E. R. & Green, G. J. (1980). Suppression of stem rust resistance in the hexaploid wheat cv. Canthatch by chromosome 7DL. *Can. J. Bot. 58*:1347-1350. https://doi.org/10.1139/b80-166

Kumar, D., Sharma, S., Sharma, R., Pundir, S., Singh, V. K., Chaturvedi D., Singh, B., Kumar, S., & Sharma, S. (2021). Genome-wide association study in hexaploid wheat identifies novel genomic regions associated with resistance to root lesion nematode (*Pratylenchus thornei*). *Sci Rep 11*:3572. https://doi.org/10.1038/s41598-021-80996-0

Linsell, K. J., Rahman, M. S., Taylor, J. D., Davey, R. S., Gogel, B. J., Wallwork, H., Forrest, K. L., Hayden, M. J., Taylor, S. P., & Oldach, K. H. (2014). QTL for resistance to root lesion nematode (*Pratylenchus thornei*) from a synthetic hexaploid wheat source. *Theor Appl Genet 127*: 1409-1421. https://doi.org/10.1007/s00122-014-2308-9

Ma, H., Hughes, G. R. (1993). Resistance to *Septoria nodorum* blotch in several *Triticum* species. *Euphytica 70*:151-157. https://doi.org/10.1007/BF00029652

Mead, R., Curnow, R. N., & Hasted, A. M. (1993). *Statistical methods in agriculture and experimental biology* (2nd ed.). Chapman & Hall: London, United Kingdom. ISBN: 0-412-35480-2.

Mendoza-de Gives, P. (2022). Soil-borne nematodes: Impact in agriculture and livestock and sustainable strategies of prevention and control with special reference to the use of nematode natural enemies. *Pathogens 11*: 640. https://doi.org/10.3390/pathogens11060640

Ogbonnaya, F. C., Imtiaz, M., Bariana, H. S., McLean, M., Shankar, M. M., Hollaway, G. J., Trethowan, R. M., Lagudah, E. S., & van Ginkel, M. (2008). Mining synthetic hexaploids for multiple disease resistance to improve bread wheat. *Aust. J. Agric. Res. 59*: 421–431. https://doi.org/10.1071/AR07227

Patterson, H. D., Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika* 58: 545–554. https://doi.org/10.1093/biomet/58.3.545

564 Potgieter, G. F., Marais, G. F., & du Toit, F. (1991). The transfer of resistance to the Russian
565 wheat aphid from *Triticum monococcum* L. to common wheat. Plant Breeding 106:284-292.
566 https://doi.org/10.1111/j.1439-0523.1991.tb00513.x

567 Rahman, M.S., Linsell, K.J., Taylor, J.D., Hayden, M. J., Collins, N. C., & Oldach, K. H. (2020).
568 Fine mapping of root lesion nematode (*Pratylenchus thornei*) resistance loci on
569 chromosomes 6D and 2B of wheat. *Theor Appl Genet 133*: 635–652.
570 https://doi.org/10.1007/s00122-019-03495-x

571 Rosyara, U., Kishii, M., Payne, T., Sansaloni, C. P., Singh, R. P., Braun, H-J., & Dreisigacker, S.
572 (2019). Genetic contribution of synthetic hexaploid wheat to CIMMYT's spring bread wheat
573 breeding germplasm. *Sci Rep 9*: 12355. https://doi.org/10.1038/s41598-019-47936-5

574 Schmidt, A. L., McIntyre, C. L., Thompson, J. P., Seymour, N. P., Liu & C. J. (2005).
575 Quantitative trait loci for root lesion nematode (*Pratylenchus thornei*) resistance in Middle-
576 Eastern landraces and their potential for introgression into Australian bread wheat. *Aust J*
577 *Agric Res 56*:1059-1068. https://doi.org/10.1071/AR05016

578 Sheedy, J. G., Robinson, N. A., Lin, J., Reen, R. A., Clewett, T. G., & Thompson, J. P. (2017).
579 Prebreeding to produce wheat cultivars that combine root-lesion nematode (*Pratylenchus*
580 *thornei* and *P. neglectus*) resistance and tolerance. Australian Wheat Breeders Assembly
581 2017. 23-25 August 2017, Sydney, Australia.

582 Sheedy, J. G., & Thompson, J. P. (2009). Resistance to the root-lesion nematode *Pratylenchus*
583 *thornei* of Iranian landrace wheat. *Aust. Plant Pathol. 38*: 478–489.
584 https://doi.org/10.1071/AP09030

585 Sheedy, J. G., Thompson, J. P. & Kelly, A. (2012). Diploid and tetraploid progenitors of wheat
586 are valuable sources of resistance to the root lesion nematode *Pratylenchus thornei*.
587 *Euphytica 186*: 377–391. https://doi.org/10.1007/s10681-011-0617-5

588 Singh, K., Chhuneja, P., Singh, I., Sharma, S. K., Garg, T., Garg, M., Keller, B., Dhaliwal, H. S.
589 (2010). Molecular mapping of cereal cyst nematode resistance in *Triticum monococcum* L.
590 and its transfer to the genetic background of cultivated wheat. *Euphytica 176*:213-222.
591 https://doi.org/10.1007/s10681-010-0227-7

592 Singh, R. P., Ma, H. & Rajaram, S. (1995). Genetic-Analysis of Resistance to Scab in Spring
593 Wheat Cultivar Frontana. *Plant Disease 79*: 238-240. https://doi.org/10.1094/PD-79-0238

594 Snape, J. W., Wright, A. J., & Simpson, E. (1984). Methods for estimating numbers for
595 quantitative characters using doubled haploid lines. *Theor Appl Genet 67*: 143-148.
596 https://doi.org/10.1007/BF00317020

597 Thompson, J. P. (1990). Treatments to eliminate root-lesion nematode (*Pratylenchus thornei*
598 Sher and Allen) from a vertisol. Nematologica 36:123-127.
599 https://doi.org/10.1163/002925990X00095

600 Thompson, J. P. (2008). Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P.*
601 *neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents.
602 *Australian Journal of Agricultural Research 59*: 432-446. https://doi.org/10.1071/AR07222

603 Thompson, J. P., Brennan, .PS., Clewett, T.G., Sheedy, J. G., & Seymour, N. P. (1999). Progress
604 in breeding wheat for tolerance and resistance to root-lesion nematode (*Pratylenchus*
605 *thornei*). *Australas Plant Pathol 28*:45-52. https://doi.org/10.1071/AP99006

606 Thompson J. P., & Haak, M.I. (1997). Resistance to root-lesion nematode (*Pratylenchus thornei*)
607 in *Aegilops tauschii* Coss., the D-genome donor to wheat. *Australian Journal of Agricultural*
608 *Research 48*: 553–559. https://doi.org/10.1071/A96167

609 Thompson, J. P., O'Reilly, M.M., & Clewett, T. G. (2009). Resistance to the root-lesion
610 nematode *Pratylenchus thornei* in wheat landraces and cultivars from the West Asia and
611 North Africa (WANA) region. *Crop & Pasture Science 60*: 1209–1217.
612 https://doi.org/10.1071/CP09159

613 Thompson, J. P., Owen, K. J., Stirling, G. R., & Bell, M. J. (2008). Root-lesion nematodes
614 (*Pratylenchus thornei* and *P. neglectus*): a review of recent progress in managing a
615 significant pest of grain crops in northern Australia. *Australasian Plant Pathology 37*: 235–
616 242. https://doi.org/10.1071/AP08021

617 Thompson, J. P., Seymour, N.P. (2011). Inheritance of resistance to root-lesion nematode
618 (*Pratylenchus thornei*) in wheat landraces and cultivars from the West Asia and North Africa
619 (WANA) region. *Crop & Pasture Science 62*:82-93. https://doi.org/10.1071/CP10309

620 Thompson, J.P.**,** Sheedy, J.G., Robinson, N.A. (2020). Resistance of wheat genotypes to root-
621 lesion nematode (*Pratylenchus thornei*) can be used to predict final nematode population
622 densities, crop greenness and grain yield in the field. *Phytopathology* **110**, 505-516.
623 https://doi.org/10.1094/PHYTO-06-19-0203-R

624 Thompson, J. P., Sheedy, J. G., Robinson, N. A., & Clewett, T.G. (2021). Tolerance of wheat
625 (*Triticum aestivum*) genotypes to root-lesion nematode (*Pratylenchus thornei*) in the
626 subtropical grain region of eastern Australia. *Euphytica 217*: 48
627 https://doi.org/10.1007/s10681-020-02761-0

628 Thompson, J., Sheedy, J., Robinson, N., Khoo, K., Chalmers, K., & Mather, D. (2015).
629 Utilization of the Watkins collection of wheat landraces for root-lesion nematode resistance.
630 Ninth International Wheat Conference, Sydney, 20-25 September. p. 149

631 Thompson, J. P., Sheedy, J. G., Seymour, N. P., Clewett, T. G., Mason, L. R., Sheppard, J. A., &
632 Banks, P. M. (2001). Advances in breeding wheat for tolerance and resistance to
633 *Pratylenchus thornei* and *P. neglectus* for the northern region. In 'Proceedings of the 10th
634 assembly of the Wheat Breeding Society of Australia Inc.'.16–21 September 2001, Mildura,
635 Australia. pp. 123–127 ISBN: 0-9579469-0-2.

636 Thompson, J. P., Zwart, R. S., Butler, D. (2012). Inheritance of resistance to root-lesion
637 nematodes (*Pratylenchus thornei* and *P. neglectus*) in five doubled-haploid populations of
638 wheat. *Euphytica 188*: 209-219. https://doi.org/10.1007/s10681-012-0689-x

639 Toktay, H., McIntyre, C. L., Nicol, J. M., Ozkan, H., & Elekcioglu, H. I. (2006). Identification of
640 common root-lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in bread wheat.
641 *Genome 49*: 1319-1323. https://doi.org/10.1139/G06-090

642 van Dijk, M., Morely, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global
643 food demand and population at risk of hunger for the period 2010–2050. *Nature Food 2*: 494-
644 501. https://doi.org/10.1038/s43016-021-00322-9

van Wart, J., Kersebaum, K. C., Peng, S., Milner, M., & Cassman, K. G. (2013). Estimating crop yield potential at regional to national scales. *Field Crops Res 143*: 34-43. https://dx.doi.org/10.1016/j.fcr.2012.11.018

VSN International (2020). Genstat Reference Manual (Release 21). VSN International, Hemel Hempstead, UK.

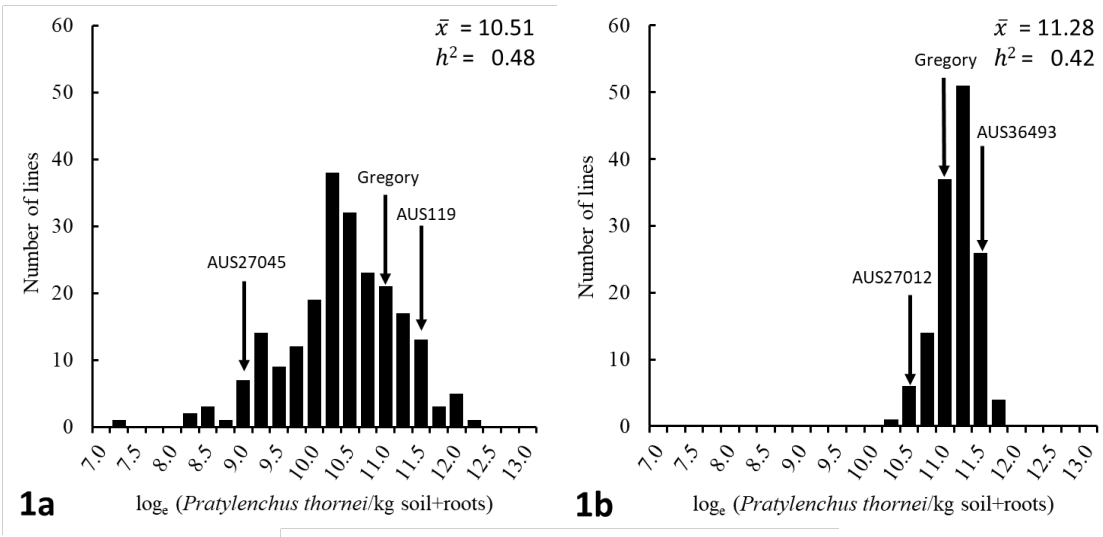
Williams, K. J., Taylor, S. P., Bogacki, P., Pallotta, M., Bariana, H. S., Wallwork, H. (2002). Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene Rlnn1 in wheat. *Theoretical and Applied Genetics 104*:874-879. https://doi.org/10.1007/s00122-001-0839-3

Wu, R., Ma, C-X., & Casella, G. (2007). *Statistical genetics of quantitative traits: Linkage, maps, and QTL*. Springer-Verlag New York, USA. https://doi.org/10.1007/978-0-387-68154-2

Zwart, R. S., Thompson, J. P., Milgate, A. W., Bansal, U. K., Williamson, P. M., Raman, H., & Bariana, H. S. (2010). QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Mol Breed 26*:107-124. https://doi.org/10.1007/s11032-009-9381-9

LIST OF FIGURES

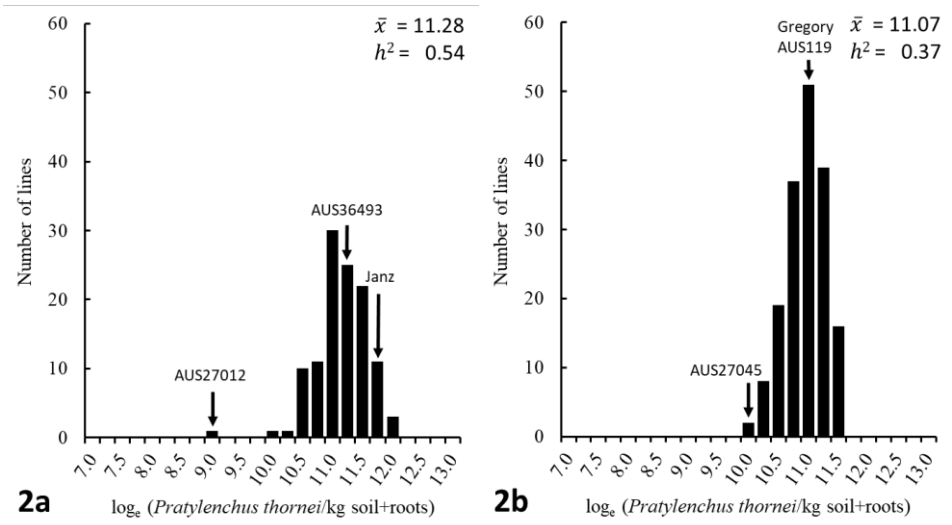
683

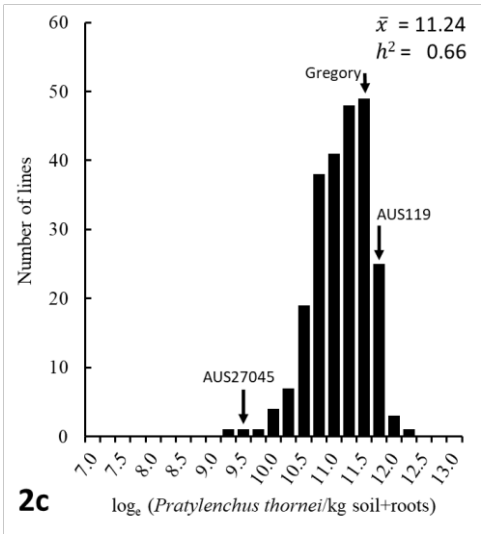


684

685 Figure 1. Distribution of BC_1F_2 recombinant inbred lines and their parents based on best linear
686 unbiased predictions (BLUPs) of *Pratylenchus thornei* final population densities for the a)
687 Tma1GRIL population, b) Tmm1GRIL population and c) Tmm1JRIL population. Low final *P.*
688 *thornei* population densities indicate resistance; high population densities indicate susceptibility.
689 Final population density values are for comparison within, but not among, experiments. AUS119
690 = Chinese Spring originally sourced from the Australian Winter Cereals Collection (now
691 Australian Grains Genebank); AUS27012 = *Triticum monococcum* ssp. *monococcum*;
692 AUS27045 = *T. monococcum* ssp. *aegilopoides*; AUS36493 = Chinese Spring originally sourced
693 from Kansas State University Wheat Genetics Resource Centre.

694





695

696 Figure 2. Distribution of recombinant inbred lines and their parents based on best linear unbiased
697 predictions (BLUPs) of *Pratylenchus thornei* final population densities for the a) Tmm1JRIL
698 population (BC_1F_6), b) Tma1GRIL population (BC_1F_6) and c) Tma3GRIL population (BC_3F_5 and
699 BC_3F_6 combined analysis). Low final *P. thornei* population densities indicate resistance; high
700 population densities indicate susceptibility. Final population density values are for comparison
701 within, but not among, experiments. AUS119 = Chinese Spring originally sourced from the
702 Australian Winter Cereals Collection (now Australian Grains Genebank); AUS27012 = *Triticum*
703 *monococcum* ssp. *monococcum*; AUS27045 = *T. monococcum* ssp. *aegilopoides*; AUS36493 =

704 Chinese Spring originally sourced from Kansas State University Wheat Genetics Resource
705 Centre.
706
707

LIST OF TABLES

718 Table 1. Description of four einkorn-derived recombinant inbred line populations characterised for

719 their resistance to root-lesion nematode (*Pratylenchus thornei*).

Recombinant Inbred Line Population	**Parentage**	**Generation tested for *Pratylenchus thornei* resistance**
Tmm1GRIL	Chinese Spring (AUS36493)/AUS27012//Gregory	BC_1F_2 (135)
Tma1GRIL	Chinese Spring (AUS119)/AUS27045//Gregory	BC_1F_2 (218); BC_1F_6 (163)
Tmm1JRIL	Chinese Spring (AUS36493)/AUS27012//Janz	BC_1F_2 (152); BC_1F_6 (107)
Tma3GRIL	Chinese Spring (AUS119)/AUS27045//3*Gregory	BC_3F_5 (226); BC_3F_6 (226)

720 *Note*. Number of RILs tested in each generation are in parenthesis.

Table 2. Single seed descent efficiency (E_{ssd}) of four einkorn-derived recombinant inbred line populations (RILPs).

RILP	**Parentage**	**E_{ssd} [a] by generation**			
		BC_xF_2	**BC_xF_3**	**BC_xF_4**	**BC_xF_5**
Tma1GRIL	AUS119/AUS27045//Gregory	0.49	0.53	0.58	0.84
Tma3GRIL	AUS119/AUS27045//3*Gregory	0.87	0.84	0.96	-
Tmm1GRIL	AUS36493/AUS27012//Gregory	0.48	-	-	-
Tmm1JRIL	AUS36493/AUS27012//Janz	0.55	0.47	0.51	0.88

Note. AUS119 = Chinese Spring originally sourced from the Australian Winter Cereals Collection (now Australian Grains Genebank); AUS27012 = *Triticum monococcum* ssp. *monococcum*; AUS27045 = *T. monococcum* ssp. *aegilopoides*; AUS36493 = Chinese Spring originally sourced from Kansas State University Wheat Genetics Resource Centre.

[a] Single seed descent efficiency (E_{ssd}) was calculated as the number of generation F_x plants that produce generation F_{x+1} seed divided by the number of F_x seeds used to establish the F_x plants.

729 Table 3. Comparison of observed segregation ratios of einkorn-derived BC_1F_2 recombinant inbred
730 line populations with predicted Mendelian segregation ratios for 1-gene and 2-gene additive and
731 dominance genetic models.

Model	**Einkorn-derived BC_1F_2 recombinant inbred line populations, resistance donor[a] and number of RILs**		
	Tma1GRIL AUS27045 218	**Tmm1GRIL AUS27012 135**	**Tmm1JRIL AUS27012 152**
Additive 1-gene			
Predicted ratio (VS:MS:R)	5:2:1	5:2:1	5:2:1
Observed ratio	142:48:28	114:18:3	127:24:1
$\chi 2$ value	1.04^{ns}	29.16***	32.99***
Additive 2-gene			
Predicted ratio (VS:S:MS:MR:R)	25:20:14:4:1	25:20:14:4:1	25:20:14:4:1
Observed ratio	91:51:48:20:8	86:28:18:2:1	90:37:24:1:0
$\chi 2$ value	13.89**	35.75***	30.67***
Dominance 1-gene			
Dominant susceptibility			
Predicted ratio (VS:R)	7:1	7:1	7:1
Observed ratio	142:76	114:21	127:25
$\chi 2$ value	99.67***	1.15^{ns}	2.17^{ns}
Dominant resistance			
Predicted ratio (VS:R)	5:3	5:3	5:3
Observed ratio	142:76	114:21	127:25
$\chi 2$ value	0.65^{ns}	27.74***	28.74***
Dominance 2-gene			
Dominant susceptibility			
Predicted ratio (VS:MS:R)	49:14:1	49:14:1	49:14:1
Observed ratio	142:48:28	114:18:3	127:24:1
$\chi 2$ value	181.29***	5.97^{ns}	4.34^{ns}
Dominant resistance			
Predicted ratio (VS:MS:R)	25:29:5	25:29:5	25:29:5
Observed ratio	142:48:28	114:18:3	127:24:1
$\chi 2$ value	64.23***	97.86***	106.21***

732 *Note*. Significant χ^2 values indicate observed ratios differed from predicted ratios and the model
733 was rejected. ^{ns}Not significantly different. *Significant at the 0.05 probability level.
734 **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

735 [a] AUS27012 = *Triticum monococcum* ssp. *monococcum*; AUS27045 = *T. monococcum* ssp.

736 *aegilopoides*.

737

738 Table 4. Quantitative genetic analysis of *Pratylenchus thornei* resistance of einkorn-derived recombinant inbred line (RIL) populations.

Traits	**Einkorn-derived Recombinant Inbred Line Populations**					
	Tmm1GRIL	**Tma1GRIL**	**Tma1GRIL**	**Tmm1JRIL**	**Tmm1JRIL**	**Tma3GRIL**
Generation tested	BC_1F_2	BC_1F_2	BC_1F_6	BC_1F_2	BC_1F_6	BC_3F_6[a]
Inbreeding coefficient (F_g)	8.00	8.00	4.27	8.00	4.27	4.27
Number of RILs	135	218	163	152	107	226
Mean of RILs	11.13	10.51	11.07	11.83	11.28	11.24
R parent mean	10.70	9.13	10.19	10.29	9.23	9.5
S parent mean	11.71	11.51	11.15	12.55	11.94	11.84
Parental phenotypic range (PR)	1.01	2.38	0.96	2.26	2.71	2.34
RIL minimum $\log_e(x)$	10.45	7.47	10.19	10.78	10.08	9.79
RIL minimum BTM	*34,659*	*1,752*	*26,594*	*48,240*	*23,939*	*8,843*
RIL maximum $\log_e(x)$	11.93	12.29	11.71	13.02	12.21	12.43
RIL maximum BTM	*152,318*	*217,188*	*122,341*	*453,234*	*201,209*	*123,479*
RIL phenotypic range (GR)	1.48	4.82	1.52	2.24	2.13	2.64
Max. *P. thornei* reduction (%)[b]	77	99	78	89	88	93
Number (& %) of ≥MR RILs[c]	7 (5%)	42 (19%)	14 (9%)	7 (5%)	1 (1%)	11 (5%)
Average standard error of differences (AvSED)	0.56	0.96	0.63	0.75	0.67	0.48
Genetic variance (σ^2_g)	0.27	0.90	0.31	0.58	0.49	0.34
Mid-parent values (BC_xF_1)						
Observed	-	-	11.37	-	11.09	11.61
Predicted	11.17	10.76	10.84	11.93	11.26	11.40
Heritability[d] (h^2)						
$h^2 = 1 - PEV/(2\sigma^2_g)$	0.42	0.48	0.37	0.52	0.54	0.66
Minimum number of effective resistance genes (*k*)						
$k = PR^2/8\sigma^2_g$[e]	0.5	0.8	0.4	1.1	1.9	2.0

$k = (GR*h^2)^2/F_g\sigma^2{}_g{}^f$	0.2	0.8	0.2	0.3	0.6	2.1

Note. *Pratylenchus thornei* final population densities are best linear unbiased predictions (BLUPs) of $\log_e(x)$ transformed data, where x = nematodes per kg of soil and roots; BTM = back-transformed mean.

[a] Data from a combined analysis of the BC_3F_5 and BC_3F_6 generations. The inbreeding coefficient (F_g) for the BC_3F_6 generation has been used to estimate the minimum number of effective resistance genes.

[b] Per cent reduction in final *P*. *thornei* population density of the most resistant RIL compared with the most susceptible RIL.

[c] Total number of RILs in each population classified as R, RMR and MR after classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/) using the method of Thompson et al., (2020).

[d] Generalised heritability (Cullis et al., 2006)

[e] Castle-Wright estimator of gene number (Wu et al., 2007)

[f] Modified Castle-Wright estimator of gene number (Singh et al., 1995)

5.1 Implications of Chapter 5

Crop wild relatives have been an important resource to increase the genetic diversity of cultivated species and to improve their resistance and tolerance to biotic and abiotic stresses. Resistance to *P. thornei* was reported on the B- and D-genomes of ILW genotypes in Chapter 3 and in synthetic hexaploid wheat populations (Zwart et al. 2010), but not on the A-genome of wheat. To address this gap in our genetic resources, two *P. thornei*-resistant einkorn accessions were used to develop four wheat RIL populations so that 1) the einkorn-derived *P. thornei* resistance could be transferred to wheat and 2) the effective number of resistance genes could be estimated using segregation and quantitative genetic analyses. Chapter 5 was the first report of *P. thornei* resistance being transferred from einkorn to wheat. The resistance was controlled by one to two genes that had an additive gene action. This knowledge, and the 26 *P. thornei* resistant RILs identified in this research, will facilitate the development of ABLs with novel A-genome *P. thornei*-resistance. It would be valuable to genotype the Tma3GRIL population and then conduct additional genetic analyses to identify QTL associated with the *P. thornei* resistance. A targeted intercrossing program among synthetic-derived ABLs that carry *P. thornei* resistance on their B- and D-genomes (Sheedy et al. 2017) and the *P. thornei* resistant RILs identified in this research will expedite the development of ABLs that combine *P. thornei* resistances on each of the A-, B-, and D-genomes of wheat. Those novel resistance combinations will likely deliver a higher level of *P. thornei* resistance than is currently available in the most resistant of common wheat cultivars.

CHAPTER 6: DISCUSSION AND CONCLUSIONS

6.1 Principal outcomes

This research has broadly endeavoured to 1) identify novel resistances to *P. thornei* and *P. neglectus*, the two most common and damaging species of root-lesion nematodes to Australian and global wheat production, 2) transfer these resistances to wheat breeding lines adapted to north-eastern Australia and 3) to improve our knowledge on the genetics of RLN resistance in wheat. To achieve this, we targeted the vast genetic diversity that exists in Iranian landrace wheats and einkorn, an A^m-genome diploid that is homoeologus with the A^u-genome of wheat. We combined traditional phenotyping and plant breeding techniques with quantitative and molecular genetic analyses to deliver the following outcomes.

The GWAS of ILWs established that seven QTL associated with *P. thornei* resistance and one associated with *P. thornei* susceptibility were present in a collection of 245 genotypes (Table 2). This is the first report for five of the resistance QTL, namely *QRlnt.usq-2B.2*, *QRlnt.usq-2B.3*, *QRlnt.usq-3B.1*, *QRlnt.usq-5B.1* and *QRlnt.usq-7B.1*. The susceptibility QTL, *QRlnt.usq-1B.1*, and two of the resistance QTL, *QRlnt.usq-2B.1* and *QRlnt.usq-6D.1*, were located near SNPs that have been reported in other landrace and/or synthetic hexaploid-derived populations (Rahman et al. 2019; Schmidt et al. 2005; Toktay et al. 2006; Zwart et al. 2005; 2010).

The maximum number of *P. thornei* resistance QTL found in any single genotype was six, indicating the polygenic nature of the resistance in this collection. Regression analysis of the reproduction factor of genotypes and the number of *P. thornei* resistance QTL per genotype established that *P. thornei* reproduction decreased exponentially as the number of resistance QTL per genotype increased. This supports the conclusion that *P. thornei* resistance was dose-dependent, as has been reported for WANA landraces (Thompson and Seymour 2011) and synthetic hexaploid wheats (Thompson 2008; Zwart et al. 2004). The evidence from this study and others supports the conclusion that *P. thornei* resistance in wheat is polygenic and dose-dependent.

This is the first report of a GWAS being used to identify novel QTL associated with resistance to root-lesion nematodes from ILW. Analysing existing phenotypic data of a germplasm collection in a GWAS framework proved an effective tool to identify putative *P. thornei* resistance QTL without the need to develop biparental populations.

Table 2. Quantitative trait loci (QTL) associated with resistance or susceptibility to the root-lesion nematodes *Pratylenchus thornei* and *P. neglectus*. Cultivars contributing resistance QTL are bolded. QTL associated with susceptibility are denoted by (S).

Population	RLN	Chromosomal Location																		Ref
ABL-derived																				
GS50a/Janz DH	Pt													6D						1
GS50a/Janz F3; **GS50a**/Batavia DH	Pt												6B	6D						2
Tammin/**Excalibur** DH	Pn															7A				3
Excalibur/Kukri DH	Pn															7A				4
Indian wheat GWAS	Pt		1B x 2	1D		2B		3B		5A			6B			7A				8
CIMMYT ABL GWAS	Pt			1D	2A						5B									12
CIMMYT ABL GWAS	Pn	1A	1B				3A	3B					6B			7A		7D		12
Landrace-derived																				
Morocco426/Janz DH	Pt					2B		3B												9
Morocco426/Janz DH	Pt					2B											7B			11
Iraq43/Janz DH	Pt							3B												9
Iraq43/Janz DH	Pt													6D			7B			11
Iraq43/**Janz** DH	Pt		1B (S)																	9
AUS49307.2/Pastor RIL	Pt		1B			2B								6D						10
Iranian landrace wheats GWAS	Pt		1B (S)			2B x 3		3B			5B			6D			7B			16
SHW-derived																				
W-7984/Opata RILs (ITMI population)	Pt					2B								6D						5
CPI133872/Janz DH	Pt													6D						6

CPI133872/Janz DH	Pn							4D				6D		6
CPI133872/Janz DH	Pt			2B								6D x 2		7
CPI133872/Janz DH	Pn			2B								6D		7
Croc_1/Ae.sq224//Opata x Pastor RIL	Pt						3B							10
CIMMYT SHW GWAS	Pn							4A		5B			7B	13
Sokoll/Krichauff DH	Pt		2A	2B x 3		2D					5D	6D x 2		14
Sokoll-derived RIL	Pt			2B								6D		15

Note: ABL = Advanced breeding line (includes released cultivars); DH = double haploid; GWAS = Genome-wide association study; RIL = Recombinant inbred line; RLN = Root-lesion nematode; Pn = *Pratylenchus neglectus*; Pt = *Pratylenchus thornei*; SHW = Synthetic hexaploid wheat. References: 1. D. Mather pers comm 2013; 2. Viccars et al. 1999; 3. Williams et al. 2002; 4. Jayatilake et al. 2013; 5. Zwart et al. 2006; 6. Zwart et al. 2005; 7. Zwart et al. 2010; 8. Kumar et al. 2021; 9. Schmidt et al. 2005; 10. Toktay et al. 2006; 11. Thompson et al. 2015; 12. Dababat et al. 2016; 13.Mulki et al. 2013; 14. Linsell et al. 2014; 15. Rahman et al. 2019; 16. Sheedy et al. unpublished data.

Validation of the QTL in active breeding populations rather than developing additional biparental populations reduced the time taken and cost of delivering novel QTL combinations in four advanced breeding lines suitable for use by plant breeding programs.

After characterising the *P. thornei*-resistant ILWs for their resistance to *P. neglectus*, seven ILW genotypes were identified that are resistant to both *P. thornei* and *P. neglectus*. Five of these genotypes do not carry the known *P. neglectus* resistance loci *Rlnn1* or *QRlnt.lrc-2B* and are likely novel sources of resistance. The five ILW with putatively novel *P. neglectus* resistance produced final *P. neglectus* population densities that were, on average, 73% less than the most susceptible genotype. This was a lower percentage reduction than genotypes that carried the *Rlnn1* resistance gene (86%) and the synthetic and synthetic-derived genotypes that carried *QRlnt.lrc-2B* (78%), but would still effectively manage *P. neglectus* population densities in cropping systems. They also offer the prospect of *P. neglectus* resistance that is not linked with the yellow flour colour defect associated with *Rlnn1*.

Subsequently, 13 ILW-derived ABL were produced that were at least moderately resistant-moderately susceptible (MRMS) to *P. thornei*, seven to *P. neglectus* and six of these had combined resistance to both *P. thornei* and *P. neglectus*. Of the seven *P. neglectus*-resistant genotypes, one likely carries novel *P. neglectus* resistance with the remaining six genotypes carrying *Rlnn1*. The six ABL that combined effective levels of resistance to *P. thornei* and *P. neglectus* have the parentage AUS28369/2*EGA Wylie where AUS28369 is an ILW rated as resistant-moderately resistant (RMR) to *P. neglectus* and moderately resistant (MR) to *P. thornei*, and EGA Wylie is an Australian wheat cultivar with moderate tolerance to *P. thornei* (Thompson et al. 2021). All six ABL have resistance to both nematode species that is phenotypically superior to current Australian commercial cultivars and all are agronomically similar to their recurrent parent EGA Wylie.

Through a program of interspecific hybridization, four einkorn-derived RILPs were developed and characterized for their resistance to *P. thornei* in the BC_1F_2, BC_1F_6, BC_3F_5 and/or BC_3F_6 generations. During this process, 26 RILs (in the BC_1F_6 and BC_3F_6 generations) were identified that likely carry novel A-genome resistance to *P. thornei*. This is the first report of *P. thornei* resistance being transferred from einkorn to wheat. Segregation analysis of the BC_1F_2 generation and the application of

quantitative genetic formulae for estimation of number of resistance genes in the BC_1F_2, BC_1F_6, and BC_3F_6 generations indicated that one to two genes controlled the *P. thornei* resistance in all four RILPs.

The development of these einkorn-derived RILPs and the selection of 26 *P. thornei* resistant RILs has broadened the useable genetic base of resistance available to breeding programs by transferring resistance from the secondary gene pool to the primary gene pool. Exploitation by plant breeders of this largely untapped pool of resistance to *P. thornei* will deliver additional genetic resources that increase diversity within their breeding programs. Combined with the knowledge that the einkorn-derived resistance is controlled by one to two genes and operates with an additive gene action, this germplasm will facilitate the efficient development of genotypes with novel *P. thornei*-resistance combinations that can better manage *P. thornei* population densities in commercial farming systems.

6.2 Future opportunities

Iranian landrace wheats are an important source of genetic diversity for wheat improvement. The identification of five novel *P. thornei* resistance QTL through the GWAS of an ILW collection will be beneficial for developing *P. thornei*-resistant ABL. However, fine mapping of these QTL would be valuable to identify SNPs that are very closely linked to the resistances. Improving our knowledge of the precise genetic location of the *P. thornei* resistances will allow the identification of candidate resistance genes and their associated mechanisms of resistance, development of ABL with novel QTL combinations that will deliver durable and improved levels of resistance, and development of the closely linked markers necessary for commercial breeding applications.

Currently there is contrasting information on the genetics of *P. neglectus* resistance in wheat. The *Rlnn1* gene was shown to be a single gene of large effect (Williams et al. 2002) and *P. neglectus* resistance derived from CIMMYT produced SHWs has been shown to be oligogenic and additive (Mulki et al. 2013) and polygenic in CIMMYT ABL (Dababat et al. 2016) and five SHW/Janz DH populations (Thompson et al. 2012). To date, there are no reports of the genetics of *P. neglectus* resistance in landrace wheats. Developing populations derived from the five ILWs that carry novel *P. neglectus* resistance identified in this research will allow genetic analyses to

improve our knowledge on the inheritance and number of genes that control the resistances and facilitate the identification of SNPs closely linked to the novel *P. neglectus* resistances. Additionally, evaluating Vikram's core set that captures 93% of the rare alleles of the entire ILW collection (Vikram et al. 2020) in a GWAS framework would identify novel *P. neglectus* resistant genotypes and SNPs associated with those resistances. Using both approaches would maximise the likelihood of identifying the genetic diversity of *P. neglectus* resistance available to wheat improvement in the ILW collection, improve our understanding of the genetics and mechanisms of the resistances, and provide the molecular tools necessary to select for the resistances in wheat breeding programs

In this research, *P. thornei* resistance was successfully transferred from einkorn to wheat and it was established that the resistance was controlled by one to two genes with an additive gene action. To maximise the benefit of this new genetic resource for plant breeders, it will be necessary to conduct additional genetic analyses to identify SNPs associated with the *P. thornei* resistances and to develop markers suited to commercial selection platforms. Those molecular tools, and those already published for other types of *P. thornei* resistance, could be used for marker-assisted selection of populations derived from a targeted intercrossing program among the synthetic-derived ABLs and the *P. thornei* resistant einkorn-derived RILs that we have identified in this research. Such a program would develop ABLs that combine *P. thornei* resistances on each of their A-, B-, and D-genomes, which will likely deliver a higher level of *P. thornei* resistance than is currently available in commercial wheat cultivars.

6.3 Conclusions

The root-lesion nematodes *P. thornei* and *P. neglectus* are serious pathogens of global wheat production and can be managed effectively through the incorporation of genetic resistance and tolerance into commercial wheat genotypes. This research has identified novel sources of resistance to both RLN, identified novel QTL for *P. thornei* resistance, determined the number of genes and gene action of einkorn-derived *P. thornei* resistance and produced 43 wheat ABL with resistance to *P. thornei*, *P. neglectus* or both *P. thornei* and *P. neglectus*. These tools, germplasm and knowledge of the genetics of resistance can be used by researchers and plant breeding programs to develop wheat genotypes that carry durable and genetically diverse RLN resistances that are superior to any level currently commercially available.

REFERENCES

Aballay E, Persson P, Mårtensson A (2009) Plant-parasitic nematodes in Chilean vineyards. *Nematropica* 39: 85-97. https://journals.flvc.org/nematropica/article/view/64470

Aballay E, Prodan S, Martensson A, Persson P (2012) Assessment of rhizobacteria from grapevine for their suppressive effect on the parasitic nematode *Xiphinema index*. *Crop Protection* 42: 36-41. https://doi.org/10.1016/j.cropro.2012.08.013

Adhikari L, Raupp J, Wu S, Wilson D, Evers B, Koo DH, Singh N, Friebe B, Poland J (2022) Genetic characterization and curation of diploid A-genome wheat species. *Plant Physiology* 188: 2101–2114. https://doi.org/10.1093/plphys/kiac006

Alipour H, Bihamta MR, Mohammadi V, Peyghambari SA, Bai G, Zhang G (2017) Genotyping-by-Sequencing (GBS) Revealed Molecular Genetic Diversity of Iranian Wheat Landraces and Cultivars. *Frontiers in Plant Science* 8: 1293. https://doi.org/10.3389/fpls.2017.01293

Anker CC, Buntjer JB and Niks RE (2001) Morphological and molecular characterisation confirm that *Triticum monococcum* s.s. is resistant to wheat leaf rust. *Theoretical and Applied Genetics* 103: 1093-1098. https://doi.org/10.1007/s001220100667

Bekal S, Domier LL, Niblack TL, Lambert KN (2011) Discovery and initial analysis of novel genomes in the soybean cyst nematode. *Journal of General Virology* 92: 1870-1879. https://doi.org/10.1099/vir.0.030585-0

Bhattacharya M, Jafari Shabestari J, Qualset CO, Corke H (1997) Diversity of starch pasting properties in Iranian hexaploid wheat landraces. *Cereal Chemistry* 74: 417-423. https://doi.org/10.1094/CCHEM.1997.74.4.417

Bos I, Caligari P (1995) Selection methods in plant breeding. (Chapman & Hall London, UK). Pp 347. ISBN: 9789401584326

Brennan PS, Martin DJ, Thompson JP, Mason LR, Sheppard JA, Keys PJ, Uebergang RW, The D, Agius PJ, Fiske ML, Ross J, Clewett TG (1994) *Triticum aestivum* ssp. *vulgare* (bread wheat) cv. Pelsart. *Australian Journal of Experimental Agriculture* 34: 864-865. https://doi.org/10.1071/EA9940864

Bucki P, Qing X, Castillo P, Gamliel A, Dobrinin S, Alon T, Miyara SB (2020) The genus *Pratylenchus* (Nematoda: Pratylenchidae) in Israel: From taxonomy to control practices. *Plants* 9: 1475. https://doi.org/10.3390/plants9111475

Cabos RYM, Wang KH, Sipes BS, Heller WP, Matsumoto TK (2013) Detection of plant-parasitic nematode DNA in the gut of predatory and omnivorous nematodes. *Nematropica* 43: 44-48. https://journals.flvc.org/nematropica/article/view/82430

Cadle MM, Murray TD, Jones SS (1997) Identification of resistance to *Pseudocercosporella herpotrichoides* in *Triticum monococcum*. *Plant Disease* 81: 1181-1186. https://doi.org/10.1094/PDIS.1997.81.10.1181

Casanas F, Simo J, Casals J, Prohens J (2017) Toward an evolved concept of landrace. *Frontiers of Plant Science* 8: 145. https://www.ncbi.nlm.nih.gov/pubmed/28228769

Castillo P, Jimenez-Diaz RM, Gomez-Barcina A, Vovlas N (1995) Parasitism of the root-lesion nematode *Pratylenchus thornei* on chickpea. *Plant Pathology* 44: 728-733. https://doi.org/10.1111/j.1365-3059.1995.tb01697.x

Castillo P, Vovlas N (2007) *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. In Nematology Monographs and Perspectives Volume 6 Series 9. Eds DJ Hunt and RN Perry. (Brill: Leiden, the Netherlands.)

Chen J, Chen J, Zhang Y, Lv Y, Qiao H, Tian M, Cheng L, Chen F, Zhang S, Guan W (2021) Effects of maternal supplementation with fully oxidised beta-carotene on the reproductive performance and immune response of sows, as well as the growth performance of nursing piglets. *British Journal of Nutrition* 125: 62-70. https://pubmed.ncbi.nlm.nih.gov/32792028/

Cho MR, Yiem MS, Jeon HY, Han HR, Kim HH, Na SY, Lim CK (2005) Occurrence of *Pasteuria* spp. associated with plant-parasitic nematodes in Korea. *Journal of Asia-Pacific Entomology* 8: 193-197. https://doi.org/10.1016/S1226-8615(08)60091-6

Corbett DCM (1982) Migratory soil Tylenchida. In 'Plant nematology'. (Ed. JF Southey) pp. 188-201. (Her Majesty's Stationery Office: London).

Cox TS (1991) 'The contribution of introduced germplasm to the development of U.S. wheat cultivars.' Use of Plant Introductions in Cultivar Development, Part 1, Crop

Science Society of America Special Publication No. 17, USA. https://doi.org/10.2135/cssaspecpub17.c3

Cox TS (1998) Deepening the wheat gene pool. *Journal of Crop Production* 1: 1-25. https://doi.org/10.1300/J144v01n01_01

Crawford AC, Stefanova K, Lambe W, McLean R, Wilson R, Barclay I, Francki MG (2011) Functional relationships of phytoene synthase 1 alleles on chromosome 7A controlling flour colour variation in selected Australian wheat genotypes. *Theoretical and Applied Genetics* 123: 95-108. https://doi.org/10.1007/s00122-011-1569-9

Cullis BR, Smith AB, Coombes NE (2006) On the design of early generation variety trials with correlated data. *Journal of Agricultural, Biological and Environmental Statistics* 11: 381-393. https://doi.org/10.1198/108571106X154443

Dababat AA, Ferney G-BH, Erginbas-Orakci G, Dreisigacker S, Imren M, Toktay H, Elekcioglu HI, Mekete T, Nicol JM, Ansari O, Ogbonnaya F (2016) Association analysis of resistance to cereal cyst nematodes (*Heterodera avenae*) and root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) in CIMMYT advanced spring wheat lines for semi-arid conditions. *Breeding Science* 66: 692-702. https://doi.org/10.1270/jsbbs.15158

da Luz WC (1982) Nematodeos fitoparasitas associados com trigo no Rio Grande do Sul (Plant parasitic nematodes associated with wheat in the state of Rio Grande do Sul, Brazil). *Pesquisa Agropecuaria Brasileira* 17: 215-217. (Portugese language with English summary). https://seer.sct.embrapa.br/index.php/pab/article/view/15600

D'Egidio MG, Nardi S, Vallega V (1993) Grain, flour and dough characteristics of selected strains of diploid wheat *Triticum monococcum* L. *Cereal Chemistry* 70: 298-303. https://www.cerealsgrains.org/publications/cc/backissues/1993/Documents/CC1993a62.html

Di Pietro JP, Caillaud CM, Chaubet B, Pierre JS, Trottet M (1998) Variation in resistance to the grain aphid, *Sitobion avenae* (*Sternorhynca*: *Aphididae*), among diploid wheat genotypes: multivariate analysis of agronomic data. *Plant Breeding* 117: 407-412. https://doi.org/10.1111/j.1439-0523.1998.tb01964.x

Doyle AD, McLeod RW, Wong PTW, Hetherington SE, Southwell RJ (1987) Evidence for the involvement of the root lesion nematode *Pratylenchus thornei* in wheat yield decline in northern New South Wales. *Australian Journal of Experimental Agriculture* 27: 563-570. https://doi.org/10.1071/EA9870563

Ehdaie B, Baker CA (1999) Inheritance and allelism for resistance to Russian wheat aphid in an Iranian spring wheat. *Euphytica* **107**: 71-78. https://doi.org/10.1023/A:1003549512216

Ehdaie B, Waines JG, Hall AE (1988) Differential responses of landrace and improved spring wheat genotypes to stress environments. *Crop Science* **28**: 838-842. https://doi.org/10.2135/cropsci1988.0011183X002800050024x

El Bouhssini M, Benlhabib O, Bentika A, Sharma HC, Lhaloui S (1998) Sources of resistance in *Triticum* and *Aegilops* species to Hessian fly (Diptera: Cecidomyiidae) in Morocco. *Arab Journal of Plant Protection* 15: 126-128. https://doi.org/10.1023/A:1008675029389

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics 4th Ed. (Addison Wesley Longman Ltd, Essex, England). Pp 480. ISBN: 9780582243026

Fanning J, Linsell K, McKay A, Gogel B, Munoz-Santa I, Davey R, Hollaway G (2018) Resistance to the root lesion nematodes *Pratylenchus thornei* and *P. neglectus* in cereals: Improved assessments in the field. *Applied Soil Ecology* 132: 146-154. https://doi.org/10.1016/j.apsoil.2018.08.023

FAO. 2021. Food Outlook – Biannual report on global food markets. Food Outlook, November 2021. Rome. https://doi.org/10.4060/cb7491en

FAO. 2022. Crop Prospects and Food Situation – Quarterly Global Report No. 1, March 2022. Rome. https://doi.org/10.4060/cb8893en

FAO. 2023. FAO Cereal supply and demand brief. Release date: 03/02/2023. Rome. https://www.fao.org/worldfoodsituation/csdb/en/

Fatemah F, Reza F-N, Reza AA, Vahe M, Frahnaz JA, Zeynab B (2012) Determination of reaction of some wheat lines/cultivars to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) under controlled conditions in southwest Iran. *International Journal of Nematology* 22: 73-80.

Feldman M, Sears ER (1981) The wild gene resources of wheat. *Scientific American* 244: 102-112. https://www.jstor.org/stable/24964263

Feuillet C, Langridge P, Waugh R (2007) Cereal breeding takes a walk on the wild side. *Trends in Genetics* 24:24-32. https://doi.org/10.1016/j.tig.2007.11.001

Fourie H, McDonald AH, Loots GC (2001) Plant-parasitic nematodes in field crops in South Africa. 6. Soybean. *Nematology* 3: 447-454. https://doi.org/10.1163/156854101753250773

Fortuner R (1977) *Pratylenchus thornei*. Commonwealth Institute of Helminthology Descriptions of Plant-parasitic Nematodes Set 7, No. 93.

France RA, Brodie BB (1995) Differentiation of two isolates of *Pratylenchus penetrans* based on their reaction to potato. *Journal of Nematology* 27: 339-345. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2619616/

France RA, Brodie BB (1996) Characterisation of *Pratylenchus penetrans* from ten geographically isolated populations based on their reaction on potato. *Journal of Nematology* 28: 520-526. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2619724/

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica* 91: 59-87. https://doi.org/10.1007/BF00035277

Gill BS, Raupp WJ, Sharma HC, Browder LE, Hatchett JH, Harvey TL, Moseman JG, Waines JG (1986) Resistance in *Aegilops squarrosa* to wheat leaf rust, wheat powdery mildew, Greenbug and Hessian fly. *Plant Disease* 70: 553-556. https://doi.org/10.1094/pd-70-553

Gorham J (1990) Salt tolerance in the *Triticeae*: K/Na discrimination in synthetic hexaploid wheats. *Journal of Experimental Botany* 41: 623-627. https://doi.org/10.1093/jxb/41.5.623

Gorham J, Bristol A, Young EM, Wyn-Jones RG (1991) The presence of the enhanced K/Na discrimination trait in diploid *Triticum* species. *Theoretical and Applied Genetics* 82: 729-739. https://doi.org/10.1007/BF00227318

GRDC (2021) 2022 Queensland Winter Crop Sowing Guide. Grains Research and Development Corporation, Canberra, Australia. Pp 32. ISSN: 2652-3590 (print); 2652-3604 (online). https://grdc.com.au/queensland-winter-crop-sowing-guide

Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. *Taxon* 20:509-517. https://doi.org/10.2307/1218252

Holeva R, Phillips MS, Neilson R, Brown DJF, Young V, Boutsika K, Blok VC (2006) Real-time PCR detection of vector trichodorid nematodes and Tobacco Rattle Virus. *Molecular and Cellular Probes* 20: 203-211. https://doi.org/10.1016/j.mcp.2005.12.004

Hussien T, Bowden RL, Gill BS, Cox TS (1998) Chromosomal locations in common wheat of three new leaf rust resistance genes from *Triticum monococcum*. *Euphytica* 101: 127-131. https://doi.org/10.1023/A:1018376315133

Jayatilake DV, Tucker EJ, Bariana H, Kuchel H, Edwards J, McKay AC, Chalmers K, Mather DE (2013) Genetic mapping and marker development for resistance of wheat against the root lesion nematode *Pratylenchus neglectus*. *BMC Plant Biology* 13: 230. https://doi.org/10.1186/1471-2229-13-230

Johnson BL, Dhaliwal HS (1976) Reproductive isolation of *Triticum boeoticum* and *Triticum urartu* and the origin of the tetraploid wheats. *American Journal of Botany* 63: 1088-1094. https://doi.org/10.2307/2441653

Jones MGK, Fosu-Nyarko J (2014) Molecular biology of root lesion nematodes (*Pratylenchus* spp.) and their interaction with host plants. *Annals of Applied Biology* 164: 163-181. https://di.org/10.1111/aab.12105

Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, Kikuchi T, Manzanilla-Lopez R, Palomares-Rius JE, Wesemael WML, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14: 946-961. https://doi.org/10.1111/mpp.12057

Keil T, Laubach E, Sharma S, Jung C (2009) Screening for resistance in the primary and secondary gene pool of barley against the root-lesion nematode *Pratylenchus neglectus*. *Plant Breeding* 128: 436-442. https://doi.org/10.1111/j.1439-0523.2009.01612.x

Kerber ER, Green GJ (1980) Suppression of stem rust resistance in the hexaploid wheat cv. Canthatch by chromosome 7DL. *Canadian Journal of Botany* 58: 1347-1350. https://doi.org/10.1139/b80-166

Khan MR, Jain RK, Singh RV, Pramanik A (2010) Economically important plant parasitic nematodes distribution atlas. Indian Council of Agricultural Research. New Delhi, India. pp145.

Khan MR, Mohidin FA, Khan U, Ahamad F (2016) Native *Pseudomonas* spp. suppressed the root-knot nematode *in vitro* and *in vivo*, and promoted the nodulation and grain yield in the field grown mungbean. *Biological Control* 101: 159-168. http://dx.doi.org/10.1016/j.biocontrol.2016.06.012

Khanna-Chopra R, Viswanathan C (1999) Evaluation of heat stress tolerance in irrigated environment of *T. aestivum* and related species. I. Stability and yield components. *Euphytica* 106: 169-180. https://doi.org/10.1023/A:1003531722420

Koenning SR, Overstreet C, Nolling JW, Donald PA, Becker JO, Fortnum BA (1999) Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. *Journal of Nematology* 31(4S): 587-618. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2620402/

Kolarov J (1988) Resistance of different wheats to the cereal leaf beetle *Oulema melanopus* L. (*Coleoptera*, *Chrysomelidae*). *Cereal Research Communications* 16: 19-23. https://www.jstor.org/stable/23782757

Kumar D, Sharma S, Sharma R, Pundir S, Kumar Singh V, Chaturvedi D, Singh B, Kumar S, Sharma S (2021) Genome-wide association study in hexaploid wheat identifies novel genomic regions associated with resistance to root lesion nematode (*Pratylenchus thornei*). *Scientific Reports* 11: 3572. https://doi.org/10.1038/s41598-021-80996-0

Linsell KJ, Rahman MS, Taylor JD, Davey RS, Gogel BJ, Wallwork H, Forrest KJ, Hayden MJ, Taylor SP, Oldach KH (2014) QTL for resistance to root lesion nematode (*Pratylenchus thornei*) from a synthetic hexaploid wheat source. *Theoretical and Applied Genetics* 127: 1409-1421. https://doi.org/10.1007/s00122-014-2308-9

Lucas H (2012) The Wheat Initiative – an international research initiative for wheat improvement. Second Global Conference on Agricultural Research for Development. Punta del Este, Uruguay, 29 October – 1 November 2012.

Ma H, Hughes GR (1993) Resistance to Septoria nodorum blotch in several Triticum species. *Euphytica* 70: 151-157. https://doi.org/10.1007/BF00029652

Martin RR, Pinkerton JN, Kraus J (2009) The use of collagenase to improve the detection of plant viruses in vector nematodes by RT-PCR. *Journal of Virological Methods* 155: 91-95. https://doi.org/10.1016/j.jviromet.2008.10.002

Marcussen T, Sandve SR, Heier L, Spannagl M, Pfeifier M, The International Wheat Genome Consortium, Jakobsen KS, Wulff BBH, Steuernagl B, Mayer KFX, Olsen O-A (2014) Ancient hybridisations among the ancestral genomes of bread wheat. *Science* 345. https://doi.org/10.1126/science.1250092

Matthews P, McCaffery D, Jenkins L (2022) Winter Crop Variety Sowing Guide 2022. New South Wales Department of Primary Industries, Sydney. Pp 187. ISSN: 1328-9535. https://www.dpi.nsw.gov.au/agriculture/broadacre-crops/guides/publications/winter-crop-variety-sowing-guide

McIntosh RA, Dubcovsky J, Rogers WJ, Xia XC, Raupp WJ (2020) Catalogue of gene symbols for wheat. https://wheat.pw.usda.gov/GG3/wgc

Meagher JW (1970) Seasonal fluctuations in numbers of larvae of the cereal cyst nematode (*Heterodera avenae*) and of *Pratylenchus minyus* and *Tylenchorhynchus brevidens* in soil. *Nematologica* 16: 333-347. https://doi.org/10.1163/187529270X00018

Mendoza-de Gives P (2022) Soil-Borne Nematodes: Impact in agriculture and livestock and sustainable strategies of prevention and control with special reference to the use of nematode natural enemies. *Pathogens* 11: 640. https://doi.org/10.3390/pathogens11060640

Mokrini F, Waeyenberger L, Viaene N, Abbad Andaloussi F, Moens M (2016) Diversity of root-lesion nematodes (*Pratylenchus* spp.) associated with wheat (*Triticum aestivum* and *T. durum*) in Morocco. *Nematology* 18: 781-801. https://doi.org/10.1163/15685411-00002993

Mokrini F, Viaene N, Waeyenberge L, Dababat AA, Moens M (2018) Root-lesion nematodes in cereal fields: importance, distribution, identification, and management strategies. *Journal of Plant Diseases and Protection* 126: 1-11. https://doi.org/10.1007/s41348-018-0195-z

Murray GM, Brennan JP (2009) Estimating disease losses to the Australian wheat industry. *Australasian Plant Pathology* 38: 558-570. https://doi.org/10.1071/AP09053

Norton DC (1978) Ecology of plant-parasitic nematodes (John Wiley and Sons: New York). Pp 268. ISBN: 9780471031888

Nabi F, Arain MA, Rajput N, Alagawany M, Soomro J, Umer M, Soomro F, Wang Z, Ye R, Liu J (2020) Health benefits of carotenoids and potential application in poultry industry: A review. *Journal of Animal Physiology and Animal Nutrition* 104: 1809-1818. https://www.ncbi.nlm.nih.gov/pubmed/32333620

Nusbaum CJ and Ferris H (1973) The role of cropping systems in nematode population management. *Annual Review of Phytopathology* 11: 423-440. https://doi.org/10.1146/annurev.py.11.090173.002231

Ogbonnaya FC, Imtiaz M, Bariana HS, McLean M, Shankar MM, Hollaway GJ, Trethowan RM, Lagudah ES, van Ginkel M (2008) Mining synthetic hexaploids for multiple disease resistance to improve bread wheat. *Australian Journal of Agricultural Research* 59: 421-431. https://doi.org/10.1071/AR07227

Owen KJ, Clewett TG, Bell KL, Thompson JP (2014) Wheat biomass and yield increased when populations of the root-lesion nematode (*Pratylenchus thornei*) were reduced through sequential rotation of partially resistant winter and summer crops. *Crop and Pasture Science* 65: 227-241. https://doi.org/10.1071/CP13295

Owen KJ, Clewett TG, Bell KL, Thompson JP (2022) Cereal and pulse crops with improved resistance to *Pratylenchus thornei* are needed to maximize wheat production and expand crop Sequence options. *Agronomy* 12: 573. https://doi.org/10.3390/agronomy12030573

Pasam RK, Bansal U, Daetwyler HD, Forrest KL, Wong D, Petkowski J, Willey N, Randhawa M, Chhetri M, Miah H, Tibbits J, Bariana H, Hayden MJ (2017) Detection and validation of genomic regions associated with resistance to rust diseases in a worldwide hexaploid wheat landrace collection using BayesR and mixed linear model approaches. *Theoretical and Applied Genetics* 130: 777-793. https://doi.org/10.1007/s00122-016-2851-7

Potgieter GF, Marais GF, du Toit F (1991) The transfer of resistance to the Russian wheat aphid from *Triticum monococcum* L. to common wheat. *Plant Breeding* 106: 284-292. https://doi.org/10.1111/j.1439-0523.1991.tb00513.x

Pourjam E, Kheiri A, Geraert E, Alizadeh A (1999) Variations in Iranian populations of *Pratylenchus neglectus* and *P. thornei* (Nematoda: Pratylenchidae). *Iranian Journal of Plant Pathology* 35: 23-27.

Quick JS, Nkongolo KK, Meyer W, Peairs FB, Weaver B (1991) Russian wheat aphid reaction and agronomic and quality traits of a resistant wheat. *Crop Science* 31: 50-53. https://doi.org/10.2135/cropsci1991.0011183X003100010012x

Ramshini H, Mirzazadeh T, Moghaddam ME, Amiri R (2016) Comparison of old and new wheat cultivars in Iran by measuring germination related traits, osmotic tolerance and ISSR diversity. *Physiology and Molecular Biology of Plants* 22: 391–398 https://doi.org/10.1007/s12298-016-0372-0

Rao MS, Kamalnath M, Umamaheswari R, Rajinikanth R, Prabu P, Priti K, Grace GN, Chaya NK, Gopalakrishnan C (2017) *Bacillus subtilis* IIHR BS-2 enriched vermicompost controls root knot nematode and soft rot disease complex in carrot. *Scientia Horticulturae* 218: 56-62. https://doi.org/10.1016/j.scienta.2017.01.051

Reen RA, Thompson JP, Clewett TG, Sheedy JG, Bell KL (2014) Yield response in chickpea cultivars and wheat following crop rotations affecting population densities of *Pratylenchus thornei* and arbuscular mycorrhizal fungi. *Crop & Pasture Science* 65: 428-441. https://doi.org/10.1071/CP13441

Rhode RA (1972) Expression of resistance in plants to nematodes. *Annual Review of Phytopathology* 10: 233-252. https://doi.org/10.1146/annurev.py.10.090172.001313

Roberts PA (2002) Concepts and consequences of resistance. In 'Plant resistance to parasitic nematodes'. (Ed J Starr, R Cook and J Bridge) pp. 23-41 (CABI Publishing: Wallingford, UK). ISBN: 0851994660.

Rosyara U, Kishii M, Payne T, Sansaloni CP, Singh RP, Braun H-J, Dreisigacker S (2019). Genetic contribution of synthetic hexaploid wheat to CIMMYT's spring bread wheat breeding germplasm. *Scientific Reports* 9:12355. https://doi.org/10.1038/s41598-019-47936-5

Saidi A (2001) Wheat breeding in Iran. In 'Proceedings of the 10th assembly of the Wheat Breeding Society of Australia inc.'. 16-21 September 2001, Mildura, Australia. pp. 63-67. ISBN: 0957946902.

Saremirad A, Bihamta MR, Malihipour A, Mostafavi K, Alipour H (2021) Genome-wide association study in diverse Iranian wheat germplasms detected several putative genomic regions associated with stem rust resistance. *Food Science and Nutrition* 9: 1357-1374. https://doi.org/10.1002/fsn3.2082

Schmidt AL, McIntyre CL, Thompson JP, Seymour NP, Liu CJ (2005) Quantitative trait loci for root lesion nematode (*Pratylenchus thornei*) resistance in Middle-Eastern landraces and their potential for introgression into Australian bread wheat. *Australian Journal of Agricultural Research* 56: 1059-1068. https://doi.org/10.1071/AR05016

Schmolke M, Mihler V, Hartl L, Zeller FJ, Hsam SLK (2012) A new powdery mildew resistance allele at the Pm4 wheat locus transferred from einkorn (*Triticum monococcum*). *Molecular Breeding* 29: 449–456. https://doi.org/10.1007/s11032-011-9561-2

Sharma HC, Foster JE, Ohm HW, Patterson FL (1992) A note on resistance to Hessian fly (*Mayetiola destructor*) (Diptera: Cecidomyiidae) biotype L in tribe *Triticeae*. *Phytoprotection* 73: 79-82. https://doi.org/10.7202/706023ar

Sheedy JG, Lin J, Thompson JP (2022) Discovery of resistance to *Pratylenchus neglectus* among *P. thornei*- resistant Iranian landrace wheats and the introgression of both resistances into advanced breeding lines. *Plant Pathology* 71: 2017-2028. https://doi.org/10.1111/ppa.13616

Sheedy JG, Thompson JP (2009) Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. *Australasian Plant Pathology* 38: 478-489. https://doi.org/10.1071/AP09030

Sheedy JG, Thompson JP, Kelly A (2012) Diploid and tetraploid progenitors of wheat are valuable sources of resistance to the root lesion nematode *Pratylenchus thornei*. *Euphytica* 186: 377-391. https://doi.org/10.1007/s10681-011-0617-5

Shiferaw B, Smale M, Braun H-J, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security 5: 291-317. https://doi.org/10.1007/s12571-013-0263-y

Sher SA, Allen MW (1953) Revision of the genus *Pratylenchus* (Nematoda: Tylenchidae). In 'University of California publications in zoology, volume 57(6)'. Eds

AH Miller, RM Eakin and C Stern) pp. 441-470. (University of California Press: Berkeley and Los Angeles).

Shewry PR, Hey SJ (2015) The contribution of wheat to human diet and health. Food and Energy Security 4:178-202. https://doi.org/10.1002/fes3.64

Shi AN, Leath S, Murphy JP (1998) A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. *Phytopathology* 86: 144-147. https://doi.org/10.1094/PHYTO.1998.88.2.144

Siddique ZA, Mahmood I (1996) Biological control of plant parasitic nematodes by fungi: A review. *Bioresource Technology* 58: 229-239. https://doi.org/10.1016/S0960-8524(96)00122-8

Siddique ZA, Mahmood I (1999) Role of bacteria in the management of plant parasitic nematodes: A review. *Bioresource Technology* 69: 167-179. https://doi.org/10.1016/S0960-8524(98)00122-9

Singh JSBK, Sharma TR (1997) Morpho-cytogenetics of *Triticum aestivum* L. x *Aegilops speltoides* Tausch. hybrids. *Wheat Information Service* 84: 51-52. http://shigen.nig.ac.jp/wheat/wis/No84/p51/1.html

Singh K, Chhuneja P, Singh I, Sharma SK, Garg T, Garg M, Keller B, Dhaliwal HS (2010) Molecular mapping of cereal cyst nematode resistance in *Triticum monococcum* L. and its transfer to the genetic background of cultivated wheat. *Euphytica* 176: 213-222. https://doi.org/10.1007/s10681-010-0227-7

Sleper DA, Poehlman JM (2006) Breeding field crops 5th Ed. (Blackwell Publishing, Hoboken, New Jersey, USA). Pp 432. ISBN: 9780813824284

Sotherton NW, Lee G (1988) Field assessments of resistance to the aphids *Sitobion avenae* and *Metopolophium dirhodum* in old and modern spring-sown wheats. *Annals of Applied Biology* 112: 239-248. https://doi.org/10.1111/j.1744-7348.1988.tb02060.x

Spiller NJ, Llewellyn M (1986) A comparison of the level of resistance in diploid *Triticum monococcum* and hexaploid *Triticum aestivum* wheat seedlings to the aphids *Metopolophium dirhodum* and *Rhopalosiphum padi*. *Annals of Applied Biology* 109: 173-177. https://doi.org/10.1111/j.1744-7348.1986.tb03196.x

Stirling GR (1991) Biological control of plant parasitic nematodes. (CAB International: Wallingford, UK). Pp 48. ISBN: 978-1-35107-146-8

Stirling GR (2014) Biological control of plant-parasitic nematodes: soil ecosystem management in sustainable agriculture. 2nd Ed. (CABI: Wallingford, United Kingdom). Pp 510. ISBN: 9781786395337

Stoskopf NC, Tomes DT, Christie BR (1993) Plant breeding theory and practice. (Westview Press Inc., Boulder, Colorado, USA). pp 531. ISBN: 9780367298470

Sturhan D, Shutova TS, Akimov VN, Subbotin SA (2005) Occurrence, hosts, morphology and molecular characterisation of *Pasteuria* bacteria parasitic in nematodes of the family Plectidae. *Journal of Invertebrate Pathology* 88: 17-26. https://doi.org/10.1016/j.jip.2004.11.001

Taylor SP, Hollaway GK, Hunt CH (2000) Effect of field crops on population densities of *Pratylenchus neglectus* and *P. thornei* in southeastern Australia; Part 1: *P. neglectus*. *Journal of Nematology* 32: 591-599. https://pubmed.ncbi.nlm.nih.gov/19271014/

Taylor SP, Vanstone VA, Ware AH, McKay AC, Szot D, Russ MH (1999) Measuring yield loss in cereals caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) with and without nematicide. *Australian Journal of Agricultural Research* 50: 617-622. https://doi.org/10.1071/A98103

Thompson JP (2008) Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. *Australian Journal of Agricultural Research* 59: 432-446. https://doi.org/10.1071/AR07222

Thompson JP, Brennan PS, Clewett TG, Sheedy JG and Seymour NP (1999) Progress in breeding wheat for tolerance and resistance to root-lesion nematode (*Pratylenchus thornei*). *Australasian Plant Pathology* 28:45-52. https://doi.org/10.1071/Ap99006

Thompson JP, Clewett TG, Sheedy JG, Reen RA, O'Reilly MM, Bell KL (2010) Occurrence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in the northern grain region of Australia. *Australasian Plant Pathology* 39: 254-264. https://doi.org/10.1071/AP09094

Thompson JP, Haak MI (1997) Resistance to root-lesion nematode (*Pratylenchus thornei*) in *Aegilops tauschii* Coss., the D-genome donor to wheat. *Australian Journal of Agricultural Research* 48: 553-559. https://doi.org/10.1071/A96167

Thompson JP, O'Reilly MM, Clewett TG (2009) Resistance to the root-lesion nematode *Pratylenchus thornei* in wheat landraces and cultivars from the West Asia and North Africa (WANA) region. *Crop & Pasture Science* 60: 1209-1217. https://doi.org/10.1071/CP09159

Thompson JP, Owen KJ, Stirling GR, Bell MJ (2008) Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*): a review of recent progress in managing a significant pest of grain crops in northern Australia. *Australasian Plant Pathology* 37: 235–242. https://doi.org/10.1071/AP08021

Thompson JP, Mackenzie J, Sheedy GH (2012a) Root-lesion nematode (*Pratylenchus thornei*) reduces nutrient response, biomass and yield of wheat in sorghum-fallow-wheat cropping systems in a subtropical environment. *Field Crops Research* 137: 126-140. https://doi.org/10.1016/j.fcr.2012.08.011

Thompson JP, Seymour NP (2011) Inheritance of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat landraces and cultivars from the West Asia and North Africa (WANA) region. *Crop & Pasture Science* 62: 82-93. https://doi.org/10.1071/CP10309

Thompson JP, Sheedy JG, Robinson NA (2020). Resistance of wheat genotypes to root-lesion nematode (*Pratylenchus thornei*) can be used to predict final nematode population densities, crop greenness, and grain yield in the field. Phytopathology 110: 505-516. https://doi.org/10.1094/PHYTO-06-19-0203-R

Thompson JP, Sheedy JG, Robinson NA, Clewett TG (2021) Tolerance of wheat (*Triticum aestivum*) genotypes to root-lesion nematode (*Pratylenchus thornei*) in the subtropical grain region of eastern Australia. *Euphytica* 217: 48 https://doi.org/10.1007/s10681-020-02761-0

Thompson J, Sheedy J, Robinson N, Khoo K, Chalmers K, Mather D (2015) Utilisation of the Watkins collection of wheat landraces for root-lesion nematode resistance. Ninth International Wheat Conference, Sydney, 20-25 September. p. 149.

Thompson JP, Sheedy JG, Seymour NP, Clewett TG, Mason LR, Sheppard JA, Banks PM (2001) Advances in breeding wheat for tolerance and resistance to *Pratylenchus thornei* and *P. neglectus* for the northern region. In 'Proceedings of the 10th assembly

of the Wheat Breeding Society of Australia inc.'. 16-21 September 2001, Mildura, Australia. pp. 123-127. ISBN: 0957946902.

Thompson JP, Zwart RS, Butler D (2012b) Inheritance of resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in five doubled-haploid populations of wheat. *Euphytica* 188: 209-219. https://doi.org/10.1007/s10681-012-0689-x

Toktay H, McIntyre CL, Nicol JM, Ozkan H, Elekcioglu HI (2006) Identification of common root-lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in wheat. *Genome* 49: 1319-1323. https://doi.org/10.1139/G06-090

Tomar SMS, Kochumadhavan M and Nambisan PNN (1988) Evaluation and utilisation of diploid species of wheat. *Wheat Information Service* 67: 9-10. http://shigen.nig.ac.jp/wheat/wis/No67/p9/1.html

Townshend JL, Anderson RV (1976) *Pratylenchus neglectus*. Commonwealth Institute of Helminthology Descriptions of Plant-parasitic Nematodes Set 6, No. 82.

Tripp R (1996) Biodiversity and modern crop varieties: Sharpening the debate. *Agriculture and Human Values* 13: 48-63. https://doi.org/10.1007/BF01530523

Trudgill DL (1991) Resistance to and tolerance of plant parasitic nematodes in plants. *Annual Review of Phytopathology* 29: 167-192. https://doi.org/10.1146/annurev.py.29.090191.001123

Valkoun JJ (2001) Wheat pre-breeding using wild progenitors. *Euphytica* 119: 17-23. https://doi.org/10.1023/A:1017562909881

Valkoun J, Kucerova D and Bartos P (1982) Genetics of resistance of cultivated einkorn wheat to stripe rust and powdery mildew. *Annals of the Research Institute for Crop production, Prague Ruzyne* 22: 6-16. (Abstract)

Valkoun J, Kucerova D and Bartos P (1989) Transfer of a new gene for stem rust resistance from *Triticum monococcum* L. to hexaploid wheat, *T. aestivum* L. *Genetika a Slechteni* 25: 209-214. (Abstract)

Vallega VE (1992) Agronomical performance and breeding value of selected strains of diploid wheat, *Triticum monococcum*. *Euphytica* 61: 13-23. https://doi.org/10.1007/BF00035542

van Slageren MW (1994) 'Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae).' (Wageningen Agricultural University: The Netherlands). Pp 512. ISBN: 9067543772.

Vanstone VA, Hollaway GJ, Stirling GR (2008) Managing nematode pests in the southern and western regions of the Australian cereal industry: continuing progress in a challenging environment. *Australasian Plant Pathology* 37: 220-234. https://doi.org/10.1071/AP08020

Vanstone VA, Rathjen AJ, Ware AH, Wheeler RD (1998) Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. *Australian Journal of Experimental Agriculture* 38: 181-188. https://doi.org/10.1071/EA97109

Vicars L, Spindler L, Haak I, Wildermuth G, Thompson J, Banks P, Appels R, Lagudah E (1999) Genetic markers for resistance to crown rot and root-lesion nematodes. In 'Proceedings of the 9th Assembly of the Wheat Breeding Society of Australia'. pp. 118–120. ISBN: 0909756384.

Vikram P, Franco J, Burgueño J, Li H, Sehgal D, Saint-Pierre C, Ortiz C, Kumar Singh V, Sneller C, Sharma A, Tattaris M, Guzman C, Pena J, Sansaloni CP, Serna JAC, Thiyagarajan K, Davila GF, Reynolds M, Sonder K, Govindan V, Ellis M, Bhavani S, Kamali MRJ, Roosatei M, Singh S, Basandrai D, Bains NS, Basandrai A, Payne T, Crossa J, Singh S (2020) Strategic use of Iranian bread wheat landrace accessions for genetic improvement: Core set formulation and validation. *Plant Breeding* 00: 1–13. https://doi.org/10.1111/pbr.12885

Waines JG, Ehdaie B, Barnhart D (1987) Variability in *Triticum* and *Aegilops* species for seed characteristics. *Genome* 29: 41-46. https://doi.org/10.1139/g87-007

Whish JPM, Thompson, JP, Clewett TG, Wood J, Rostad HE (2017) Predicting the slow decline of root lesion nematodes (*Pratylenchus thornei*) during host-free fallows to improve farm management decisions. *European Journal of Agronomy* 91: 44-53. https://doi.org/10.1016/j.eja.2017.09.012

Williams KJ, Taylor SP, Bogacki P, Pallotta M, Bariana HS, Wallwork H (2002) Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1*

in wheat. *Theoretical and Applied Genetecs* 104: 874-879. https://doi.org/10.1007/s00122-001-0839-3

Yeates GW (2010) Nematodes in Ecological Webs. In: Encyclopaedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. https://doi.org/10.1002/9780470015902.a0021913

Yanushevich ZV, Harris DR and Hillman GC (1989) Agricultural evolution north of the Black Sea from Neolithic to the Iron Age. *Foraging and Farming: The Evolution of Plant Exploitation* 13: 607-619. ISBN: 9781315746425.

Yu Q (2008) Species of *Pratylenchus* (Nematoda: Pratylenchidae) in Canada: description, distribution, and identification. *Canadian Journal of Plant Pathology* 30: 477-485. https://doi.org/10.1080/07060660809507545

Zhang W, Dubcovsky J (2008) Association between allelic variation at the *Phytoene synthase 1* gene and yellow pigment content in the wheat grain. *Theoretical and Applied Genetics* 116: 635-645. https://doi.org/10.1007/s00122-007-0697-8

Zwart RS, Thompson JP, Godwin ID (2004) Genetic analysis of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat. *Plant Breeding* 123: 209-212. https://doi.org/10.1111/j.1439-0523.2004.00986.x

Zwart RS, Thompson JP, Godwin ID (2005) Identification of quantitative trait loci for resistance to two species of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in wheat. *Australian Journal of Agricultural Research* 56: 345-352. https://doi.org/10.1071/AR04223

Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, Bariana HS (2010) QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Molecular Breeding* 26: 107-124. https://doi.org/10.1007/s11032-009-9381-9

Zwart RS, Thompson JP, Sheedy JG, Nelson JC (2006) Mapping quantitative trait loci for resistance to *Pratylenchus thornei* from synthetic hexaploid wheat in the International Triticeae Mapping Initiative (ITMI) population. *Australian Journal of Agricultural Research* 57: 525-530. https://doi.org/10.1071/AR05177

APPENDIX A: SUPPLEMENTARY INFORMATION FOR CHAPTER 3

Accessory Table 1. The 24 KASP markers and their corresponding loci information used to validate GWAS-derived QTL in BC_1F_4 breeding populations.

Source[1]	KASP ID	QTL	Position in new 90K consensus map	Index on iSelect 90K SNP bead chip	SNP id	SNP Name	Outcome
GWAS	77218_1B	*QRlnt.usq-1B.1*	136	77218	IWA2667	wsnp_Ex_c22006_31180883	enriched for resistant allele partly in population AUS28451
GWAS	10642_1B	*QRlnt.usq-1B.1*	136	10642	IWB10642	BS00073094_51	enriched for resistant allele partly in population AUS28451; and in population AUS28677
GWAS	36300_2B	*QRlnt.usq-2B.1*	68	36300	IWB36300	IACX8446	enriched for resistant allele in population AUS28369 and AUS28677
GWAS	72375_2B	*QRlnt.usq-2B.1*	75	72375	IWB72375	Tdurum_contig54649_915	**Monomorphic**
GWAS	32838_2B	*QRlnt.usq-2B.2*	172	32838	IWB32838	GENE-2192_463	enriched for resistant allele in AUS28451; and in AUS28470

GWAS	75885_2B	*QRlnt.usq-2B.2*	174	75885	IWA780	wsnp_CAP11_c5554_2580044	enriched for resistant allele in AUS28451; and in AUS28470
GWAS	27473_2B	*QRlnt.usq-2B.3*	252	27473	IWB27473	Excalibur_c5438_274	enriched for resistant allele in AUS28645
GWAS	30421_2B	*QRlnt.usq-2B.3*	253	30421	IWB30421	Excalibur_rep_c109577_698	**Monomorphic**
GWAS	10783_3B	*QRlnt.usq-3B.1*	189	10783	IWB10783	BS00075108_51	**Monomorphic**
GWAS	76275_3B	*QRlnt.usq-3B.1*	189	76275	IWA1459	wsnp_Ex_c11246_18191331	enriched for resistant allele in AUS28372
GWAS	27185_5B	*QRlnt.usq-5B.1*	105	27185	IWB27185	Excalibur_c50887_231	**Monomorphic**
GWAS	73671_5B	*QRlnt.usq-5B.1*	108	73671	IWB73671	Tdurum_contig84745_267	enriched for resistant allele in AUS28677
GWAS	31346_6D	*QRlnt.usq-6D.1*	32	31346	IWB31346	Excalibur_rep_c84264_67	enriched for resistant allele in population AUS28369; and AUS28645
GWAS	49821_6D	*QRlnt.usq-6D.1*	39	49821	IWB49821	Kukri_rep_c68823_696	**Monomorphic**
GWAS	8724_7B	*QRlnt.usq-7B.1*	186	8724	IWB8724	BS00057649_51	enriched for resistant allele in population AUS28369; and in AUS28470; not in linkage with other uat markers

GWAS	40270_7B	*QRlnt.usq-7B.1*	199	40270	IWB40270	Kukri_c10172_396	**Monomorphic**
AWBMMP	5813_2B		91	.	.	BS00002660_51	**Monomorphic**
AWBMMP	7407_2B		61	.	.	BS00023068_51	**Monomorphic except for 2 parents**
Linsell_2B	49736_2B		60	.	.	Kukri_rep_c117487_334	**Monomorphic**
AWBMMP	uat14_6D		.	.	.	uat14	Different from the 6D locus; enriched for resistant allele in population AUS28677
Linsell_6D	49086_6D		37	.	.	Kukri_rep_c105352_281	Similar to GWAS_6D locus; enriched for resistant allele in population AUS28369; AUS28645 ; AUS28372
AWBMMP	uat6_7B		.	.	.	uat6	**Monomorphic**
AWBMMP	uat7_7B		.	.	.	uat7	**Monomorphic**
AWBMMP	uat8_7B		.	.	.	uat8	enriched for resistant allele in population AUS28369; similar to 7B-locus

1 GWAS: Genome wide association study of Sheedy et al. presented in chapter 2; AWBMMP: Australian wheat and barley molecular marker project (http://www.markers.net.au/); Linsell_2B & Linsell 6D: Linsell KJ, Rahman MS, Taylor JD, Davey RS, Gogel BJ, Wallwork H, Forrest

KL, Hayden MJ, Taylor SP, Oldach KH (2014) QTL for resistance to root lesion nematode (*Pratylenchus thornei*) from a synthetic hexaploid wheat source. Theor Appl Genet 127:1409-1421.

APPENDIX B: SUPPLEMENTARY INFORMATION FOR CHAPTER 4

Source article:

Sheedy JG, Lin J, Thompson JP (2022) Discovery of resistance to *Pratylenchus neglectus* among *P. thornei*-resistant Iranian landrace wheats and the introgression of both resistances into advanced breeding lines. *Plant Pathology*, *00*, 1-12. https://doi.org/10.1111/ppa.13616

Accessory Table 1. Iranian landrace wheat genotypes evaluated for their resistance to *Pratylenchus neglectus* and *P. thornei*

#	Genotype	Crop	Iranian Province of Origin	Evaluated for resistance: *Pratylenchus neglectus*[1]	Evaluated for resistance: *Pratylenchus thornei*[2]	Synonyms[3] CIMMYT	Synonyms[3] USDA-ARS	Synonyms[3] UCD	Vikram's Core Set[4]
1	AUS28280	Wheat	Esfahan		Yes	CWI55533	PI 627712	IWA8603156	
2	AUS28281	Wheat	Esfahan		Yes	CWI55534	PI 627713	IWA8603157	
3	AUS28283	Wheat	Yazd		Yes	CWI55538	PI 627719	IWA8603174	
4	AUS28284	Wheat	East Azerbaijan	Yes	Yes	CWI55542	PI 627787	IWA8603258	
5	AUS28285	Wheat	Razavi Khorasan		Yes	CWI55550	PI 627824	IWA8603306	
6	AUS28286	Wheat	Esfahan		Yes	CWI55592	PI 627864	IWA8604067	
7	AUS28289	Durum	Kermanshah		Yes	CWI55595	PI 627867	IWA8604077	
8	AUS28290	Wheat	Markazi	Yes	Yes	CWI55599	PI 627880	IWA8604094	
9	AUS28291	Wheat	Kordestan	Yes	Yes	CWI55629	PI 627947	IWA8604259	
10	AUS28293	Wheat	Hamadan		Yes	CWI55634	PI 627953	IWA8604268	
11	AUS28294	Wheat	Qazvin	Yes	Yes	CWI55635	PI 627954	IWA8604270	
12	AUS28295	Wheat	Markazi	Yes	Yes	CWI55636	PI 623425	IWA8604272	
13	AUS28297	Wheat	East Azerbaijan		Yes	CWI55665	PI 623428	IWA8604394	
14	AUS28298	Wheat	Markazi		Yes	CWI55668	PI 623433	IWA8604409	
15	AUS28299	Durum	Mazandaran		Yes	CWI55680	PI 628022	IWA8604484	
16	AUS28300	Wheat	Esfahan		Yes	CWI55686	PI 628031	IWA8604526	

17	AUS28301	Wheat	Razavi Khorasan	Yes	Yes	CWI55694	PI 628040	IWA8604559	
18	AUS28302	Wheat	Esfahan	Yes	Yes	CWI55698	PI 628045	IWA8604568	
19	AUS28303	Wheat	Kermanshah		Yes	CWI55700	PI 628047	IWA8604571	
20	AUS28304	Wheat	Razavi Khorasan	Yes	Yes	CWI55733	PI 628100	IWA8604686	
21	AUS28305	Wheat	Kordestan	Yes	Yes	CWI55735	PI 628102	IWA8604688	
22	AUS28306	Wheat	East Azerbaijan		Yes	CWI55741	PI 628109	IWA8604697	
23	AUS28307	Wheat	Esfahan	Yes	Yes	CWI55748	PI 628116	IWA8604710	
24	AUS28308	Wheat	Esfahan	Yes	Yes	CWI55751	PI 628119	IWA8604715	
25	AUS28309	Wheat	Esfahan	Yes	Yes	CWI55752	PI 628120	IWA8604716	
26	AUS28310	Wheat	Esfahan		Yes	CWI55753		IWA8604717	
27	AUS28311	Wheat	Esfahan	Yes	Yes	CWI55754		IWA8604718	
28	AUS28314	Wheat	Kerman		Yes	CWI55757	PI 628129	IWA8604735	
29	AUS28315	Wheat	Kerman	Yes	Yes	CWI55758	PI 628132	IWA8604740	
30	AUS28318	Wheat	Esfahan		Yes	CWI55761	PI 628135	IWA8604744	
31	AUS28319	Wheat	North Khorasan		Yes	CWI55762	PI 628137	IWA8604752	
32	AUS28321	Wheat	Razavi Khorasan	Yes	Yes	CWI55769	PI 628144	IWA8604765	
33	AUS28322	Wheat	Razavi Khorasan	Yes	Yes	CWI55770	NSGC 12570	IWA8604766	
34	AUS28323	Wheat	Zanjan	Yes	Yes	CWI55776	PI 628150	IWA8604782	
35	AUS28324	Wheat	East Azerbaijan		Yes	CWI55777	PI 628151	IWA8604784	
36	AUS28325	Wheat	Razavi Khorasan		Yes	CWI55781	PI 628158	IWA8604794	
37	AUS28326	Wheat	Yazd	Yes	Yes	CWI55787	PI 628167	IWA8604807	
38	AUS28327	Wheat	Razavi Khorasan		Yes	CWI55789	PI 628178	IWA8604829	
39	AUS28329	Wheat	Razavi Khorasan	Yes	Yes	CWI55814	PI 623452	IWA8604895	
40	AUS28330	Wheat	Razavi Khorasan		Yes	CWI55816	PI 628219	IWA8604898	
41	AUS28331	Wheat	Razavi Khorasan		Yes	CWI55821	NSGC 12578	IWA8604911	Yes
42	AUS28332	Wheat	Hamadan	Yes	Yes	CWI55873	PI 623459	IWA8606031	
43	AUS28333	Wheat	Hamadan	Yes	Yes	CWI55874	PI 628248	IWA8606032	
44	AUS28334	Wheat	Kermanshah	Yes	Yes	CWI55889	PI 623467	IWA8606074	
45	AUS28335	Wheat	Ilam		Yes	CWI55890	PI 623472	IWA8606080	

46	AUS28336	Wheat	Ilam	Yes	Yes	CWI55891	PI 623473	IWA8606081	
47	AUS28337	Wheat	Ilam		Yes	CWI55892	PI 623474	IWA8606082	Yes
48	AUS28338	Wheat	Ilam	Yes	Yes	CWI55893	PI 623475	IWA8606083	
49	AUS28339	Wheat	Kermanshah		Yes	CWI55895	PI 623481	IWA8606091	
50	AUS28340	Wheat	Kermanshah		Yes	CWI55896	PI 623482	IWA8606092	Yes
51	AUS28341	Durum	Kermanshah		Yes	CWI55897	PI 623484	IWA8606095	
52	AUS28342	Wheat	Ilam	Yes	Yes	CWI55909	PI 623501	IWA8606134	
53	AUS28343	Wheat	Ilam		Yes	CWI55910	PI 623502	IWA8606135	
54	AUS28344	Wheat	West Azerbaijan		Yes	CWI55924	PI 623509	IWA8606158	
55	AUS28345	Wheat	West Azerbaijan		Yes	CWI55927	PI 623510	IWA8606161	
56	AUS28346	Wheat	West Azerbaijan		Yes	CWI55928	PI 620905	IWA8606163	
57	AUS28347	Wheat	West Azerbaijan		Yes	CWI55929	PI 623511	IWA8606164	
58	AUS28348	Wheat	West Azerbaijan		Yes	CWI55931	PI 623512	IWA8606171	
59	AUS28349	Wheat	West Azerbaijan		Yes	CWI55939	PI 623514	IWA8606188	
60	AUS28350	Wheat	West Azerbaijan		Yes	CWI55940	PI 623515	IWA8606190	
61	AUS28351	Wheat	West Azerbaijan		Yes	CWI55941	PI 623516	IWA8606194	
62	AUS28352	Wheat	West Azerbaijan		Yes	CWI55942	PI 623517	IWA8606195	
63	AUS28353	Wheat	West Azerbaijan		Yes	CWI55944	PI 623518	IWA8606205	
64	AUS28354	Wheat	West Azerbaijan		Yes	CWI55945	PI 623520	IWA8606207	Yes
65	AUS28355	Wheat	West Azerbaijan		Yes	CWI55953	PI 623522	IWA8606229	
66	AUS28356	Wheat	West Azerbaijan		Yes	CWI55954	PI 623524	IWA8606232	Yes
67	AUS28357	Wheat	West Azerbaijan		Yes	CWI55955	PI 623525	IWA8606233	
68	AUS28358	Wheat	West Azerbaijan		Yes	CWI55966	PI 623526	IWA8606249	
69	AUS28359	Wheat	West Azerbaijan		Yes	CWI55967	PI 623527	IWA8606250	
70	AUS28360	Wheat	West Azerbaijan		Yes	CWI55968	PI 623528	IWA8606251	
71	AUS28361	Wheat	West Azerbaijan		Yes	CWI55969	PI 623529	IWA8606252	
72	AUS28362	Wheat	West Azerbaijan		Yes	CWI55970	PI 623530	IWA8606255	
73	AUS28363	Wheat	West Azerbaijan		Yes	CWI55971	PI 623531	IWA8606256	
74	AUS28364	Wheat	West Azerbaijan		Yes	CWI55972	PI 623532	IWA8606257	

75	AUS28365	Wheat	West Azerbaijan		Yes	CWI55973	PI 623533	IWA8606258	Yes
76	AUS28366	Wheat	West Azerbaijan	Yes	Yes	CWI55975	PI 623534	IWA8606263	
77	AUS28367	Wheat	West Azerbaijan		Yes	CWI55976	PI 623535	IWA8606264	
78	AUS28368	Wheat	West Azerbaijan		Yes	CWI55977	PI 623536	IWA8606265	
79	AUS28369	Wheat	West Azerbaijan	Yes	Yes	CWI55979	PI 623538	IWA8606267	
80	AUS28370	Wheat	West Azerbaijan		Yes	CWI55980	PI 623539	IWA8606268	
81	AUS28371	Wheat	West Azerbaijan		Yes	CWI55981	PI 623540	IWA8606269	
82	AUS28372	Wheat	West Azerbaijan	Yes	Yes	CWI55982	PI 623541	IWA8606270	
83	AUS28373	Wheat	West Azerbaijan		Yes	CWI55983	PI 623542	IWA8606271	
84	AUS28374	Wheat	West Azerbaijan		Yes	CWI55984	PI 623543	IWA8606272	Yes
85	AUS28375	Wheat	Kermanshah	Yes	Yes	CWI56750	PI 623946	IWA8607438	Yes
86	AUS28376	Wheat	Kermanshah		Yes	CWI56751	PI 623947	IWA8607440	
87	AUS28377	Wheat	Kermanshah		Yes	CWI56752	PI 623948	IWA8607441	
88	AUS28378	Wheat	Kermanshah		Yes	CWI56753	PI 623949	IWA8607442	
89	AUS28379	Wheat	Hamadan		Yes	CWI56787	PI 623977	IWA8607483	
90	AUS28380	Wheat	Hamadan		Yes	CWI56789	PI 623978	IWA8607485	
91	AUS28381	Wheat	Hamadan		Yes	CWI56793	PI 621335	IWA8607493	
92	AUS28382	Wheat	Hamadan		Yes	CWI56794		IWA8607494	
93	AUS28383	Wheat	Hamadan		Yes	CWI56798	PI 623980	IWA8607499	
94	AUS28384	Wheat	Kermanshah	Yes	Yes	CWI56806	PI 623986	IWA8607511	
95	AUS28385	Wheat	Kermanshah		Yes	CWI56808	PI 623987	IWA8607513	
96	AUS28386	Durum	Kermanshah		Yes	CWI56826	PI 621344	IWA8607539	
97	AUS28387	Wheat	Kermanshah	Yes	Yes	CWI56829	PI 624007	IWA8607542	
98	AUS28388	Wheat	Kermanshah		Yes	CWI56830	PI 624008	IWA8607545	
99	AUS28389	Wheat	Kermanshah	Yes	Yes	CWI56831	PI 624009	IWA8607547	
100	AUS28390	Wheat	Kermanshah		Yes	CWI56850	PI 624023	IWA8607570	
101	AUS28391	Wheat	Kermanshah	Yes	Yes	CWI56853	PI 624026	IWA8607575	
102	AUS28392	Wheat	Kermanshah	Yes	Yes	CWI56854	PI 624027	IWA8607576	Yes
103	AUS28394	Wheat	Kermanshah		Yes	CWI56869	PI 624044	IWA8607601	

104	AUS28395	Wheat	Kermanshah		Yes	CWI56870	PI 624047	IWA8607604	
105	AUS28396	Wheat	Kermanshah		Yes	CWI56878	PI 624060	IWA8607624	
106	AUS28397	Wheat	Kermanshah		Yes	CWI56880	PI 624062	IWA8607627	
107	AUS28398	Wheat	Hamadan	Yes	Yes	CWI56944	PI 624143	IWA8607763	
108	AUS28399	Wheat	Hamadan	Yes	Yes	CWI56946	PI 624144	IWA8607766	
109	AUS28400	Wheat	Hamadan	Yes	Yes	CWI56952	PI 624145	IWA8607776	
110	AUS28401	Wheat	Kermanshah	Yes	Yes	CWI56969	PI 624162	IWA8607818	
111	AUS28402	Wheat	Kermanshah	Yes	Yes	CWI56970	PI 624163	IWA8607820	
112	AUS28403	Durum	Kermanshah		Yes	CWI56978	PI 624173	IWA8607837	
113	AUS28404	Wheat	Kermanshah		Yes	CWI56979	PI 624183	IWA8607849	
114	AUS28405	Wheat	Hamadan		Yes	CWI56983	PI 624189	IWA8607855	
115	AUS28406	Durum	Kermanshah		Yes	CWI56988	PI 621414	IWA8607861	
116	AUS28407	Wheat	Kermanshah	Yes	Yes	CWI56991	PI 624194	IWA8607866	
117	AUS28408	Wheat	Kermanshah	Yes	Yes	CWI56996	PI 624198	IWA8607871	
118	AUS28409	Wheat	Kermanshah		Yes	CWI57039	PI 624229	IWA8607923	
119	AUS28410	Wheat	Ilam		Yes	CWI57066	PI 624249	IWA8607958	
120	AUS28411	Wheat	Ilam		Yes	CWI57067	PI 624250	IWA8607959	
121	AUS28412	Wheat	Ilam		Yes	CWI57068	PI 624251	IWA8607960	
122	AUS28413	Wheat	Hamadan	Yes	Yes	CWI57069	PI 624252	IWA8607961	
123	AUS28414	Wheat	Hamadan		Yes	CWI57070	PI 624253	IWA8607962	
124	AUS28415	Wheat	Hamadan	Yes	Yes	CWI57071	PI 624254	IWA8607963	
125	AUS28416	Wheat	Hamadan		Yes	CWI57072	PI 624255	IWA8607964	
126	AUS28417	Wheat	Hamadan	Yes	Yes	CWI57073	PI 624256	IWA8607967	
127	AUS28418	Wheat	Kermanshah		Yes	CWI57075	PI 624257	IWA8607971	
128	AUS28420	Wheat	Kermanshah		Yes	CWI57080	PI 624262	IWA8607981	Yes
129	AUS28421	Wheat	Kermanshah		Yes	CWI57081	PI 624263	IWA8607982	
130	AUS28422	Wheat	Kermanshah		Yes	CWI57083	PI 624265	IWA8607984	
131	AUS28423	Wheat	Kermanshah	Yes	Yes	CWI57084	PI 624266	IWA8607985	
132	AUS28424	Wheat	Kermanshah	Yes	Yes	CWI57086	PI 621438	IWA8607987	

133	AUS28425	Wheat	Kermanshah		Yes	CWI57088	PI 624270	IWA8607990	
134	AUS28426	Wheat	Kermanshah	Yes	Yes	CWI57091	PI 624274	IWA8607995	
135	AUS28428	Wheat	Kermanshah		Yes	CWI57038	PI 624228	IWA8607922	
136	AUS28429	Khorasan wheat	Kermanshah		Yes	CWI56999	PI 624208	IWA8607886	
137	AUS28430	Wheat	Kermanshah	Yes	Yes	CWI57099	PI 624282	IWA8608010	
138	AUS28431	Durum	Kermanshah		Yes	CWI57100	PI 624283	IWA8608011	
139	AUS28432	Wheat	Kermanshah		Yes	CWI57101	PI 624284	IWA8608012	Yes
140	AUS28433	Wheat	Kermanshah	Yes	Yes	CWI57102	PI 624286	IWA8608014	
141	AUS28434	Wheat	Hamadan	Yes	Yes	CWI57108	PI 621446	IWA8608025	
142	AUS28435	Wheat	East Azerbaijan		Yes	CWI57110	PI 624291	IWA8608032	
143	AUS28436	Wheat	East Azerbaijan		Yes	CWI57112	PI 624292	IWA8608045	
144	AUS28437	Wheat	East Azerbaijan		Yes	CWI57113	PI 624293	IWA8608046	
145	AUS28438	Wheat	East Azerbaijan		Yes	CWI57114	PI 624294	IWA8608047	
146	AUS28439	Wheat	East Azerbaijan		Yes	CWI57115	PI 624296	IWA8608051	
147	AUS28440	Wheat	East Azerbaijan		Yes	CWI57120	PI 624298	IWA8608059	
148	AUS28441	Wheat	East Azerbaijan		Yes	CWI57121	PI 624299	IWA8608060	
149	AUS28442	Wheat	East Azerbaijan	Yes	Yes	CWI57123	PI 624300	IWA8608064	
150	AUS28443	Wheat	East Azerbaijan	Yes	Yes	CWI57124	PI 621454	IWA8608065	
151	AUS28444	Wheat	East Azerbaijan		Yes	CWI57125	PI 624301	IWA8608066	
152	AUS28445	Wheat	East Azerbaijan		Yes	CWI57126	PI 624302	IWA8608068	
153	AUS28446	Wheat	East Azerbaijan		Yes	CWI57129	PI 624303	IWA8608072	
154	AUS28447	Wheat	East Azerbaijan		Yes	CWI57130	PI 624304	IWA8608073	
155	AUS28448	Wheat	East Azerbaijan		Yes	CWI57131	PI 624305	IWA8608074	
156	AUS28450	Wheat	East Azerbaijan		Yes	CWI57133	PI 624306	IWA8608076	
157	AUS28451	Wheat	East Azerbaijan	Yes	Yes	CWI57134	PI 621458	IWA8608077	
158	AUS28452	Wheat	East Azerbaijan	Yes	Yes	CWI57136	PI 624307	IWA8608080	
159	AUS28453	Wheat	East Azerbaijan		Yes	CWI57137	PI 624308	IWA8608082	
160	AUS28454	Wheat	East Azerbaijan		Yes	CWI57138	PI 624309	IWA8608083	
161	AUS28455	Wheat	East Azerbaijan		Yes	CWI57139	PI 624310	IWA8608084	

162	AUS28456	Wheat	East Azerbaijan		Yes	CWI57140	PI 624311	IWA8608085	
163	AUS28457	Wheat	East Azerbaijan	Yes	Yes	CWI57141	PI 624312	IWA8608086	Yes
164	AUS28458	Wheat	Kordestan		Yes	CWI57142	PI 624313	IWA8608087	
165	AUS28459	Wheat	Kordestan		Yes	CWI57143	PI 624314	IWA8608089	
166	AUS28460	Wheat	Kordestan		Yes	CWI57161	PI 624315	IWA8608123	
167	AUS28461	Wheat	Kordestan		Yes	CWI57162	PI 624316	IWA8608124	
168	AUS28462	Wheat	Kordestan	Yes	Yes	CWI57163	PI 624317	IWA8608125	
169	AUS28463	Wheat	Kordestan		Yes	CWI57164	PI 624318	IWA8608127	
170	AUS28464	Wheat	Kordestan		Yes	CWI57166	PI 624319	IWA8608130	
171	AUS28466	Wheat	Kordestan		Yes	CWI57169	PI 624322	IWA8608135	
172	AUS28468	Wheat	Kordestan		Yes	CWI57173	PI 624324	IWA8608146	
173	AUS28469	Wheat	Kordestan		Yes	CWI57174	PI 624325	IWA8608147	
174	AUS28470	Wheat	Kordestan	Yes	Yes	CWI57176	PI 624327	IWA8608152	
175	AUS28471	Wheat	Kordestan		Yes	CWI57185	PI 624332	IWA8608171	
176	AUS28472	Wheat	Kordestan		Yes	CWI57186	PI 624333	IWA8608172	Yes
177	AUS28473	Wheat	Kordestan		Yes	CWI57188	PI 624334	IWA8608175	
178	AUS28474	Wheat	Kordestan		Yes	CWI57189	PI 624335	IWA8608176	
179	AUS28475	Wheat	Kordestan		Yes	CWI57190	PI 624336	IWA8608177	
180	AUS28476	Durum	Kordestan		Yes	CWI57191	PI 624337	IWA8608188	
181	AUS28477	Durum	Kordestan		Yes	CWI57195	PI 624338	IWA8608190	
182	AUS28478	Durum	Kordestan		Yes	CWI57196	PI 621479	IWA8608191	
183	AUS28629	Wheat	Kordestan		Yes	CWI57545	PI 624661	IWA8608763	
184	AUS28630	Wheat	Kordestan	Yes	Yes	CWI57546	PI 624662	IWA8608766	
185	AUS28631	Wheat	Kordestan	Yes	Yes	CWI57547	PI 624663	IWA8608767	
186	AUS28632	Wheat	Kordestan	Yes	Yes	CWI57548	PI 624664	IWA8608769	
187	AUS28633	Wheat	Kermanshah		Yes	CWI57550	PI 624667	IWA8608772	
188	AUS28634	Wheat	Kermanshah		Yes	CWI57555	PI 624671	IWA8608778	
189	AUS28635	Wheat	Kermanshah	Yes	Yes	CWI57559	PI 624674	IWA8608782	
190	AUS28636	Wheat	Kermanshah		Yes	CWI57560	PI 624675	IWA8608783	

191	AUS28637	Wheat	Hamadan		Yes	CWI57570	PI 624683	IWA8608799
192	AUS28638	Wheat	Kermanshah	Yes	Yes	CWI57573	PI 624686	IWA8608802
193	AUS28639	Wheat	Kermanshah		Yes	CWI57575	PI 624688	IWA8608804
194	AUS28640	Wheat	Kermanshah		Yes	CWI57576	PI 624689	IWA8608805
195	AUS28642	Wheat	Kermanshah	Yes	Yes	CWI57586	PI 624699	IWA8608819
196	AUS28643	Durum	Kermanshah		Yes	CWI57590	PI 624702	IWA8608823
197	AUS28644	Wheat	Kermanshah	Yes	Yes	CWI57596	PI 624708	IWA8608830
198	AUS28645	Wheat	Kermanshah	Yes	Yes	CWI57601	PI 624713	IWA8608838
199	AUS28647	Wheat	Kermanshah		Yes	CWI57603	PI 624715	IWA8608840
200	AUS28648	Wheat	Kermanshah		Yes	CWI57606	PI 624717	IWA8608845
201	AUS28649	Wheat	Kermanshah	Yes	Yes	CWI57607	PI 624718	IWA8608846
202	AUS28650	Wheat	Kermanshah		Yes	CWI57608	PI 624720	IWA8608848
203	AUS28651	Durum	Kermanshah		Yes	CWI57615	PI 624726	IWA8608857
204	AUS28652	Durum	Kermanshah		Yes	CWI57616	PI 624727	IWA8608858
205	AUS28653	Wheat	Kermanshah		Yes	CWI57621	PI 624732	IWA8608865
206	AUS28654	Wheat	Kermanshah		Yes	CWI57623	PI 624734	IWA8608868
207	AUS28656	Wheat	Kermanshah		Yes	CWI57625	PI 624736	IWA8608870
208	AUS28657	Wheat	Kermanshah	Yes	Yes	CWI57626	PI 624737	IWA8608871
209	AUS28658	Wheat	Kermanshah		Yes	CWI57627	PI 624738	IWA8608872
210	AUS28659	Wheat	Kermanshah		Yes	CWI57630	PI 624742	IWA8608877
211	AUS28660	Wheat	Kermanshah		Yes	CWI57634	PI 624746	IWA8608882
212	AUS28661	Wheat	Kermanshah		Yes	CWI57636	PI 624748	IWA8608886
213	AUS28662	Wheat	Kermanshah		Yes	CWI57637	PI 624750	IWA8608888
214	AUS28664	Wheat	Kermanshah		Yes	CWI57649	PI 624760	IWA8608904
215	AUS28665	Wheat	Kermanshah		Yes	CWI57652	PI 624762	IWA8608908
216	AUS28666	Wheat	Kermanshah	Yes	Yes	CWI57653	PI 624763	IWA8608909
217	AUS28667	Wheat	Kermanshah	Yes	Yes	CWI57655	PI 624765	IWA8608911
218	AUS28668	Wheat	Kermanshah	Yes	Yes	CWI57657	PI 624767	IWA8608915
219	AUS28669	Wheat	Kermanshah		Yes	CWI57658	PI 624768	IWA8608916

220	AUS28670	Durum	Kermanshah		Yes	CWI57660	PI 624771	IWA8608920
221	AUS28671	Wheat	Kermanshah	Yes	Yes	CWI57662	PI 624773	IWA8608923
222	AUS28672	Wheat	Kermanshah		Yes	CWI57663	PI 624774	IWA8608924
223	AUS28674	Wheat	Kermanshah		Yes	CWI57666	PI 624776	IWA8608928
224	AUS28675	Wheat	Kermanshah		Yes	CWI57668	PI 624778	IWA8608931
225	AUS28676	Wheat	Kermanshah		Yes	CWI57669	PI 624779	IWA8608934
226	AUS28677	Wheat	Kermanshah	Yes	Yes	CWI57672	PI 624782	IWA8608938
227	AUS28678	Persian wheat	Kermanshah		Yes	CWI57674	PI 624784	IWA8608940
228	AUS28679	Wheat	Kermanshah		Yes	CWI57676	PI 624786	IWA8608943
229	AUS28681	Wheat	Kermanshah	Yes	Yes	CWI57692	PI 624804	IWA8608963
230	AUS28682	Wheat	Kermanshah		Yes	CWI57693	PI 624805	IWA8608964
231	AUS28683	Wheat	Kermanshah		Yes	CWI57698	PI 624810	IWA8608974
232	AUS28684	Wheat	Ilam		Yes	CWI57700	PI 624814	IWA8608981
233	AUS28685	Wheat	Ilam		Yes	CWI57701	PI 624815	IWA8608982
234	AUS28686	Wheat	Ilam	Yes	Yes	CWI57702	PI 624816	IWA8608983
235	AUS28687	Wheat	Ilam	Yes	Yes	CWI57706	PI 624821	IWA8608990
236	AUS28688	Wheat	Ilam		Yes	CWI57707	PI 624822	IWA8608991
237	AUS28689	Wheat	Ilam	Yes	Yes	CWI57708	PI 624823	IWA8608992
238	AUS28690	Wheat	Ilam	Yes	Yes	CWI57710	PI 624825	IWA8608994
239	AUS28691	Wheat	Ilam		Yes	CWI57712	PI 624827	IWA8608996
240	AUS28692	Wheat	Ilam		Yes	CWI57721	PI 624837	IWA8609011
241	AUS28693	Wheat	Ilam	Yes	Yes	CWI57722	PI 624838	IWA8609012
242	AUS28694	Wheat	Ilam		Yes	CWI57723	PI 624839	IWA8609013
243	AUS28695	Wheat	Ilam		Yes	CWI57726	PI 624842	IWA8609016
244	AUS28696	Wheat	Ilam		Yes	CWI57727	PI 624843	IWA8609017
245	AUS28697	Wheat	Ilam		Yes	CWI57729	PI 624845	IWA8609019
246	AUS28698	Wheat	Ilam		Yes	CWI57730	PI 624846	IWA8609020
247	AUS28699	Wheat	Ilam	Yes	Yes	CWI57731	PI 624847	IWA8609021
248	AUS28700	Wheat	Ilam	Yes	Yes	CWI57732	PI 624848	IWA8609022

249	AUS28701	Wheat	Ilam	Yes	Yes	CWI57733	PI 624849	IWA8609023
250	AUS28702	Wheat	Ilam		Yes	CWI57740	PI 624856	IWA8609030
251	AUS28703	Wheat	Ilam	Yes	Yes	CWI57741	PI 624857	IWA8609031
252	AUS28704	Wheat	Ilam		Yes	CWI57742	PI 624858	IWA8609032
253	AUS28705	Wheat	Ilam		Yes	CWI57743	PI 624859	IWA8609033
254	AUS28706	Wheat	Ilam	Yes	Yes	CWI57744	PI 624860	IWA8609035
255	AUS28707	Wheat	Ilam		Yes	CWI57745	PI 624861	IWA8609036
256	AUS28708	Wheat	Ilam		Yes	CWI57746	PI 624863	IWA8609038
257	AUS28709	Wheat	Ilam		Yes	CWI57747	PI 624864	IWA8609039
258	AUS28710	Wheat	Ilam		Yes	CWI57748	PI 624865	IWA8609040
259	AUS28711	Wheat	Ilam		Yes	CWI57749	PI 624866	IWA8609041
260	AUS28712	Wheat	Ilam	Yes	Yes	CWI57751	PI 624867	IWA8609045
261	AUS28713	Wheat	Kordestan		Yes	CWI57754	PI 624870	IWA8609048
262	AUS28714R[5]	Wheat	Kordestan	Yes		CWI57755	PI 624871	IWA8609049
263	AUS28714W[6]	Wheat	Kordestan	Yes	Yes	CWI57755	PI 624871	IWA8609049
264	AUS28715	Wheat	Kordestan		Yes	CWI57756	PI 624873	IWA8609051
265	AUS28716	Wheat	Kordestan		Yes	CWI57757	PI 624874	IWA8609053
266	AUS28717	Wheat	Kordestan		Yes	CWI57758	PI 624875	IWA8609055
267	AUS28718	Wheat	Kordestan	Yes	Yes	CWI57759	PI 624876	IWA8609056
268	AUS28721	Wheat	Kordestan		Yes	CWI57762	PI 624877	IWA8609059
269	AUS28722	Wheat	Kordestan		Yes	CWI57763	PI 624878	IWA8609060
270	AUS28723	Wheat	Kordestan	Yes	Yes	CWI57764	PI 624879	IWA8609061
271	AUS28724	Wheat	Kordestan		Yes	CWI57765	PI 624880	IWA8609062
272	AUS28725	Wheat	Kordestan		Yes	CWI57766	PI 624882	IWA8609064
273	AUS28726	Wheat	Kordestan		Yes	CWI57767	PI 624883	IWA8609067
274	AUS28727	Wheat	Kordestan	Yes	Yes	CWI57768	PI 624884	IWA8609068
275	AUS28728	Wheat	Kordestan	Yes	Yes	CWI57773	PI 624890	IWA8609076
276	AUS36669[7]	Wheat	East Azerbaijan	Yes		CWI57134	PI 621458	IWA8608077

[1] Genotypes evaluated in this study for resistance to *Pratylenchus neglectus*

[2] Genotypes evaluated by Sheedy and Thompson (2009) for resistance to *Pratylenchus thornei*

[3] Genotype synonyms from the International Maize and Wheat Improvement Center (CIMMYT), United States Department of Agriculture - Agricultural Research Service (USDA-ARS) and the University of California, Davis (UCD)

[4] Genotypes present in the Iranian landrace wheat genetic diversity core set collated by Vikram et al. 2020

[5] Red-grained reselection of AUS28714

[6] White-grained reselection of AUS28714

[7] Reselection of AUS28451 reported by Sheedy et al. 2007; 2008. Also known as AUS28154R

References

Sheedy JG, Smiley RW, Easley SA, Thompson AL (2007) Resistance reaction of Pacific Northwest spring wheat and barley cultivars to root-lesion nematode (*P. neglectus*), 2007. Plant Disease Management Reports (Online). Report 1:CF022. The American Phytopathological Society, St. Paul, MN. https://doi.org/10.1094/PDMR01

Sheedy JG, Smiley RW, Easley SA, Thompson AL (2008) Resistance of Pacific Northwest spring wheat and barley cultivars to root-lesion nematode (*P. thornei*), 2007. Plant Disease Management Reports (Online). Report 2:N007. The American Phytopathological Society, St. Paul, MN. https://doi.org/10.1094/PDMR02

Sheedy JG, Thompson JP (2009) Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. Australasian Plant Pathology 38:478-489. https://doi.org/10.1071/AP09030

Vikram P, Franco J, Burgueño J, Li H, Sehgal D, Saint-Pierre C, Ortiz C, Kumar Singh V, Sneller C, Sharma A, Tattaris M, Guzman C, Pena J, Paola Sansaloni C, Amador J, Serna C, Thiyagarajan K, Fuentes Davila G, Reynolds M, Sonder K, Govindan V, Ellis M, Bhavani S, Jalal Kamali MR, Roosatei M, Singh S, Basandrai D, Singh Bains N, Basandrai A, Payne T, Crossa J, Singh S (2020) Strategic use of Iranian bread wheat landrace accessions for genetic improvement: Core set formulation and validation. Plant Breeding 2020:00:1–13. https://doi.org/10.1111/pbr.12885

Accessory Table 2. *Pratylenchus neglectus* resistance rating of standard genotypes used in the characterisation of a collection of Iranian landrace wheats

#	Genotype	Crop	*Pratylenchus neglectus* resistance		
			Rating[1,2]	n[3]	Gene/QTL
1	Brookton	Wheat	SVS	67	
2	CPI133842	Wheat (synthetic hexaploid)	MS	8	QRlnn.lrc-2B/QRlnt.lrc-2B
3	CPI133859	Wheat (synthetic hexaploid)	MS	10	QRlnn.lrc-2B/QRlnt.lrc-2B
4	CPI133872	Wheat (synthetic hexaploid)	MR	10	QRlnn.lrc-2B/QRlnt.lrc-2B
5	CPI133872_Janz DH001	Wheat	MS	6	QRlnn.lrc-2B/QRlnt.lrc-2B
6	CPI133872_Janz DH010	Wheat	MSS	10	QRlnn.lrc-2B/QRlnt.lrc-2B
7	CPI133872_Janz DH024	Wheat	MRMS	10	QRlnn.lrc-2B/QRlnt.lrc-2B
8	CPI133872_Janz DH043	Wheat	MRMS	10	QRlnn.lrc-2B/QRlnt.lrc-2B
9	CPI133872_Janz DH074	Wheat	MRMS	6	QRlnn.lrc-2B/QRlnt.lrc-2B
10	CPI133872_Janz DH083	Wheat	MR	10	QRlnn.lrc-2B/QRlnt.lrc-2B
11	Cunningham	Wheat	S	67	
12	EGA Wylie	Wheat	MSS	56	
13	Excalibur	Wheat	MS	16	Rlnn1
14	GBA Ruby	Wheat	S	9	
15	GBA Sapphire	Wheat	S	10	
16	Janz	Wheat	S	19	
17	Machete	Wheat	S	70	
18	Petrie	Wheat	SVS	19	
19	TAMD870167/AUS18913	Wheat (synthetic hexaploid)	MRMS	7	QRlnn.lrc-2B/QRlnt.lrc-2B
20	Wyalkatchem	Wheat	MRMS	67	Rlnn1

21	Yallaroi/AUS24152	Wheat (synthetic hexaploid)	MSS	7	QRlnn.lrc-2B/QRlnt.lrc-2B
22	Yenda	Wheat	MR	66	Rlnn1

[1] Classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/).

[2] Long-term classifications may vary from the classifications based on the restricted data set reported in the manuscript text

[3] Number of experiments used to determine *P. neglectus* resistance rating

Accessory Table 3. *Pratylenchus thornei* resistance rating of standard genotypes and treatments and Iranian landrace-derived advanced breeding lines (ABL)

Genotype	Parentage	Crop	Type	Experiments		*Pratylenchus thornei* resistance	
						Rating[1,2]	n[3]
Batavia	Brochis 'S'/Banks	Wheat	Standard	2017x09		SVS	82
Catalina	VI-184/Silverstar	Wheat	Standard	2017x09		MSS	58
Chara	BD225/CD87	Wheat	Standard	2017x09		MS	58
CPI133872	CPI133821 (68.111/Rugby//Ward/3/Flamingo/4/Rabicorno) x AUS24132 (*A. tauschii* ssp. *strangulata*)	Wheat (synthetic hexaploid)	Standard	2017x09	2018x06	RMR	96
EGA Kidman	Pelsart/2*Batavia DH	Wheat	Standard		2018x06	MSS	32
Gauntlet	Kukri/Sunvale	Wheat	Standard		2018x06	MRMS	26
GS50a	Gather Selection 50a	Wheat	Standard	2017x09	2018x06	MR	108
Inoculated/Unplanted		Treatment	Standard	2017x09	2018x06	R[4]	100
Merinda	Janz/2*SUN129A	Wheat	Standard	2017x09		MSS	63
Petrie	Vasco/Batavia	Wheat	Standard	2017x09	2018x06	SVS	57
QT8447	GS50a/3*Cunningham//Janz	Wheat	Standard		2018x06	MRMS	47
Strzelecki	Vicam/4*Batavia	Wheat	Standard		2018x06	SVS	48
Sunguard	SUN289E/Sr2Janz	Wheat	Standard		2018x06	S	40
Sunzell	Sunbrook*3/Sunstate	Wheat	Standard		2018x06	MS	27
Tamaroi	Kamilaroi/3/Wells/56111//Guillemot/4/Altar 84	Durum	Standard	2017x09		MR	48

Yallaroi	Guillemont Seln No.3/Kamilaroi Sib	Durum	Standard	2017x09		MR	59
Yandanooka	Calingiri/WAWHT-1137//38-W-386443	Wheat	Standard	2017x09		SVS	64
2016FL072	AUS28369/2*Wylie	Wheat	ABL	2017x09	2018x06	RMR	2
2016FL073	AUS28369/2*Wylie	Wheat	ABL	2017x09	2018x06	RMR	2
2016FL076	AUS28369/2*Wylie	Wheat	ABL	2017x09	2018x06	R	2
2016FL077	AUS28369/2*Wylie	Wheat	ABL	2017x09	2018x06	RMR	2
2016FL079	AUS28369/2*Wylie	Wheat	ABL	2017x09	2018x06	RMR	2
2016FL080	AUS28645/2*Gregory	Wheat	ABL	2017x09	2018x06	MS	2
2016FL081	AUS28645/2*Gregory	Wheat	ABL	2017x09	2018x06	MRMS	2
2016FL082	AUS28645/2*Gregory	Wheat	ABL	2017x09	2018x06	MSS	2
2016FL083	AUS28677/2*Wylie	Wheat	ABL	2017x09	2018x06	S	2
2016FL084	AUS28677/2*Wylie	Wheat	ABL	2017x09	2018x06	MS	2
2016FL085	AUS28677/2*Wylie	Wheat	ABL	2017x09	2018x06	S	2
2016FL086	AUS28372/2*Gregory	Wheat	ABL	2017x09	2018x06	MRMS	2
2016FL087	AUS28372/2*Gregory	Wheat	ABL	2017x09	2018x06	MRMS	2
2016FL094	AUS28451/2*Gregory	Wheat	ABL	2017x09	2018x06	MRMS	2
2016FL183	AUS28470/2*Gregory	Wheat	ABL	2017x09	2018x06	MS	2
2016FL184	AUS28470/2*Gregory	Wheat	ABL	2017x09	2018x06	MSS	2
2016FL185	AUS28470/2*Gregory	Wheat	ABL	2017x09	2018x06	MSS	2
2016FL186	AUS28470/2*Gregory	Wheat	ABL	2017x09	2018x06	MSS	2
USQW19030	AUS28369/2*Wylie	Wheat	ABL	2017x09	2018x06	RMR	2
USQW19031	AUS28369/2*Wylie	Wheat	ABL	2017x09	2018x06	RMR	2

USQW19032	AUS28369/2*Wylie	Wheat	ABL	2017x09	2018x06	RMR	2
USQW19033	AUS28451/2*Gregory	Wheat	ABL	2017x09	2018x06	RMR	2

[1] Classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/).

[2] Long-term classifications may vary from the classifications based on the restricted data set reported in the manuscript text

[3] Number of experiments used to determine *P. thornei* resistance rating

[4] For comparison purposes only. The inoculated/unplanted treatment simulates a weed-free fallow and can be used to estimate the performance of a completely resistant genotype. The authors do not contend that the unplanted treatment carries genetic resistance.

Accessory Table 4. *Pratylenchus neglectus* resistance ratings of standard genotypes and treatments and Iranian landrace-derived advanced breeding lines (ABL)

Genotype	Parentage	Crop	Type	Experiments		*Pratylenchus neglectus* resistance	
						Rating[1,2]	n[3]
Abacus	K875/Snoopy//T2898/3/Currency	Triticale	Standard	2017x13	2021x14	RMR	67
Baxter	QT2327/Cook//QT2804	Wheat	Standard		2021x14	MSS	27
Bremer	DM02-25-SB02-167/Correll//Mace	Wheat	Standard		2021x14	VS	7
Brookton	Torres/Cranbrook/Emblen.P1640..Nuri70/Cranbrook	Wheat	Standard	2017x13	2021x14	SVS	67
Cunningham	3Ag3/4*Condor/Cook	Wheat	Standard	2017x13	2021x14	S	67
EGA Wylie	QT2327/Cook//QT2804	Wheat	Standard	2017x13	2021x14	MSS	56
Inoculated/Unplanted		Treatment	Standard	2017x13	2021x14	R[4]	66
Longsword	SUN435G/2*Mace	Wheat	Standard		2021x14	R	6
Machete	RAC177/Madden	Wheat	Standard	2017x13	2021x14	S	70
Tahara	Drira//Maya/Armadillo	Triticale	Standard	2017x13		MR	45
Tamaroi	Kamilaroi/3/Wells/56111//Guillemot/4/Altar 84	Durum	Standard	2017x13	2021x14	MS	44
USQW15012	CPI133872_Janz DH083/Wylie	Wheat	Standard		2021x14	RMR	15
Wyalkatchem	Machete/W84-129*504	Wheat	Standard	2017x13	2021x14	MRMS	67
Yallaroi	Guillemont Seln No.3/Kamilaroi Sib	Durum	Standard	2017x13		MRMS	48
Yenda	Bindawarra/Bowie//3Ag3/Wyuna	Wheat	Standard	2017x13	2021x14	MR	66
2016FL072	AUS28369/2*Wylie	Wheat	ABL	2017x13	2021x14	MR	2

2016FL073	AUS28369/2*Wylie	Wheat	ABL	2017x13	2021x14	MRMS	2
2016FL076	AUS28369/2*Wylie	Wheat	ABL	2017x13	2021x14	RMR	2
2016FL077	AUS28369/2*Wylie	Wheat	ABL	2017x13		MS	1
2016FL079	AUS28369/2*Wylie	Wheat	ABL	2017x13		MS	1
2016FL080	AUS28645/2*Gregory	Wheat	ABL	2017x13		MS	1
2016FL081	AUS28645/2*Gregory	Wheat	ABL	2017x13		S	1
2016FL082	AUS28645/2*Gregory	Wheat	ABL	2017x13		MS	1
2016FL083	AUS28677/2*Wylie	Wheat	ABL	2017x13		MS	1
2016FL084	AUS28677/2*Wylie	Wheat	ABL	2017x13		S	1
2016FL085	AUS28677/2*Wylie	Wheat	ABL	2017x13		MRMS	1
2016FL086	AUS28372/2*Gregory	Wheat	ABL	2017x13		MSS	1
2016FL087	AUS28372/2*Gregory	Wheat	ABL	2017x13		MS	1
2016FL094	AUS28451/2*Gregory	Wheat	ABL	2017x13		MS	1
2016FL183	AUS28470/2*Gregory	Wheat	ABL	2017x13		MSS	1
2016FL184	AUS28470/2*Gregory	Wheat	ABL	2017x13		MS	1
2016FL185	AUS28470/2*Gregory	Wheat	ABL	2017x13		MS	1
2016FL186	AUS28470/2*Gregory	Wheat	ABL	2017x13		MS	1
USQW19030	AUS28369/2*Wylie	Wheat	ABL	2017x13	2021x14	MR	2
USQW19031	AUS28369/2*Wylie	Wheat	ABL	2017x13	2021x14	MRMS	2
USQW19032	AUS28369/2*Wylie	Wheat	ABL	2017x13	2021x14	MRMS	2
USQW19033	AUS28451/2*Gregory	Wheat	ABL	2017x13		MS	1

[1] Classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/).

[2] Long-term classifications may vary from the classifications based on the restricted data set reported in the manuscript text

[3] Number of experiments used to determine *P. neglectus* resistance rating

[4] For comparison purposes only. The inoculated/unplanted treatment simulates a weed-free fallow and can be used to estimate the performance of a completely resistant genotype. The authors do not contend that the unplanted treatment carries genetic resistance.

Accessory Table 5. Parentage and resistance ratings of Iranian landrace-derived advanced breeding lines (ABL)

Genotype	Pseudonym	Parentage	Crop	*Pratylenchus thornei* resistance		*Pratylenchus neglectus* resistance	
				Rating[1]	n[2]	Rating[1]	n[2]
2016FL072	2016FL072	AUS28369/2*Wylie	Wheat	RMR	2	MR	2
2016FL073	2016FL073	AUS28369/2*Wylie	Wheat	RMR	2	MRMS	2
USQW19030	2016FL074	AUS28369/2*Wylie	Wheat	RMR	2	MR	2
USQW19031	2016FL075	AUS28369/2*Wylie	Wheat	RMR	2	MRMS	2
2016FL076	2016FL076	AUS28369/2*Wylie	Wheat	R	2	RMR	2
2016FL077	2016FL077	AUS28369/2*Wylie	Wheat	RMR	2	MS	1
USQW19032	2016FL078	AUS28369/2*Wylie	Wheat	RMR	2	MRMS	2
2016FL079	2016FL079	AUS28369/2*Wylie	Wheat	RMR	2	MS	1
2016FL080	2016FL080	AUS28645/2*Gregory	Wheat	MS	2	MS	1
2016FL081	2016FL081	AUS28645/2*Gregory	Wheat	MRMS	2	S	1
2016FL082	2016FL082	AUS28645/2*Gregory	Wheat	MSS	2	MS	1
2016FL083	2016FL083	AUS28677/2*Wylie	Wheat	S	2	MS	1
2016FL084	2016FL084	AUS28677/2*Wylie	Wheat	MS	2	S	1
2016FL085	2016FL085	AUS28677/2*Wylie	Wheat	S	2	MRMS	1
2016FL086	2016FL086	AUS28372/2*Gregory	Wheat	MRMS	2	MSS	1
2016FL087	2016FL087	AUS28372/2*Gregory	Wheat	MRMS	2	MS	1
USQW19033	2016FL093	AUS28451/2*Gregory	Wheat	RMR	2	MS	1
2016FL094	2016FL094	AUS28451/2*Gregory	Wheat	MRMS	2	MS	1

2016FL183	2016FL183	AUS28470/2*Gregory	Wheat	MS	2	MSS	1
2016FL184	2016FL184	AUS28470/2*Gregory	Wheat	MSS	2	MS	1
2016FL185	2016FL185	AUS28470/2*Gregory	Wheat	MSS	2	MS	1
2016FL186	2016FL186	AUS28470/2*Gregory	Wheat	MSS	2	MS	1

[1] Classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/).

[2] Number of experiments used to determine P. thornei and P. neglectus resistance ratings

APPENDIX C: SUPPLEMENTARY INFORMATION FOR CHAPTER 5

Accessory Table 1. *Pratylenchus thornei* resistance ratings of parents, standard genotypes and treatments used to characterise einkorn-derived recombinant inbred line populations (RILP)

Genotype	Crop	Type	Experiment Year, RILP and Generation							*Pratylenchus thornei* resistance	
			2015	2015	2015	2019	2019	2019	2020	Rating[1,2]	n[3]
AUS119	Wheat	Parent	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)	Tmm1J (TC_1F_6)	Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	SVS	7
AUS27012	Einkorn	Parent		Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)				Tma3G (BC_3F_6)	RMR	6
AUS27045	Einkorn	Parent	Tma1G (TC_1F_2)				Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	R	5
AUS36493	Wheat	Parent	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)	Tmm1J (TC_1F_6)			Tma3G (BC_3F_6)	SVS	7
EGA Gregory	Wheat	Parent	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)			Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	MSS	27
Janz	Wheat	Parent			Tmm1J (TC_1F_2)	Tmm1J (TC_1F_6)			Tma3G (BC_3F_6)	S	36
Arapiles	Barley	Standard	Tma1G (TC_1F_2)							MS	34

Batavia	Wheat	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)					SVS	82
Catalina	Wheat	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)					MSS	58
Chara	Wheat	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)						MS	58
CPI133872	Wheat (synthetic hexaploid)	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)	Tmm1J (TC_1F_6)	Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	RMR	96
EGA Kidman	Wheat	Standard					Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	MSS	32
Gauntlet	Wheat	Standard					Tma1G (TC_1F_6)			MRMS	26
GS50a	Wheat	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)	Tmm1J (TC_1F_6)	Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	MR	108
Inoculated/ Unplanted	Treatment	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)	Tmm1J (TC_1F_6)	Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	R^4	100
Merinda	Wheat	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)					MSS	63
Petrie	Wheat	Standard				Tmm1J (TC_1F_6)	Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	SVS	57
QT8447	Wheat	Standard				Tmm1J (TC_1F_6)	Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	MRMS	47

Seri 82	Wheat	Standard					Tma1G (TC_1F_6)			SVS	3
Strzelecki	Wheat	Standard				Tmm1J (TC_1F_6)	Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	SVS	48
Sunguard	Wheat	Standard					Tma1G (TC_1F_6)	Tma3G (BC_3F_5)	Tma3G (BC_3F_6)	S	40
Sunzell	Wheat	Standard					Tma1G (TC_1F_6)			MS	27
Tamaroi	Durum	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)					MR	48
Yallaroi	Durum	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)					MR	59
Yandanooka	Wheat	Standard	Tma1G (TC_1F_2)	Tmm1G (TC_1F_2)	Tmm1J (TC_1F_2)					SVS	64

[1] Genotype classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/) using the method of Thompson et al. (2020).

[2] Long-term classifications may vary from the classifications based on the restricted data set reported in the manuscript text.

[3] Number of experiments used to determine P. thornei resistance rating

[4] For comparison purposes only. The inoculated/unplanted treatment simulates a weed-free fallow and can be used to estimate the performance of a completely resistant genotype. The authors do not contend that the unplanted treatment carries genetic resistance.

Accessory Table 2. Alpha classifications of BC_1F_2 genotypes used to form categories to compare the segregation ratios of recombinant inbred line populations with predicted Medelian segregation ratios for 1-gene and 2-gene additive and dominance genetic models

Model	Predicted Ratio	Genotype alpha classifications used to form segregation categories[1]				
		Category 1	Category 2	Category 3	Category 4	Category 5
Additive						
1-gene	5:2:1	VS, SVS, S, MSS, MS	MRMS, MR	RMR, R		
2-gene	25:20:14:4:1	VS, SVS, S, MSS	MS	MRMS, MR	RMR	R
Dominance						
1-gene						
Dominant susceptibility	7:1	VS, SVS, S, MSS, MS	MRMS, MR, RMR, R			
Dominant resistance	5:3	VS, SVS, S, MSS, MS	MRMS, MR, RMR, R			
2-gene						
Dominant susceptibility	49:14:1	VS, SVS, S, MSS, MS	MRMS, MR	RMR, R		
Dominant resistance	25:29:5	VS, SVS, S, MSS, MS	MRMS, MR	RMR, R		

[1] Genotype classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/) using the method of Thompson et al. (2020).

Accessory Table 3. *Pratylenchus thornei* population densities (best linear unbiased predictions) and resistance classifications of the Tmm1JRIL recombinant inbred line population

Genotype	Parentage	Crop	Generation when tested	*Pratylenchus thornei*/kg soil+roots			
				$log_e(x)$	se	BTM[1]	Rating[2,3]
AUS27012		Einkorn	F∞	9.23	0.46	10,170	R
Inoculated/Unplanted		Unplanted		9.26	0.46	10,474	R[4]
CPI133872		Wheat (synthetic hexaploid)	F∞	9.77	0.46	17,467	RMR
GS50a		Wheat	F∞	9.98	0.46	21,557	MR
Tmm1JRIL047	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.08	0.51	23,939	MR
QT8447		Wheat	F∞	10.24	0.46	28,105	MRMS
Tmm1JRIL003	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.40	0.46	32,991	MRMS
Tmm1JRIL091	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.56	0.46	38,688	MS
Tmm1JRIL048	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.60	0.46	40,169	MS
Tmm1JRIL112	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.61	0.46	40,637	MS
Tmm1JRIL004	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.66	0.46	42,436	MS
Tmm1JRIL085	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.67	0.46	43,052	MS
Tmm1JRIL015	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.70	0.46	44,217	MS
Tmm1JRIL049	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.71	0.46	44,715	MS
Tmm1JRIL088	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.71	0.46	44,823	MS

Tmm1JRIL102	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.72	0.46	45,259	MS
Tmm1JRIL045	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.73	0.46	45,687	MS
Tmm1JRIL001	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.78	0.46	48,019	MS
Tmm1JRIL084	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.78	0.46	48,086	MS
Tmm1JRIL030	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.78	0.46	48,108	MS
Tmm1JRIL121	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.92	0.46	55,496	MSS
Tmm1JRIL006	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.93	0.46	55,575	MSS
Tmm1JRIL046	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.95	0.46	57,104	MSS
Tmm1JRIL078	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.96	0.46	57,364	MSS
Tmm1JRIL002	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.96	0.46	57,537	MSS
Tmm1JRIL094	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.96	0.51	57,611	MSS
Tmm1JRIL062	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.98	0.46	58,451	MSS
Tmm1JRIL024	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	10.98	0.51	58,872	MSS
Tmm1JRIL065	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.02	0.58	61,306	MSS
Tmm1JRIL053	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.03	0.46	61,703	MSS
Tmm1JRIL119	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.04	0.46	62,460	MSS
Tmm1JRIL095	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.04	0.46	62,616	MSS
Tmm1JRIL076	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.05	0.46	62,818	MSS
Tmm1JRIL093	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.05	0.46	63,056	MSS
Tmm1JRIL036	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.06	0.46	63,524	MSS

Tmm1JRIL031	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.07	0.46	64,005	MSS
Tmm1JRIL100	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.08	0.46	65,083	MSS
Tmm1JRIL038	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.09	0.51	65,291	MSS
2013-383	AUS36493/AUS27012//Janz	Wheat	BC_1F_1	11.09	0.51	65,433	MSS
Tmm1JRIL111	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.10	0.46	66,282	MSS
Tmm1JRIL056	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.11	0.46	67,107	MSS
Tmm1JRIL018	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.11	0.46	67,154	MSS
Tmm1JRIL057	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.12	0.46	67,565	MSS
Tmm1JRIL083	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.13	0.46	68,470	MSS
Tmm1JRIL068	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.14	0.46	68,747	MSS
Tmm1JRIL120	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.14	0.46	68,893	MSS
Tmm1JRIL021	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.14	0.51	69,115	MSS
Tmm1JRIL073	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.14	0.46	69,166	MSS
Tmm1JRIL072	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.14	0.51	69,208	MSS
Tmm1JRIL012	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.15	0.46	69,660	MSS
Tmm1JRIL064	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.16	0.46	70,135	MSS
Tmm1JRIL017	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.17	0.46	71,104	MSS
Tmm1JRIL043	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.18	0.51	71,344	MSS
Tmm1JRIL029	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.19	0.46	72,668	MSS
Tmm1JRIL028	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.21	0.51	74,022	MSS

Tmm1JRIL066	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.23	0.58	75,060	S
Tmm1JRIL116	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.24	0.46	76,280	S
AUS36493	Chinese Spring (KSU)	Wheat	F∞	11.25	0.46	76,729	S
Tmm1JRIL034	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.27	0.46	78,793	S
Tmm1JRIL008	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.28	0.58	78,979	S
Tmm1JRIL063	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.28	0.46	79,424	S
Tmm1JRIL096	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.29	0.46	79,875	S
Tmm1JRIL019	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.30	0.46	80,916	S
Tmm1JRIL026	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.31	0.46	81,416	S
Tmm1JRIL059	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.31	0.51	81,567	S
Tmm1JRIL058	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.32	0.46	82,781	S
Tmm1JRIL016	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.32	0.46	82,799	S
Tmm1JRIL123	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.33	0.58	82,921	S
Tmm1JRIL082	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.33	0.46	83,575	S
Strzelecki		Wheat	F∞	11.34	0.58	83,890	S
Tmm1JRIL117	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.34	0.51	84,444	S
Tmm1JRIL077	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.34	0.46	84,481	S
Tmm1JRIL014	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.37	0.46	86,894	S
Tmm1JRIL107	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.37	0.46	86,958	S
Tmm1JRIL104	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.37	0.46	86,990	S

Tmm1JRIL109	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.41	0.46	90,001	S
Tmm1JRIL099	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.43	0.46	92,069	S
Tmm1JRIL097	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.44	0.46	93,158	S
Tmm1JRIL040	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.46	0.51	94,787	S
Tmm1JRIL086	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.47	0.46	95,614	S
Tmm1JRIL101	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.48	0.46	96,356	S
Tmm1JRIL013	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.49	0.46	97,639	S
Tmm1JRIL075	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.51	0.46	100,114	S
Tmm1JRIL060	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.53	0.46	101,406	S
Tmm1JRIL106	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.54	0.46	103,184	S
Tmm1JRIL051	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.55	0.46	103,685	S
Tmm1JRIL011	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.55	0.51	103,990	S
Tmm1JRIL092	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.57	0.46	105,791	SVS
Tmm1JRIL110	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.58	0.46	107,198	SVS
Tmm1JRIL023	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.60	0.46	108,895	SVS
Tmm1JRIL108	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.61	0.46	109,915	SVS
Tmm1JRIL052	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.62	0.46	111,764	SVS
Tmm1JRIL050	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.63	0.51	112,021	SVS
Tmm1JRIL010	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.63	0.46	112,242	SVS
Tmm1JRIL022	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.65	0.46	114,810	SVS

Tmm1JRIL080	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.67	0.46	117,460	SVS
Tmm1JRIL069	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.68	0.46	117,752	SVS
Tmm1JRIL032	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.71	0.51	121,666	SVS
Tmm1JRIL079	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.71	0.46	121,840	SVS
Tmm1JRIL067	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.71	0.46	122,343	SVS
Tmm1JRIL007	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.72	0.46	122,533	SVS
Tmm1JRIL113	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.72	0.46	122,660	SVS
Tmm1JRIL090	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.73	0.58	124,408	SVS
Tmm1JRIL071	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.75	0.46	126,202	SVS
Tmm1JRIL115	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.78	0.46	130,083	SVS
Tmm1JRIL020	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.79	0.46	131,488	SVS
Tmm1JRIL118	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.85	0.51	140,062	SVS
Tmm1JRIL055	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.87	0.46	142,451	SVS
Tmm1JRIL098	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.88	0.46	143,781	SVS
Tmm1JRIL035	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.88	0.46	143,857	SVS
Tmm1JRIL044	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.93	0.46	151,586	VS
Janz		Wheat	$F\infty$	11.94	0.46	152,773	VS
Tmm1JRIL114	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.96	0.46	156,932	VS
Tmm1JRIL041	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	11.99	0.46	160,492	VS
Tmm1JRIL033	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	12.04	0.46	170,046	VS

Petrie		Wheat	F∞	12.05	0.51	170,372	VS
Tmm1JRIL054	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	12.21	0.46	200,613	VS
Tmm1JRIL103	AUS36493/AUS27012//Janz	Wheat	BC_1F_6	12.21	0.46	201,209	VS
Mean				**11.23**			
LSD$_{(P=0.05)}$				**1.32**			
h^2				**0.54**			

[1] Back-transformed means.

[2] Genotype classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/) using the method of Thompson et al. (2020).

[3] Long-term classifications may vary from the classifications based on the restricted data set reported in this table.

[4] For comparison purposes only. The inoculated/unplanted treatment simulates a weed-free fallow and can be used to estimate the performance of a completely resistant genotype. The authors do not contend that the unplanted treatment carries genetic resistance.

Accessory Table 4. *Pratylenchus thornei* population densities (best linear unbiased predictions) and resistance classifications of the Tma1GRIL recombinant inbred line population

Genotype	Parentage	Crop	Generation	*Pratylenchus thornei*/kg soil+roots			
			When tested	$\log_e(x)$	se	BTM[1]	Rating[2,3]
Inoculated/Unplanted		Unplanted		10.06	0.47	23,463	R[4]
CPI133872		Wheat (synthetic hexaploid)	F∞	10.17	0.44	26,028	R
QT8447		Wheat	F∞	10.17	0.44	26,236	R
Tma1GRIL042	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.19	0.44	26,594	R
AUS27045		Einkorn	F∞	10.19	0.44	26,687	R
Tma1GRIL172	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.38	0.44	32,348	RMR
Tma1GRIL098	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.39	0.47	32,683	RMR
Tma1GRIL139	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.44	0.51	34,102	MR
Tma1GRIL147	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.45	0.44	34,538	MR
Tma1GRIL142	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.45	0.47	34,609	MR
Tma1GRIL004	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.46	0.47	34,985	MR
Tma1GRIL060	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.47	0.44	35,385	MR
Tma1GRIL188	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.48	0.44	35,445	MR
Tma1GRIL143	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.52	0.51	37,011	MR
Tma1GRIL047	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.58	0.44	39,212	MR

Tma1GRIL173	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.58	0.47	39,517	MR
Tma1GRIL186	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.60	0.44	40,185	MR
Tma1GRIL193	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.61	0.47	40,535	MR
GS50a		Wheat	F_∞	10.62	0.44	40,805	MRMS
Tma1GRIL003	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.62	0.44	40,826	MRMS
Tma1GRIL040	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.62	0.44	40,868	MRMS
Tma1GRIL175	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.62	0.47	40,979	MRMS
Tma1GRIL187	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.64	0.44	41,661	MRMS
Tma1GRIL110	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.64	0.47	41,746	MRMS
Tma1GRIL141	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.65	0.44	42,098	MRMS
Tma1GRIL108	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.65	0.44	42,139	MRMS
Sunzell		Wheat	F_∞	10.65	0.44	42,145	MRMS
Tma1GRIL148	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.70	0.51	44,379	MRMS
Tma1GRIL135	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.71	0.44	44,795	MRMS
Tma1GRIL046	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.72	0.47	45,246	MRMS
Tma1GRIL117	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.72	0.44	45,298	MRMS
Tma1GRIL132	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.74	0.44	46,180	MRMS
Tma1GRIL034	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.75	0.44	46,447	MRMS
Tma1GRIL146	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.75	0.44	46,619	MRMS
Tma1GRIL189	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.76	0.44	47,255	MRMS

Tma1GRIL180	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.76	0.44	47,271	MRMS
Tma1GRIL013	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.77	0.44	47,379	MRMS
Tma1GRIL103	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.79	0.44	48,307	MRMS
Tma1GRIL027	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.79	0.44	48,382	MRMS
Tma1GRIL133	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.82	0.44	49,815	MS
Tma1GRIL121	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.83	0.44	50,352	MS
Tma1GRIL182	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.83	0.47	50,365	MS
Tma1GRIL005	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.83	0.44	50,455	MS
Petrie		Wheat	F_∞	10.83	0.44	50,536	MS
Tma1GRIL086	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.83	0.44	50,584	MS
Tma1GRIL081	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.83	0.44	50,699	MS
Tma1GRIL125	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.84	0.44	51,207	MS
Tma1GRIL058	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.86	0.47	51,936	MS
Tma1GRIL160	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.86	0.44	51,991	MS
Tma1GRIL109	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.87	0.44	52,819	MS
Tma1GRIL167	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.88	0.44	52,948	MS
Gauntlet		Wheat	F_∞	10.89	0.44	53,455	MS
Tma1GRIL190	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.89	0.44	53,727	MS
Tma1GRIL128	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.90	0.47	53,927	MS
Tma1GRIL151	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.90	0.44	53,965	MS

Tma1GRIL009	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.90	0.44	54,146	MS
Tma1GRIL020	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.91	0.47	54,704	MS
Tma1GRIL131	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.91	0.44	54,743	MS
Tma1GRIL061	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.92	0.44	55,284	MS
Tma1GRIL076	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.92	0.44	55,442	MS
Tma1GRIL154	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.92	0.47	55,545	MS
Sunguard		Wheat	F_∞	10.93	0.44	55,872	MS
Tma1GRIL105	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.94	0.44	56,126	MS
Tma1GRIL015	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.94	0.47	56,285	MS
Tma1GRIL138	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.94	0.44	56,389	MS
Tma1GRIL192	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.94	0.44	56,431	MS
Tma1GRIL037	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.95	0.44	56,994	MS
Tma1GRIL178	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.96	0.44	57,801	MS
Tma1GRIL006	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.97	0.47	58,232	MS
Tma1GRIL194	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.98	0.44	58,526	MS
EGA Kidman		Wheat	F_∞	10.98	0.44	58,961	MS
Tma1GRIL166	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	10.99	0.44	59,000	MSS
Tma1GRIL174	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.00	0.44	59,926	MSS
Tma1GRIL101	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.01	0.44	60,238	MSS
Tma1GRIL036	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.01	0.44	60,361	MSS

Tma1GRIL070	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.01	0.44	60,500	MSS
EGA Gregory		Wheat	F∞	11.01	0.44	60,532	MSS
Tma1GRIL118	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.01	0.44	60,551	MSS
Tma1GRIL016	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.01	0.51	60,676	MSS
Tma1GRIL024	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.01	0.44	60,687	MSS
Tma1GRIL083	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.02	0.47	60,826	MSS
Tma1GRIL102	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.03	0.44	61,889	MSS
Tma1GRIL168	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.04	0.44	62,148	MSS
Tma1GRIL023	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.04	0.44	62,430	MSS
Tma1GRIL183	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.05	0.44	62,728	MSS
Tma1GRIL149	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.06	0.44	63,690	MSS
Tma1GRIL056	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.06	0.47	63,814	MSS
Tma1GRIL184	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.07	0.47	64,103	MSS
Tma1GRIL052	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.07	0.44	64,521	MSS
Tma1GRIL033	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.08	0.44	64,590	MSS
Tma1GRIL140	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.08	0.44	64,846	MSS
Tma1GRIL025	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.08	0.44	64,892	MSS
Tma1GRIL011	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.08	0.44	64,896	MSS
Tma1GRIL026	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.08	0.44	64,965	MSS
Tma1GRIL169	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.09	0.44	65,488	MSS

Tma1GRIL043	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.10	0.44	65,913	MSS
Tma1GRIL165	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.10	0.44	66,070	MSS
Tma1GRIL094	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.10	0.51	66,356	MSS
Tma1GRIL028	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.11	0.44	67,130	MSS
Tma1GRIL099	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.12	0.44	67,337	MSS
Tma1GRIL191	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.12	0.47	67,401	MSS
Tma1GRIL104	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.12	0.44	67,531	MSS
Tma1GRIL114	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.14	0.44	68,593	MSS
Seri		Wheat	F_∞	11.14	0.44	68,780	MSS
Tma1GRIL197	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.15	0.44	69,278	MSS
AUS119	Chinese Spring (AWCC)	Wheat	F_∞	11.15	0.44	69,789	MSS
Tma1GRIL127	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.16	0.44	70,121	MSS
Tma1GRIL195	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.17	0.44	70,971	S
Tma1GRIL077	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.17	0.44	71,159	S
Tma1GRIL054	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.18	0.47	71,668	S
Tma1GRIL085	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.19	0.44	72,197	S
Tma1GRIL075	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.20	0.44	73,116	S
Tma1GRIL007	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.20	0.44	73,141	S
Tma1GRIL066	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.20	0.44	73,147	S
Tma1GRIL039	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.20	0.44	73,425	S

Tma1GRIL164	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.21	0.47	73,833	S
Tma1GRIL031	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.22	0.44	74,279	S
Tma1GRIL053	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.22	0.44	74,284	S
Tma1GRIL113	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.22	0.47	74,594	S
Tma1GRIL069	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.23	0.47	75,207	S
Tma1GRIL088	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.23	0.44	75,246	S
Tma1GRIL008	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.24	0.51	76,236	S
Tma1GRIL045	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.24	0.44	76,397	S
Tma1GRIL170	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.25	0.44	76,518	S
Tma1GRIL137	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.26	0.44	77,335	S
Tma1GRIL057	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.26	0.47	77,359	S
Tma1GRIL134	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.26	0.44	77,377	S
Tma1GRIL177	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.26	0.51	77,538	S
Tma1GRIL095	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.26	0.44	77,559	S
Tma1GRIL176	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.27	0.47	78,622	S
Tma1GRIL022	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.28	0.47	79,106	S
Tma1GRIL179	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.28	0.44	79,259	S
Tma1GRIL059	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.28	0.44	79,593	S
Tma1GRIL153	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.29	0.51	80,144	S
Tma1GRIL163	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.30	0.44	80,686	S

Tma1GRIL158	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.31	0.47	81,537	S
Tma1GRIL107	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.32	0.44	82,734	S
Tma1GRIL084	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.33	0.51	83,628	S
Tma1GRIL144	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.34	0.44	83,761	S
Tma1GRIL152	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.34	0.44	83,871	S
Tma1GRIL071	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.34	0.44	84,165	S
Tma1GRIL162	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.34	0.44	84,375	S
Tma1GRIL010	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.35	0.47	85,192	SVS
Tma1GRIL082	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.37	0.44	86,453	SVS
2013-370	AUS119/AUS27045//Gregory	Wheat	BC_1F_1	11.37	0.47	86,689	SVS
Tma1GRIL126	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.37	0.44	87,100	SVS
Tma1GRIL030	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.38	0.44	87,733	SVS
Tma1GRIL123	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.39	0.47	88,221	SVS
Tma1GRIL157	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.39	0.44	88,266	SVS
Tma1GRIL115	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.40	0.47	88,950	SVS
Tma1GRIL112	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.40	0.44	89,459	SVS
Tma1GRIL129	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.40	0.44	89,645	SVS
Tma1GRIL029	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.41	0.44	89,779	SVS
Tma1GRIL017	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.42	0.44	91,283	SVS
Tma1GRIL155	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.42	0.44	91,518	SVS

Tma1GRIL001	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.43	0.44	92,455	SVS
Tma1GRIL002	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.44	0.44	93,263	SVS
Tma1GRIL100	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.45	0.44	93,864	SVS
Tma1GRIL130	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.47	0.44	95,656	SVS
Tma1GRIL161	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.48	0.44	96,742	SVS
Tma1GRIL196	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.49	0.44	98,157	SVS
Tma1GRIL156	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.50	0.44	98,314	SVS
Tma1GRIL078	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.50	0.44	99,096	SVS
Tma1GRIL072	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.51	0.44	99,865	SVS
Tma1GRIL018	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.51	0.44	99,901	SVS
Tma1GRIL181	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.52	0.44	100,564	SVS
Tma1GRIL038	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.52	0.47	101,020	SVS
Tma1GRIL055	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.53	0.44	101,217	SVS
Strzelecki		Wheat	$F\infty$	11.53	0.44	101,583	SVS
Tma1GRIL120	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.55	0.44	103,486	VS
Tma1GRIL073	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.58	0.44	107,106	VS
Tma1GRIL122	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.58	0.44	107,260	VS
Tma1GRIL041	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.61	0.44	109,810	VS
Tma1GRIL012	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.61	0.44	110,694	VS
Tma1GRIL136	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.62	0.44	111,124	VS

Tma1GRIL106	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.63	0.47	112,198	VS
Tma1GRIL014	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.67	0.44	117,132	VS
Tma1GRIL093	AUS119/AUS27045//Gregory	Wheat	BC_1F_6	11.71	0.44	122,341	VS
Mean				**11.05**			
$LSD_{(P=0.05)}$				**1.23**			
h^2				**0.37**			

[1] Back-transformed means.

[2] Genotype classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/) using the method of Thompson et al. (2020).

[3] Long-term classifications may vary from the classifications based on the restricted data set reported in this table.

[4] For comparison purposes only. The inoculated/unplanted treatment simulates a weed-free fallow and can be used to estimate the performance of a completely resistant genotype. The authors do not contend that the unplanted treatment carries genetic resistance.

Accessory Table 5. *Pratylenchus thornei* population densities (best linear unbiased predictions) and resistance classifications of the Tma3GRIL recombinant inbred line population after combined analysis of the BC_3F_5 and BC_3F_6 generations

Genotype	Parentage	Crop	Generation(s) when tested	*Pratylenchus thornei*/kg soil+roots			
				$log_e(x)$	se	BTM[1]	Rating[2,3]
AUS27045	AUS27045	Einkorn	F∞	9.50	0.89	6,653	R
Tma3GRIL142	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	9.79	0.89	8,843	R
Unplanted	Unplanted	Unplanted		9.87	0.89	9,625	RMR[4]
GS50a	GS50a	Wheat	F∞	9.96	0.89	10,483	RMR
Tma3GRIL079	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.11	0.89	12,190	RMR
Tma3GRIL126	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.20	0.90	13,293	MR
Tma3GRIL134	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.21	0.89	13,425	MR
Tma3GRIL160	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.23	0.89	13,686	MR
AUS27012	AUS27012	Einkorn	F∞	10.32	0.93	15,006	MR
CPI133872	CPI133872	Wheat	F∞	10.34	0.89	15,412	MR
Tma3GRIL133	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.36	0.89	15,690	MR
Tma3GRIL025	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.37	0.89	15,731	MR
Tma3GRIL138	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.38	0.90	15,944	MR

Tma3GRIL100	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.40	0.89	16,284	MR
Tma3GRIL161	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.41	0.89	16,511	MR
QT8447	QT8447	Wheat	F∞	10.45	0.89	17,157	MR
Tma3GRIL124	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.47	0.89	17,394	MR
Tma3GRIL023	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.49	0.89	17,890	MRMS
Tma3GRIL036	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.51	0.89	18,187	MRMS
Tma3GRIL209	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.55	0.89	18,922	MRMS
Tma3GRIL028	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.56	0.89	19,024	MRMS
Tma3GRIL197	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.56	0.89	19,196	MRMS
Tma3GRIL193	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.57	0.91	19,302	MRMS
Tma3GRIL108	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.61	0.89	20,005	MRMS
Tma3GRIL141	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.61	0.89	20,099	MRMS
Tma3GRIL073	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.62	0.89	20,329	MRMS
Tma3GRIL145	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.65	0.89	20,989	MRMS
Tma3GRIL090	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.66	0.89	21,167	MRMS
Tma3GRIL211	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.67	0.89	21,272	MRMS
Tma3GRIL006	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.68	0.89	21,532	MRMS
Tma3GRIL033	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.69	0.89	21,757	MRMS

Tma3GRIL195	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.71	0.89	22,167	MRMS
Tma3GRIL205	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.72	0.89	22,420	MRMS
Tma3GRIL012	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.73	0.90	22,575	MRMS
Tma3GRIL072	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.73	0.89	22,672	MRMS
Tma3GRIL213	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.74	0.89	22,873	MRMS
Tma3GRIL111	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.74	0.89	22,944	MRMS
Tma3GRIL183	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.78	0.89	23,725	MRMS
Tma3GRIL125	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.78	0.89	23,761	MRMS
Tma3GRIL210	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.78	0.89	23,825	MRMS
Tma3GRIL060	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.81	0.90	24,451	MS
Tma3GRIL131	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.81	0.90	24,606	MS
Tma3GRIL148	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.82	0.90	24,805	MS
Tma3GRIL202	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.82	0.89	24,866	MS
Tma3GRIL191	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.83	0.90	25,084	MS
Tma3GRIL165	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.84	0.89	25,225	MS
Tma3GRIL198	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.84	0.89	25,308	MS
Tma3GRIL034	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.84	0.89	25,345	MS
Tma3GRIL121	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.86	0.89	25,797	MS

Tma3GRIL074	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.86	0.89	25,833	MS
Tma3GRIL084	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.87	0.89	26,059	MS
Tma3GRIL176	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.87	0.89	26,064	MS
Tma3GRIL066	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.87	0.89	26,091	MS
Tma3GRIL076	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.89	0.89	26,692	MS
Tma3GRIL185	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.90	0.89	26,749	MS
Tma3GRIL004	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.92	0.90	27,358	MS
Tma3GRIL219	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.92	0.90	27,531	MS
Tma3GRIL075	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.93	0.89	27,673	MS
Tma3GRIL186	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.93	0.89	27,787	MS
Tma3GRIL057	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.93	0.89	27,798	MS
Tma3GRIL070	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.94	0.89	27,884	MS
Tma3GRIL225	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.94	0.90	27,888	MS
Tma3GRIL168	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.94	0.89	27,907	MS
Tma3GRIL182	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.94	0.89	28,051	MS
Tma3GRIL140	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.94	0.89	28,068	MS
Tma3GRIL218	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.94	0.89	28,069	MS
EGA Kidman	EGA Kidman	Wheat	$F\infty$	10.95	0.89	28,348	MS

Tma3GRIL010	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.96	0.89	28,392	MS
Tma3GRIL035	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.96	0.89	28,527	MS
Tma3GRIL177	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.97	0.90	28,887	MS
Tma3GRIL091	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.97	0.89	28,926	MS
Tma3GRIL061	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.98	0.89	28,971	MS
Tma3GRIL009	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.98	0.90	29,026	MS
Tma3GRIL024	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.99	0.90	29,364	MS
Tma3GRIL146	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.99	0.89	29,417	MS
Tma3GRIL001	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	10.99	0.89	29,471	MS
Tma3GRIL069	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.01	0.89	30,035	MS
Tma3GRIL029	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.03	0.89	30,553	MS
Tma3GRIL192	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.03	0.89	30,567	MS
Tma3GRIL169	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.03	0.89	30,599	MS
Janz	Janz	Wheat	$F\infty$	11.03	0.93	30,701	MS
Tma3GRIL166	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.03	0.90	30,726	MS
Tma3GRIL051	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.04	0.89	30,929	MS
Tma3GRIL184	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.04	0.89	31,030	MS
Tma3GRIL097	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.05	0.89	31,288	MS

Tma3GRIL156	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.06	0.89	31,363	MS
Tma3GRIL050	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.07	0.89	31,730	MS
Tma3GRIL127	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.08	0.89	32,181	MS
Tma3GRIL083	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.09	0.89	32,393	MS
Tma3GRIL058	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.09	0.90	32,565	MS
Tma3GRIL128	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.09	0.89	32,600	MS
Tma3GRIL200	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.09	0.89	32,624	MS
Tma3GRIL093	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.10	0.89	32,666	MS
Tma3GRIL173	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.10	0.89	32,726	MS
Tma3GRIL044	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.10	0.90	32,895	MS
Tma3GRIL081	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.11	0.90	33,195	MS
Tma3GRIL135	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.11	0.89	33,205	MS
Tma3GRIL080	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.13	0.89	33,661	MS
Tma3GRIL021	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.13	0.89	33,695	MS
Tma3GRIL005	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.13	0.90	33,726	MS
Tma3GRIL030	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.13	0.89	33,915	MS
Tma3GRIL120	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.14	0.91	34,086	MSS
Tma3GRIL046	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.14	0.89	34,142	MSS

Tma3GRIL136	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.15	0.89	34,541	MSS
Tma3GRIL098	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.15	0.89	34,649	MSS
Tma3GRIL153	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.16	0.89	34,971	MSS
Tma3GRIL101	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.16	0.89	34,988	MSS
Tma3GRIL150	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.17	0.89	35,300	MSS
Tma3GRIL088	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.18	0.89	35,504	MSS
Tma3GRIL107	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.18	0.90	35,561	MSS
Tma3GRIL147	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.19	0.89	35,949	MSS
Tma3GRIL180	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.19	0.89	36,056	MSS
Tma3GRIL056	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.20	0.89	36,391	MSS
Tma3GRIL068	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.21	0.89	36,589	MSS
Tma3GRIL154	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.21	0.90	36,644	MSS
Tma3GRIL158	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.22	0.91	37,100	MSS
Tma3GRIL164	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.23	0.89	37,539	MSS
Tma3GRIL189	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.24	0.89	37,630	MSS
Tma3GRIL038	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.26	0.89	38,477	MSS
Tma3GRIL181	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.26	0.89	38,508	MSS
Tma3GRIL014	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.27	0.89	39,070	MSS

Tma3GRIL013	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.28	0.89	39,187	MSS
2014-100	AUS119/AUS27045//2*Gregory	Wheat	BC_2F_1	11.28	0.93	39,378	MSS
Tma3GRIL227	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.29	0.89	39,728	MSS
Tma3GRIL123	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.29	0.90	39,762	MSS
Tma3GRIL106	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.30	0.89	39,893	MSS
Tma3GRIL170	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.30	0.93	40,101	MSS
Tma3GRIL087	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.31	0.89	40,348	MSS
Tma3GRIL027	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.31	0.89	40,494	MSS
Tma3GRIL129	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.31	0.89	40,567	MSS
Tma3GRIL151	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.31	0.89	40,597	MSS
Tma3GRIL214	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.34	0.90	41,499	MSS
Tma3GRIL220	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_6	11.34	0.93	41,513	MSS
Tma3GRIL039	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.34	0.89	41,525	MSS
Tma3GRIL113	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.34	0.90	41,559	MSS
Tma3GRIL055	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.34	0.89	41,637	MSS
Tma3GRIL201	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.34	0.90	41,657	MSS
Tma3GRIL077	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.34	0.89	41,761	MSS
Tma3GRIL002	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.36	0.89	42,604	MSS

Tma3GRIL144	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.37	0.89	42,862	MSS
Tma3GRIL053	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.38	0.90	43,289	MSS
Tma3GRIL172	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.38	0.89	43,403	MSS
Tma3GRIL095	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.38	0.90	43,581	MSS
Tma3GRIL139	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.39	0.89	43,848	MSS
Tma3GRIL199	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.39	0.89	43,930	MSS
Tma3GRIL110	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.40	0.89	44,094	MSS
Tma3GRIL003	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.40	0.89	44,345	MSS
Tma3GRIL041	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.41	0.90	44,616	MSS
Tma3GRIL130	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.41	0.89	44,932	MSS
Tma3GRIL026	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.42	0.89	45,081	MSS
Tma3GRIL049	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.43	0.89	45,475	MSS
Tma3GRIL162	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.43	0.89	45,798	MSS
Tma3GRIL062	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.44	0.89	46,171	MSS
Tma3GRIL116	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.44	0.89	46,222	MSS
Tma3GRIL132	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.45	0.91	46,623	MSS
Tma3GRIL020	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.45	0.89	46,766	MSS
Tma3GRIL215	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.46	0.89	46,853	S

Tma3GRIL047	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.46	0.89	46,983	S
Tma3GRIL015	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.46	0.89	47,187	S
Tma3GRIL137	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.48	0.89	48,197	S
Tma3GRIL122	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.49	0.90	48,528	S
Tma3GRIL203	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.50	0.89	48,753	S
Tma3GRIL223	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.50	0.89	48,801	S
EGA Gregory	EGA Gregory	Wheat	$F\infty$	11.50	0.89	48,949	S
Tma3GRIL109	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.50	0.89	49,108	S
Tma3GRIL092	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.50	0.89	49,141	S
Tma3GRIL031	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.50	0.89	49,155	S
Tma3GRIL117	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.51	0.89	49,280	S
Tma3GRIL190	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.52	0.89	49,894	S
Tma3GRIL063	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.53	0.89	50,201	S
Tma3GRIL043	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.53	0.89	50,400	S
Tma3GRIL112	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.53	0.89	50,406	S
Tma3GRIL221	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.53	0.89	50,465	S
Tma3GRIL032	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.53	0.89	50,559	S
Tma3GRIL119	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.53	0.89	50,596	S

Tma3GRIL179	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.53	0.89	50,648	S
Tma3GRIL114	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.54	0.89	50,930	S
Tma3GRIL008	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.54	0.89	50,956	S
Tma3GRIL067	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.54	0.89	51,065	S
Tma3GRIL171	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.54	0.89	51,140	S
Tma3GRIL007	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.56	0.89	51,775	S
Sunguard	Sunguard	Wheat	$F\infty$	11.56	0.89	51,967	S
Tma3GRIL204	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.56	0.89	52,009	S
Tma3GRIL011	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.57	0.89	52,334	S
Tma3GRIL208	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.57	0.89	52,424	S
Tma3GRIL149	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.59	0.89	53,368	S
Tma3GRIL174	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.59	0.89	53,656	S
Tma3GRIL045	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.60	0.89	53,899	S
Tma3GRIL103	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.60	0.89	54,105	S
2015-065	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_1	11.61	0.94	54,361	S
Tma3GRIL207	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.61	0.89	54,369	S
Tma3GRIL054	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.62	0.89	55,053	S
Tma3GRIL040	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.63	0.89	55,991	S

Tma3GRIL022	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.64	0.89	56,088	S
Tma3GRIL105	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.64	0.89	56,427	S
Tma3GRIL216	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.66	0.89	57,636	S
Tma3GRIL078	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.67	0.89	57,745	S
Tma3GRIL071	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.67	0.89	57,950	S
Tma3GRIL188	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.67	0.89	58,235	S
Tma3GRIL155	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.68	0.89	58,525	S
Tma3GRIL094	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.68	0.89	58,589	S
Tma3GRIL037	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.68	0.89	58,869	S
Tma3GRIL089	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.70	0.89	59,979	S
Tma3GRIL178	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.72	0.89	60,939	S
Tma3GRIL159	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.72	0.89	61,044	S
Tma3GRIL217	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.73	0.89	61,397	S
Tma3GRIL143	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.74	0.89	62,004	S
Tma3GRIL194	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.74	0.89	62,196	S
Chinese Spring (KSU)	AUS36493	Wheat	$F\infty$	11.74	0.93	62,256	S
Tma3GRIL099	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.74	0.90	62,411	S
Tma3GRIL163	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.74	0.89	62,450	S

Tma3GRIL175	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.75	0.89	62,555	S
Tma3GRIL115	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.75	0.89	62,993	S
Tma3GRIL118	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.76	0.89	63,215	S
Tma3GRIL018	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.76	0.89	63,737	S
Tma3GRIL065	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.78	0.89	64,521	S
Tma3GRIL226	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.78	0.89	64,854	S
Tma3GRIL059	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.79	0.89	65,355	SVS
Tma3GRIL212	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.81	0.89	66,835	SVS
Tma3GRIL224	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.82	0.89	67,060	SVS
Tma3GRIL104	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.82	0.89	67,382	SVS
Chinese Spring (AWCC)	AUS119	Wheat	$F\infty$	11.84	0.89	68,668	SVS
Tma3GRIL082	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.84	0.89	68,987	SVS
Tma3GRIL052	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.84	0.89	69,072	SVS
Tma3GRIL086	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.86	0.90	70,333	SVS
Tma3GRIL042	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.87	0.89	70,978	SVS
Tma3GRIL048	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.88	0.89	71,480	SVS
Tma3GRIL152	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.88	0.90	71,490	SVS
Tma3GRIL206	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.88	0.89	71,563	SVS

Tma3GRIL019	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.90	0.89	72,794	SVS
Tma3GRIL064	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.90	0.89	72,913	SVS
Tma3GRIL102	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.92	0.90	74,398	SVS
Tma3GRIL167	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.92	0.89	74,764	SVS
Tma3GRIL222	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.96	0.89	77,332	SVS
Tma3GRIL016	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.96	0.90	77,724	SVS
Tma3GRIL196	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	11.97	0.89	77,921	SVS
Petrie	Petrie	Wheat	F∞	11.97	0.89	78,441	SVS
Strzelecki	Strzelecki	Wheat	F∞	11.98	0.89	79,209	SVS
Tma3GRIL017	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	12.01	0.89	81,103	SVS
Tma3GRIL096	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	12.06	0.89	85,751	SVS
Tma3GRIL157	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	12.20	0.89	98,665	VS
Tma3GRIL085	AUS119/AUS27045//3*Gregory	Wheat	BC_3F_5 & BC_3F_6	12.43	0.89	123,479	VS
Mean				**11.22**			
LSD5%				**0.94**			
h^2				**0.66**			

[1] Back-transformed means.

[2] Genotype classification according to the Australian National Variety Trial (NVT) standard disease rating scale (https://nvt.grdc.com.au/) using the method of Thompson et al. (2020).

[3] Long-term classifications may vary from the classifications based on the restricted data set reported in this table.

[4] For comparison purposes only. The inoculated/unplanted treatment simulates a weed-free fallow and can be used to estimate the performance of a completely resistant genotype. The authors do not contend that the unplanted treatment carries genetic resistance.