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

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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

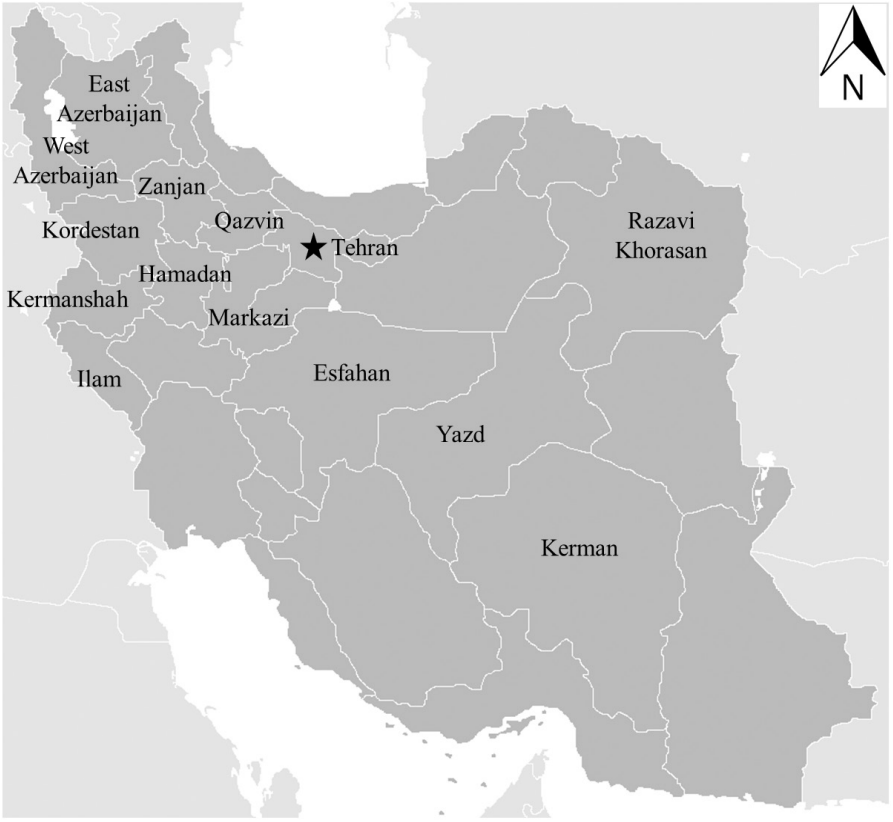

FIGURE 1 Iranian provinces from which the landrace wheat genotypes evaluated in this study originated. The number of genotypes evaluated per province, in parentheses after the province name, were as follows: East Azerbaijan (7); Esfahan (5); Hamadan (9); Ilam (14); Kerman (1); Kermanshah (29); Kordestan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

	Mean *Pratylenchus neglectus*/kg of soil + roots (*x*)		Variance components			
Trial name	$Log_e(x)$	BTM	Genetic	Error	Rotated loadings	VAF (%)
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.lrc-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.lrc-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.lrc-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.lrc-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.

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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.

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SUPPORTING INFORMATION

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

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