# **Resistance to the root-lesion nematode** *Pratylenchus thornei* **in wheat landraces and cultivars from the West Asia and North Africa (WANA) region**

# *J. P. Thompson*<sup>A,B</sup>, *M. M. O'* Reilly<sup>A</sup>, and *T. G. Clewett*<sup>A</sup>

<sup>A</sup>Queensland Primary Industries and Fisheries, Department of Employment, Economic Development and Innovation, Leslie Research Centre, PO Box 2282, Toowoomba, Qld 4350, Australia.

<sup>B</sup>Corresponding author. Email: john.thompson@dpi.qld.gov.au

**Abstract.** Resistance to the root-lesion nematode *Pratylenchus thornei* was sought in wheat from the West Asia and North Africa (WANA) region in the Watkins Collection (148 bread and 139 durum wheat accessions) and the McIntosh Collection (59 bread and 43 durum wheat accessions). It was considered that landraces from this region, encompassing the centres of origin of wheat and where *P. thornei* also occurs, could be valuable sources of resistance for use in wheat breeding. Resistance was determined by number of *P. thornei*/kg soil after the growth of the plants in replicated glasshouse experiments. On average, durum accessions produced significantly lower numbers of *P. thornei* than bread wheat accessions in both the Watkins and McIntosh Collections. Selected accessions with low *P. thornei* numbers were re-tested and 13 bread wheat and 10 durum accessions were identified with nematode numbers not significantly different from GS50a, a partially resistant bread wheat line used as a reference standard. These resistant accessions, which originated in Iran, Iraq, Syria, Egypt, Sudan, Morocco, and Tunisia, represent a resource of resistance genes in the primary wheat gene pool, which could be used in Australian wheat breeding programs to reduce the economic loss from *P. thornei.*

**Additional keywords:** *Triticum aestivum*, *Triticum turgidum* spp. *durum*, *Pratylenchus neglectus*, CCN, *Heterodera avenae*, *Cre* genes.

# **Introduction**

The root-lesion nematode *Pratylenchus thornei* causes considerable economic loss to the Australian wheat industry in the northern ([Thompson](#page-8-0) *et al*. 2008) and southern grain production regions ([Vanstone](#page-8-0) *et al*. 2008). Targeted selection in the northern region has resulted in several tolerant bread wheat cultivars ([Thompson](#page-8-0) *et al*. 2008). However, only 1 out of 27 commercial cultivars suitable for growing in Queensland is moderately resistant to *P. thornei* ([DPIF 2009](#page-7-0)). Although tolerant wheat cultivars reduce yield loss from *P. thornei* they may still allow the nematodes to multiply. On the other hand, resistant wheat cultivars reduce the multiplication rate, resulting in fewer nematodes in the roots of the current crop and lower residual nematode populations in the soil to attack subsequent crops ([Thompson](#page-8-0) *et al*. 1999). Concerted wheat breeding is required to produce a suite of resistant wheat cultivars, and for this purpose, better and more diverse sources of resistance would be of value.

Sources of resistance to various diseases in modern crops can be found among wild progenitors and domesticated landraces in the respective centres of origin or gene centres of crop species [\(Leppik 1970; Cook and Veseth 1991\)](#page-7-0) where hosts and pathogens have co-evolved (Allen *et al*[. 1999\)](#page-7-0). Wheat originated in the Fertile Crescent of the Middle East evolving through successive hybridisation of wild diploid species to

produce fertile tetraploid and hexaploid allopolyploids [\(Harlan and Zohary 1966](#page-7-0); [Feldman and Sears 1981;](#page-7-0) Claude *et al*[. 1986](#page-7-0); [Cox 1997\)](#page-7-0). First, *Triticum urartu*  $(2n = 2x = 14; A<sup>u</sup>A<sup>u</sup>$  genome) hybridised with *Aegilops speltoides*  $(2n = 2x = 14; SS = BB$  genome) to produce tetraploid wild emmer wheat (*Triticum turgidum* ssp. dicoccoides;  $2n = 4x = 28$ ;  $A^u A^u BB$  genomes). Human selection for more desirable agricultural attributes resulted in cultivated emmer wheat (*Triticum turgidum* spp. *dicoccon*). Further natural hybridisation of cultivated emmer wheat with *Aegilops tauschii*  $(2n = 2x = 14; DD$  genome) resulted in hexaploid wheat (*Triticum aestivum*; A<sup>u</sup>A<sup>u</sup>BBDD genomes), which was selected by Neolithic cultivators ~7000 years ago in south-western Asia ([Feldman and Sears 1981](#page-7-0); [Cox and Wood](#page-7-0) [1999](#page-7-0)). Continual human selection of locally adapted types has resulted in a great diversity of farmers' cultivars, known as landraces, of both tetraploid durum wheat (*Triticum turgidum* ssp. *durum* A<sup>u</sup>A<sup>u</sup>BB genomes) and hexaploid bread or common wheat. In the modern era, plant breeding through targeted hybridisation and selection of superior progeny, has resulted in further accelerated and directed evolution to produce the cultivars used in modern agriculture.

Because *P. thornei* is known to occur in Middle-Eastern countries [\(Fortuner 1977](#page-7-0); Nicol *et al*[. 2003](#page-7-0)) it is likely that the region is a rich source of resistance genes in wild wheat relatives and wheat landraces. [Thompson and Haak \(1997\)](#page-8-0) found resistance to *P. thornei* in accessions of *Ae. tauschii* from Iran, and [Thompson \(2008\)](#page-8-0) reported resistance in synthetic hexaploid wheat accessions derived from various durum and *Ae. tauschii* parents. In this paper, we report experiments designed to find resistance to *P. thornei* in landraces and some more recent wheat cultivars from the West Asia and North Africa (WANA) region. Landraces belong to the primary gene pool of wheat and therefore can be readily crossed with modern Australian wheat cultivars in order to transfer resistance genes.

# **Materials and methods**

# *Wheat accessions*

#### *Watkins Collection*

A selection of spring types of bread wheat (148 accessions) and durum wheat (139 accessions) from the WANA region was provided by the Australian Winter Cereals Collection (AWCC), Tamworth, from the landraces of the Watkins Collection. The original Watkins Collection was made up of wheat accessions from 32 countries in the late 1920s and early 1930s by A. E. Watkins of the Plant Breeding Institute, University of Cambridge, UK, and is now held at the John Innes Centre, Norwich, UK (Miller *et al*[. 2001\)](#page-7-0). These accessions are identified by AUS numbers of the AWCC.

# *McIntosh Collection*

The second collection comprised bread (59 accessions) and durum wheat cultivars (43 accessions) from the WANA region and was provided by Prof. R. A. McIntosh, University of Sydney. The collection was made in 1993 by Prof. McIntosh through cooperation with Dr O. F. Mamluk of the International Centre for Agricultural Research in Dry Areas (ICARDA), based in Syria. The aim of the collection was to obtain widely grown wheat cultivars from each wheat-growing country in the WANA region for studies on rust and flag smut resistance. These accessions are identified by ISR (Imported Seed Register) serial numbers of the University of Sydney.

A categorisation of the accessions from both the Watkins and McIntosh Collections by country of origin, and whether they are bread or durum wheat, is given in Table 1.

### *Standard and other Australian wheat cultivars*

In each experiment, wheat cultivars with known responses to *P. thornei* from previous experiments were included as reference standards. These were the partially resistant line of bread wheat GS50a [\(Thompson](#page-8-0) *et al*. 1999), the susceptible Australian bread wheat cvv. Gatcher and Suneca, and the susceptible Mexican cv. Potam 70 ([Brennan](#page-7-0) *et al*. 1994). Additional Australian wheat cultivars from the northern grain region included in the final experiment were the bread wheat cvv. Janz, Vasco, Batavia and Sunbri, and the durum wheat cvv. Kamilaroi and Yallaroi.

#### *Resistance tests*

*Experiments 1 (Watkins Collection) and 2 (McIntosh Collection)*

The 287 accessions of wheat from the Watkins Collection and 88 accessions from the McIntosh Collection were tested along

#### **Table 1. Country of origin of bread and durum wheat accessions tested from the Watkins and McIntosh Collections**



with the 4 standard wheat cultivars and an unplanted control treatment in 2 separate experiments. Plants were grown singly in pots (15 cm diameter by 15 cm high) containing 1 kg Vertosolic soil of the Irving series [\(Thompson and Beckmann 1959\)](#page-8-0), which had been pasteurised by aerated steam at  $70^{\circ}$ C for 30 min ([Thompson 1990](#page-8-0)). Nutrients were added from solution to provide 200 mg NO<sub>3</sub>-N, 25 mg P, 88 mg K, 36 mg S, 285 mg Ca, and 5 mg Zn/kg soil. Inoculum, consisting of a mixture of soil and roots containing a pure culture of *P. thornei*, was obtained from open-pot cultures of wheat ([Thompson](#page-8-0) *et al*. [1999](#page-8-0)). The population density of *P. thornei* was determined by extraction of subsamples of the mixed soil and roots in Whitehead trays (described below) to determine the quantity to mix into each pot to supply 2500 *P. thornei*/kg soil. Each wheat accession and an inoculated unplanted control treatment were replicated 3 times. Each experiment was laid out as randomised blocks in an evaporatively cooled (temperature kept below 25°C) glasshouse in Toowoomba (27.55°S, 151.95°E), and grown from June to October 1994. The soil was initially watered to

<span id="page-2-0"></span>pF 2 (56% gravimetric moisture content) and returned to that moisture content by watering to weight as required during plant growth. After 16 weeks, the wheat growth stage [\(Zadoks](#page-8-0) *et al*. 1974) was recorded and one longitudinal half of the soil and roots was cut away and removed for nematode analysis. This was replaced with new soil and the plants were grown on for seed harvest.

The removed soil was manually broken into aggregates <5 mm and any separated roots were cut into pieces <10 mm, and all was mixed together. A subsample of 150 g was extracted for nematodes by the Whitehead tray method [\(Whitehead and Hemming 1965\)](#page-8-0) in a constant-temperature room at 22°C for 48 h. Nematodes were collected on a 20-um sieve, concentrated in ~15 mL of water, and counted in a 1-mL Hawksley slide under a compound microscope. Soil moisture was determined by oven-drying a 100-g subsample at 105°C for 48 h, and nematode counts were expressed as number of *P. thornei*/kg soil (oven-dry equivalent). Data were normalised by  $ln(x+1)$  transformation before analysis of variance (ANOVA), and where statistically significant, an *F*-protected l.s.d. (*F* l.s.d.) was calculated. The lower the number of *P. thornei* in the soil and roots after growth of a wheat cultivar, the greater the level of resistance that is inferred. The reproduction factor (RF) of *P. thornei* for each accession was calculated as (back-transformed final no. of *P. thornei* per kg soil)/(no. of *P. thornei* inoculated per kg soil). A 2-tailed *t* test was used to test the significance of the 2 group means for durum and bread wheat accessions in both experiments (Payne *et al*[. 2008\)](#page-8-0). Chi-square  $(\chi^2)$  was used to test the significance of proportions of bread and durum wheat cultivars that produced fewer nematodes than the partially resistant bread wheat standard GS50a in the two collections [\(Steel and Torrie](#page-8-0) [1960](#page-8-0)).

# *Experiment 3: Re-test of resistance of selected wheat accessions from Experiment 1 (Watkins Collection) and Expt 2 (McIntosh Collection) along with some additional accessions*

From the results of the first two experiments, some accessions that produced lower numbers of *P. thornei* than GS50a were selected for further testing in an experiment conducted from July to October 1996. These accessions comprised 31 bread and 8 durum accessions from the Watkins Collection, and 15 bread and 7 durum accessions from the McIntosh Collection. Additionally, 9 bread and 5 durum accessions from the McIntosh Collection, for which seed was unavailable for the initial experiment, were included. Also tested in Expt 3 were 3 bread and 2 durum accessions from the WANA region found previously to be resistant to cereal cyst nematode (CCN, *Heterodera avenae*, Ha13) (the late F. Green, pers. comm. 1995). The numbered *Cre* symbols for CCN resistance genes used in this paper are from [McIntosh](#page-7-0) *et al*. (2008). Two other inclusions in this experiment were AUS 5205 (Persia 20) and AUS 11984 (Virest), an Italian wheat found to be resistant to *P. neglectus* (Farsi *et al*[. 1994;](#page-7-0) [Vanstone](#page-8-0) *et al*. 2001). For this experiment, plants were grown in cylindrical polythene pots (10 cm diam. by 15.5 cm high) without drainage holes, containing 650 g soil which was kept at a constant  $22^{\circ}$ C in waterbaths within the glasshouse. Otherwise, the methods used were similar to those for the first two experiments.

# **Results**

# *Experiments 1 (Watkins Collection) and 2 (McIntosh Collection)*

Figure 1 shows the frequency distribution of the bread and durum wheat accessions in the Watkins Collection for number



**Fig. 1.** Frequency distribution of bread  $(n = 149)$  and durum  $(n = 138)$  wheat cultivars in the Watkins Collection in classes determined by the number of *P. thornei*/kg soil in ln(*x*+1) units from ANOVA. Lower numbers of *P. thornei* imply greater levels of resistance than higher numbers. Significance of difference between durum and wheat means from *t*-test is *P* < 0.001.

of *P. thornei*/kg soil in  $ln(x+1)$  units. As a group, the bread wheat accessions were significantly  $(P < 0.001)$  more susceptible to *P. thornei* than the durum accessions, with back-transformed means of *P. thornei/*kg soil of 52 051 for bread wheat and 38 560 for durum wheat.

Figure 2 shows the frequency distribution of the bread and durum wheat accessions in the McIntosh Collection for number of *P. thornei*/kg soil in ln(*x*+1) units. In the McIntosh Collection also, the bread wheat accessions were significantly  $(P<0.001)$ more susceptible to *P. thornei* than the durum wheat accessions, with back-transformed means of *P. thornei/*kg soil of 34 200 for bread wheat and 21 268 for durum wheat.

Most of the WANA wheat accessions in both collections produced lower numbers of *P. thornei* than the Australian wheat cvv. Suneca and Gatcher or the Mexican cv. Potam 70 [\(Figs 1](#page-2-0) and 2). In both collections, a greater proportion of durum than bread wheat accessions produced fewer *P. thornei* than the partially resistant Australian selection GS50a [\(Table 2\)](#page-4-0). There was no significant effect of country of origin in the WANA region on proportions of accessions producing fewer *P. thornei* than GS50a (results not shown). Also, there was no significant difference in number of *P. thornei* between West Asia and North Africa for either durum or bread wheat (data not shown).

Full results for individual bread and durum wheat accessions in the Watkins and McIntosh Collections from the first 2 experiments are available as an Accessory Publication.

#### *Experiment 3*

The results of Expt 3 are given in [Table 3](#page-5-0) as mean transformed number of *P. thornei/*kg soil with back-transformed values together with the appropriate *F* l.s.d. Reproduction factor and growth stage are also given. The results are ordered by ascending number of *P. thornei*, with accessions in categories of standard wheat cultivars, WANA bread wheat, and WANA durum accessions.

The named Australian bread wheat cultivars, included as reference standards, all produced high populations of *P. thornei* ranging from 84 900/kg soil (RF 34) for Sunbri up to 406 000/kg soil (RF 162) for Suneca. In comparison, GS50a produced 11 600 *P. thornei*/kg soil (RF 4.6). Two bread wheat accessions from the WANA region [AUS 4981 (Morocco 422) and AUS 1312 (Morocco 426)] produced lower nematode numbers than GS50a, while 11 others produced numbers that did not differ significantly from GS50a [\(Table 3](#page-5-0)). Out of these 13 more resistant bread wheat accessions, the 11 from the Watkins Collection originated in Iran (8), Morocco (2), and Iraq (1), and the 2 from the McIntosh Collection originated in Iran (1) and Sudan (1). All 13 of these resistant bread wheat accessions were resistant in both Expts 1 and 3.

There were 6 WANA durum wheat accessions that produced lower numbers of *P. thornei* than GS50a and 6 others that produced numbers that did not differ significantly from GS50a. Out of these 12 more resistant durum accessions, 4 from the Watkins Collection originated in Egypt and 8 from the McIntosh Collection originated in Morocco (6), Tunisia (1), and Syria (1). Ten of these resistant WANA durum wheat accessions were found to be resistant in both Expts 2 and 3, while 2 were tested for the first time in Expt 3.

The two Australian durum cultivars, Yallaroi and Kamilaroi, had comparable levels of resistance to GS50a and to the resistant WANA wheat accessions. Several of the resistant WANA accessions were considerably slower to mature [\(Table 3\)](#page-5-0) than the slowest of the Australian cultivars tested (Suneca with Zadoks growth stage of 68 at 16 weeks). One of the more resistant accessions, AUS 5203 (Persia 18), was particularly slow reaching only growth stage 28 by 16 weeks.

The sources of resistance to CCN, namely, the bread wheat accessions AUS 4930 (Iraq 48), AUS 7639 (Iraq), and AUS 10938 (Iran 28357), and the durum wheat accessions AUS 195 (Egyptian D) and AUS 1458 (Tunisia), produced moderate to high numbers of *P. thornei*. Likewise, the 2 bread wheat sources



**Fig. 2.** Frequency distribution of bread  $(n=50)$  and durum  $(n=38)$  wheat cultivars in the McIntosh Collection shown in classes determined by the number of *P. thornei*/kg soil in ln(*x*+1) units from ANOVA. Lower numbers of *P. thornei* imply greater levels of resistance than higher numbers. Significance of difference between durum and wheat means from *t*-test is *P* < 0.001.

<span id="page-4-0"></span>**Table 2. Percentage of bread and durum wheat accessions tested from the Watkins and McIntosh Collections, which produced fewer** *Pratylenchus thornei* **than the partially resistant bread wheat GS50a in Expts 1 and 2 respectively**

 $\chi^2$  = 31.98 with 3 *d.f.*, *P* < 0.005



of resistance to *P. neglectus*, namely, AUS 5205 (Persia 20) and AUS 11984 (Virest), also produced moderate to high numbers of *P. thornei.*

#### **Discussion**

# *Comparison of bread and durum wheat for resistance to* P. thornei

Wheat landraces tend to be a genetically diverse group having evolved under both environmental and farmer selection pressures [\(Cox and Wood 1999\)](#page-7-0). Landraces from both primary and secondary gene centres can be a rich source of resistance genes to a range of co-occurring pathogens including fungi and nematodes [\(Leppik 1970](#page-7-0)). Exploring these 2 collections of wheat from the WANA region proved worthwhile for identifying sources of resistance to *P. thornei* in both bread and durum wheat. The results indicate a higher proportion of accessions of durum than bread wheat from the WANA region with a useful level of resistance.

The commercial durum cvv. Yallaroi and Kamilaroi from the Australian northern grain region showed levels of resistance comparable to the best found in the WANA durums in contrast to the susceptibility of the commercial Australian bread wheat cultivars tested in this and other investigations [\(Thompson](#page-8-0) *et al*. [1999](#page-8-0)). Similarly, modern durum wheat lines from CIMMYT (the International Centre for Maize and Wheat Improvement in Mexico) used with *Ae. tauschii* as parents of synthetic hexaploid wheat, generally produced lower numbers of *P. thornei* than their synthetic hexaploid progeny ([Thompson](#page-8-0) [2008](#page-8-0)). If both the durum and *Ae. tauschii* parents were partially resistant then the synthetic hexaploid progeny had a comparable level of resistance ([Thompson 2008](#page-8-0)). Therefore, the higher ploidy level of hexaploid wheat appears generally to result in greater reproduction of*P. thornei* than the lower ploidy level of tetraploid wheat.

#### *Resistance to* P. thornei *in bread wheat*

Most wheat production in the Australian northern grain region is from bread wheat cultivars. Therefore the finding of 13 bread wheat accessions from the WANA region with comparable levels of resistance to GS50a is of much interest. Many of these have comparable maturity to wheat cultivars from the Australian northern grain region and will be suitable for use in breeding programs. Most of these bread wheat accessions were from Iran,

which may reflect that mainly bread wheat is grown there, and were numerically well represented in the tests. It may also reflect that the centre of origin of bread wheat (and possibly of*P. thornei*) is in Iran [\(Zohary](#page-8-0) *et al*. 1969; [Nakai 1979](#page-7-0); [Feldman and Sears](#page-7-0) [1981](#page-7-0)) or nearby Azerbaijan [\(Nakai 1979\)](#page-7-0) or the south-western coastal area of the Caspian Sea running from Armenia to Iran [\(Dubcovsky and Dvorak 2007\)](#page-7-0).

The cultivation of wheat spread beyond the Fertile Crescent to other countries and continents beginning in the Neolithic Period [\(Smith 1995](#page-8-0)). In the thousands of years that wheat has been cultivated and traded in the WANA region there has been ample time for *P. thornei* to have spread and for resistant landraces to have been selected in other localities. In the present study, good levels of resistance to *P. thornei* were also found in bread wheat accessions from Sudan and Morocco in North Africa, which are relatively distant from the centre of wheat origin, and where *P. thornei* also occurs [\(Fortuner 1977](#page-7-0); Nicol *et al*[. 2003\)](#page-7-0).

In Queensland, wheat growing commenced only 150 years ago. Evidence indicates that *P. thornei* was inadvertently introduced and has spread from the older to the newer wheatgrowing subregions of Queensland with increasing period of intense wheat culture [\(Thompson](#page-8-0) *et al*. 2008). Most of the bread wheat cultivars that have been grown in the Australian northern grain region are susceptible to *P. thornei*, and many have also been intolerant [\(Thompson](#page-8-0) *et al*. 1999), which was not a problem in fields that were initially free of *P. thornei*. Given the introduction and build up of *P. thornei* to damaging levels over time, its presence in fields was first noted in the poor growth of extremely intolerant local cultivars such as Gatcher. The severity of the damage appears to have resulted from the reencounter of 2 separated components of an evolutionary system as a result of intercontinental movement (as described by [Allen](#page-7-0) *et al*. [\(1999\)](#page-7-0) for new-encounter disease), first of wheat to Queensland, followed later by *P. thornei.* It was from within a field of badly affected Gatcher that the tolerant and resistant variant GS50a stood out for its better growth and was selected ([Thompson](#page-8-0) *et al*. [1999](#page-8-0)) in a comparable way to how farmers have selected tolerant and resistant landraces in the past ([Cox and Wood 1999](#page-7-0)).

# *Resistance to* Heterodera avenae *or to* Pratylenchus neglectus *does not convey resistance to* P. thornei

The sources of resistance to CCN did not produce low numbers of *P. thornei* in this study. One of these sources (AUS 7639 from Iraq) has the *Cre1* gene for CCN resistance ([de Majnik](#page-7-0) *et al*. [2003](#page-7-0)), supporting other evidence of a general lack of association between single resistance genes to CCN and resistance to *P. thornei.* For example, no resistance to *P. thornei* was found in bread wheat lines with the CCN resistance genes *Cre2* from *Aegilops ventricosa* ([Nombela and Romero 1999\)](#page-7-0), *Cre7* from *Ae. triuncialis* ([Nombela and Romera 2001\)](#page-8-0), and *Cre3* from *Ae. tauschii* ([Thompson 2008\)](#page-8-0). However, one source of CCN resistance, AUS 4930 (Iraq 48), was found resistant to *P. thornei* in glasshouse ([Nicol 1996\)](#page-7-0) and field experiments ([Nicol](#page-7-0) *et al*. [1999](#page-7-0)). Nicol *et al*[. \(1999\)](#page-7-0) later found variation within AUS 4930 and subsequently reselected within the accession to obtain consistently resistant lines. This variation could explain why AUS 4930 was not found to be resistant to *P. thornei* in our experiments.

#### <span id="page-5-0"></span>**Table 3. Number of** *Pratylenchus thornei***/kg soil after 16 weeks growth of bread and durum wheat accessions from the Watkins and McIntosh Collections in Expt 3**

Values are ln(*x*+1) transformed means with *F* l.s.d. from ANOVA, and with back-transformed means given. RF, Reproduction factor (final population of *P. thornei*/initial population). Growth stage of wheat after Zadoks *et al*. (1974). Serial No.: AUS, Australian Winter Cereals Collection, Tamworth; ISR, Imported Seed Register of the University of Sydney; Group designation: S, standard; AW, additional Australian bread wheat; AD, additional Australian durum wheat; WB1 and WB2, Watkins bread wheat first or second test; WD1 and WD2, Watkins durum wheat first or second test; MB1 and MB2, McIntosh bread wheat first or second test; MD1 and MD2, McIntosh durum wheat first or second test; (CCN), accession previously found to be resistant to CCN; (Pneg), accession previously found to be resistant to *P. neglectus*



Accession no.	Name	Group	Country	P. thornei/kg soil		<b>RF</b>	Growth stage
			of origin	$ln(x+1)$	Back-transf.		at 16 wk
ISR 466.5	Dogo	MB1	Turkey	11.73	124 292	49.7	64
<b>AUS 5078</b>	Morocco 59	WB <sub>2</sub>	Morocco	11.85	140 023	56.0	68
ISR 466.2	Bolal	MB <sub>2</sub>	Turkey	11.88	144 047	57.6	70
ISR 484.19	$W-70-6$	MB1	Iran	11.90	146756	58.7	51
AUS 5665	Smyrna 13	WB <sub>2</sub>	Turkey	11.93	151369	60.5	73
AUS 11984	Virest (Pneg)	WB1	Italy	11.94	153 298	61.3	24
ISR 466.7	<b>CTK/UEE</b>	MB1	Turkey	11.99	161253	64.5	67
AUS 5267	Persia 104	WB <sub>2</sub>	Iran	12.06	171988	68.8	80
ISR 484.10	$M-70-4$	MB <sub>2</sub>	Iran	12.10	179 153	71.7	56
ISR 454.5	Giza 164	MB <sub>2</sub>	Egypt	12.16	190757	76.3	51
ISR 484.12	$M-70-12$	MB <sub>2</sub>	Iran	12.16	191043	76.4	52
ISR 454.2	Sakha 69	MB <sub>2</sub>	Egypt	12.25	208 431	83.4	39
ISR 457.6	Bohouth 4	MB <sub>2</sub>	Syria	12.26	211428	84.6	44
AUS 5033	Morocco 30	WB <sub>2</sub>	Morocco	12.48	262 333	104.9	73
ISR 466.3	Gerek	MB1	Turkey	12.53	277691	111.1	68
ISR 466.9	Omid	MB1	Syria	12.74	341 030	136.4	69
ISR 466.1	Bezostaya TP 91	MB1	Turkey	12.79	359003	143.6	71
ISR 466.4	Atay	MB <sub>2</sub>	Turkey	12.92	410133	164.1	70
			<b>WANA Durum wheat accessions</b>				
<b>AUS 4472</b>	Egypt 5	WD <sub>2</sub>	Egypt	8.34	4201	1.7	81
<b>AUS 4474</b>	Egypt 7	WD <sub>2</sub>	Egypt	8.89	7272	2.9	68
ISR 467.11	<b>BD-Isly</b>	MD <sub>2</sub>	Morocco	9.09	8824	3.5	51
<b>AUS 4471</b>	Egypt 3	WD <sub>2</sub>	Egypt	9.22	10140	4.1	69
ISR 467.6	<b>BD-Sarif</b>	MD1	Morocco	9.23	10225	4.1	50
ISR 457.1	Cham 1	MD <sub>2</sub>	Syria	9.29	10775	4.3	42
ISR 467.7	BD-Sebou	MD <sub>2</sub>	Morocco	9.44	12528	5.0	55
ISR 467.10	BD-O.Rabia	MD <sub>2</sub>	Morocco	9.83	18544	7.4	48
ISR 467.2	BD-Acsad 65	MD1	Morocco	9.98	21559	8.6	57
ISR 467.3	<b>BD-Tassaout</b>	MD <sub>2</sub>	Morocco	10.15	25 6 21	10.2	53
<b>AUS 4512</b>	Egypt 47	WD <sub>2</sub>	Egypt	10.18	26332	10.5	79
ISR 481.5	Khiar	MD <sub>2</sub>	Tunisia	10.23	27647	11.1	54
ISR 460.2	Karpasia	MD1	Cyprus	10.43	33977	13.6	53
AUS 5645	Smyrna 2	WD <sub>2</sub>	Turkey	10.45	34473	13.8	53
<b>AUS 4520</b>	Egypt 54	WD <sub>2</sub>	Egypt	10.76	47055	18.8	70
ISR 460.3	Cyprus 2	MD1	Cyprus	10.78	48048	19.2	48
<b>AUS 195</b>	Egyptian (CCN)	WD1	Egypt	10.84	51119	20.4	73
ISR 460.1	Kyperounda	MD1	Cyprus	10.91	54883	22.0	60
<b>AUS 1458</b>	Tunisia (CCN)	WD1	Tunisia	11.04	62 2 2 7	24.9	72
<b>AUS 4496</b>	Egypt 33	WD <sub>2</sub>	Egypt	11.20	73 361	29.3	76
<b>ISR 457.8</b>	Jori C 69	MD2	Syria	11.21	73842	29.5	56
AUS 4330	Cyprus 13	WD <sub>2</sub>	Cyprus	11.57	105688	42.3	68
<b>AUS 4932</b>	Iraq 52	WD <sub>2</sub>	Iraq	11.8	132774	53.1	26
	$F$ l.s.d. ( $P = 0.05$ )			1.05			11.2
	$C.V.$ $(\%)$			6.8			9.4

**Table 3.** (*continued* )

The 2 sources of resistance to *P. neglectus* (AUS 5205 Persia 20 and AUS 11984 Virest) did not produce low numbers of *P. thornei* in our experiments, indicating that resistance to *P. neglectus* does not convey resistance to *P. thornei.* Previously, Farsi *et al*[. \(1995\)](#page-7-0) showed that GS50a was susceptible to *P. neglectus*, indicating that resistance to *P. thornei* does not convey resistance to *P. neglectus.*

# *Genetic analysis of* P. thornei *resistance in WANA wheat accessions based on molecular markers*

The identification of several sources of resistance to *P. thornei* permits the possibility of combining resistance genes to obtain cultivars that produce even lower numbers of nematodes. Molecular marker studies with resistant WANA and other wheat accessions indicate a polygenic form of resistance to *P. thornei* in wheat. Two of the resistant wheat accessions identified in this investigation [AUS 4926 (Iraq 43) and AUS 13124 (Morocco 426)] were selected to produce doubled haploids with Janz for use in molecular marker studies [\(Schmidt](#page-8-0) *et al*. 2005). By quantitative trait locus (QTL) analysis, a novel resistance locus was identified on chromosome 3B in these 2 WANA wheat accessions, which explained up to 24% of the phenotypic variation in the AUS  $13124 \times \text{Janz}$  population and up to  $12\%$  in the AUS  $4926 \times$  Janz population. This study also identified a novel <span id="page-7-0"></span>susceptibility locus present in Janz (and conversely absent in AUS 4926) on chromosome 1B, which explained up to 21% of the phenotypic variation in the doubled haploid population from this cross. A QTL for resistance to *P. thornei* on chromosome 2B, which was previously found in synthetic hexaploid wheat (Zwart *et al.* 2004, [2005](#page-8-0)), was detected in the AUS  $13124 \times$  Janz population. Molecular markers to this QTL and another on chromosome 6D, which had been identified in both GS50a (Vicars *et al*[. 1999\)](#page-8-0) and synthetic hexaploid wheat (Zwart *et al*[. 2004](#page-8-0), [2005\)](#page-8-0), were detected in both WANA wheat accessions by single marker regression analysis of the 2 doubled haploid populations ([Schmidt](#page-8-0) *et al*. 2005). [Toktay](#page-8-0) *et al.* (2006) screened an  $F_9$  population from a cross between the resistant reselection AUS 4930 7.2 and the susceptible bread wheat Pastor for previously published microsatellite molecular markers to *P. thornei* resistance, and detected resistance loci on chromosomes 1B, 2B, and 6D coming from AUS 4930 7.2.

# *Utilisation of the durum sources of resistance to* P. thornei

Nine of the 10 durum wheat accessions that were found to be resistant in 2 experiments were from North African countries and one was from Syria. These represent new sources of resistance that could be used in breeding programs to produce commercial durum wheat accessions with even greater resistance to *P. thornei*. They could also be used for bread wheat improvement either by direct crossing or by first producing synthetic hexaploids.

# **Conclusions**

Several bread and durum wheat landraces and cultivars from the WANA region have been identified with useful levels of resistance to the root-lesion nematode *Pratylenchus thornei.* These offer a diversity of resistance genes for use in wheat breeding. The bread wheat sources of resistance are particularly valuable because Australian bread wheat cultivars are mostly susceptible to *P. thornei*, whereas some Australian durum cultivars have partial resistance.

#### **Acknowledgments**

This work was financially supported by the Grains Research and Development Corporation. We thank M. I. Haak, formerly of DPI&F Leslie Research Centre, for technical assistance. We also thank M. C. Mackay (former curator) and G. R. Grimes of the Australian Winter Cereals Collection, Tamworth, for provision of the seed and information on the Watkins Collection, R. A. McIntosh, University of Sydney, for provision of seed, and H. S. Bariana and U. K. Bansal for further information on its provenance. M. Farsi and the late F. Green provided seed of wheat accessions resistant to *P. neglectus* and CCN, respectively.

# **References**

- Allen DJ, Lenné JM, Waller JM (1999) Pathogen biodiversity: its nature, characterization and consequences. Ch. 6. In 'Agrobiodiversity: characterization, utilization and management'. pp. 123–153. (CABI Publishing: Wallingford, UK)
- Brennan PS, Martin DJ, Thompson JP (1994) *Triticum aestivum* ssp. *vulgare* (bread wheat) cv. Pelsart. *Australian Journal of Experimental Agriculture* **34**, 864–865. doi: 10.1071/EA9940864
- Claude PP, Dyck PL, Evans LE (1986) An evaluation of 391 spring wheat introductions for resistance to stem rust and leaf rust. *Canadian Journal of Plant Pathology* **8**, 132–139.
- Cook RJ, Veseth RJ (1991) How wheat manages its own health: wheats in the wild. Ch. 1. In 'Wheat health management'. pp. 1–7. (APS Press (The American Phytopathological Society): St Paul, MN)
- Cox TS (1997) Deepening the wheat gene pool. *Journal of Crop Production* **1**, 1–25. doi: 10.1300/J144v01n01\_01
- Cox TS, Wood D (1999) The nature and role of crop biodiversity. Ch. 3. In 'Agrobiodiversity: characterization, utilization and management'. pp. 35–37. (CABI Publishing: Wallingford, UK)
- de Majnik J, Ogbonnaya FC, Moullet O, Lagudah ES (2003) The *Cre1* and *Cre3* nematode resistance genes are located on homologous loci in the wheat genome. *Molecular Plant-Microbe Interactions* **16**, 1129–1134. doi: 10.1094/MPMI.2003.16.12.1129
- DPIF (2009) Wheat varieties for Queensland 2009. The State of Queensland, Department of Primary Industries and Fisheries, Brisbane. Available at: www.dpi.qld.gov.au/documents/PlantIndustries/FieldCropsAndPasture/ Wheat\_Variety\_Guide\_09.pdf
- Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science* **316**, 1862–1866. doi: 10.1126/science.1143986
- Farsi M, Vanstone VA, Fisher JM, Rathjen AJ (1994) Genetic variation for resistance to a root-lesion nematode (*Pratylenchus neglectus*). In 'Abstracts, 2nd International Triticeae Symposium'. Logan, Utah, 20–24 June 1994.
- Farsi M, Vanstone VA, Fisher JM, Rathjen AJ (1995) Genetic variation in resistance to *Pratylenchus neglectus* in wheat and triticales. *Australian Journal of Experimental Agriculture* **35**, 597–602. doi: 10.1071/ EA9950597
- Feldman M, Sears ER (1981) The wild gene resources of wheat. *Scientific American* **244**, 102–112.
- Fortuner R (1977) *Pratylenchus thornei*. C.I.H. Description of Plantparasitic Nematodes Set 7, No. 93. Commonwealth Institute of Helminthology, St Albans, UK.
- Harlan JR, Zohary D (1966) Distribution of wild wheats and barley. *Science* **15**, 170–180.
- Leppik EE (1970) Gene centers of plants as sources of disease resistance. *Annual Review of Phytopathology* **8**, 323–344. doi: 10.1146/annurev. py.08.090170.001543
- McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Somers DJ, Appels R, Devos KM (2008) Catalogue of gene symbols for wheat. In '11th International Wheat Genetics Symposium'. 24–29 August 2008, Brisbane, Australia. Available at: http://wheat.pw.usda.gov/GG2/ Triticum/wgc/2008/
- Miller TE, Ambrose MJ, Reader SM (2001) The Watkins Collection. In 'Wheat Taxonomy: The Legacy of John Percival'. (Eds PDS Caligari, PE Brandham) pp. 113–120. (Academic Press: London)
- Nakai Y (1979) Isozyme variations in *Aegilops* and *Triticum.* IV. The origin of the common wheats revealed from the study of esterase isozymes in synthesized hexaploid wheats. *Journal of Genetics* **54**, 175–189.
- Nicol J, Rivoal R, Taylor S, Zaharieva M (2003) Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: distribution, yield loss, use of host resistance and integration of molecular tools. *Nematology Monographs and Perspectives* **2**, 1–19.
- Nicol JM (1996) The distribution, pathogenicity, and population dynamics of *Pratylenchus thornei* on wheat in South Australia. PhD Thesis, University of Adelaide, SA, Australia.
- Nicol JM, Davies KA, Hancock TW, Fisher JM (1999) Yield loss caused by *Pratylenchus thornei* on wheat in South Australia. *Journal of Nematology* **31**, 367–376.
- Nombela G, Romero D (1999) Host response to *Pratylenchus thornei* of a wheat line carrying the *Cre2* gene for resistance to *Heterodera avenae. Nematology* **1**, 381–388. doi: 10.1163/156854199508379
- <span id="page-8-0"></span>Nombela G, Romera MD (2001) Field response to *Pratylenchus thornei* of a wheat line with the *CreAet* gene for resistance to *Heterodera avenae. European Journal of Plant Pathology* **107**, 749–755. doi: 10.1023/ A:1011923400460
- Payne RW, Harding SA, Murray DA, Soutar DM, Baird DB, Welham SJ, Kane AF, Gilmour AR, Thompson R, Webster R, Tunnicliffe Wilson G (2008) 'GENSTAT for Windows.' 11th edn (VSN International: Hemel Hemstead, UK)
- 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. doi: 10.1071/AR05016
- Smith CW (1995) 'Crop production.' pp. 60–62. (John Wiley and Sons: New York)
- Steel GD, Torrie JH (1960) 'Principles and procedures of statistics.' (McGraw-Hill Book Company Inc.: New York)
- Thompson CH, Beckmann GG (1959) Soils and land use in the Toowoomba area, Darling Downs, Queensland. Soils and Land Use Series No. 28. Division of Soils, CSIRO, Melbourne.
- Thompson JP (1990) Treatments to eliminate root-lesion nematode (*Pratylenchus thornei* Sher and Allen) from a vertisol. *Nematologica* **36**, 123–127.
- 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. doi: 10.1071/AR07222
- Thompson JP, Brennan PS, Clewett TG, Sheedy JG, Seymour NP (1999) Progress in breeding wheat for tolerance and resistance to root-lesion nematode (*Pratylenchus thornei*). *Australasian Plant Pathology* **28**, 45–52. doi: 10.1071/AP99006
- 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. doi: 10.1071/A96167
- 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. doi: 10.1071/AP08021
- Toktay H, McIntyre CL, Nicol JM, Ozkan H, Elekcloglu HI (2006) Identification of common root-lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in bread wheat. *Genome* **49**, 1319–1323. doi: 10.1139/ G06-090
- 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**, 220–224. doi: 10.1071/AP08020
- Vanstone VA, Russ MH, Das RK, Rathjen AJ (2001) Development of wheat with resistance to *Pratylenchus neglectus*. In 'Proceedings, 13th Biennial Conference of the Australasian Plant Pathology Society'. Cairns, Qld, September 2001, p. 307. (Australasian Plant Pathology Society: Cairns)
- 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 rootlesion nematodes. In 'Proceedings of the 9th Assembly of the Wheat Breeding Society of Australia'. pp. 118–120.
- Whitehead AG, Hemming JR (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* **55**, 25–38. doi: 10.1111/j.1744-7348.1965.tb07864.x
- Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421. doi: 10.1111/j.1365- 3180.1974.tb01084.x
- Zohary D, Harlan JR, Vardi A (1969) The wild diploid progenitors of wheat and their breeding value. *Euphytica* **18**, 58–65.
- Zwart RS, Thompson JP, Godwin ID (2004) Genetic analysis of resistance to root-lesion nematode (*Pratylenchus thornei* Sher and Allen) in wheat. *Plant Breeding* **123**, 209–212. doi: 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 nematode (*Pratylenchus thornei* and *P. neglectus*) in wheat. *Australian Journal of Agricultural Research* **56**, 345–352. doi: 10.1071/AR04223

Manuscript received 2 June 2009, accepted 25 August 2009