Genetic Characterization of Resistance in Wheat to the Root-Lesion Nematode *Pratylenchus Thornei*

Guiping Yan, Richard W. Smiley, Jennifer Gourlie, and Alison L. Thompson

Abstract

The root-lesion nematodes *Pratylenchus neglectus* and *P. thornei* are economically damaging pathogens of wheat in the Pacific Northwest (PNW). Growing resistant and tolerant varieties is the best approach to minimize yield loss. All PNW wheat varieties were found to be susceptible. Crosses were made between the PNW-adapted varieties (Alpowa Louise, Otis) and the Australian wheat GS50a with resistance to *P. thornei*. Resistance evaluations were performed under controlled greenhouse conditions using six check varieties, parents, 203 F_2 and 194 F_3 recombinant inbred lines (RILs) of GS50a x Alpowa, 102 F_2 RILs of GS50a x Otis, and 120 F_2 RILs of GS50a x Louise, each of which was inoculated with 1,500 or 2,000 *P. thornei*/kg of soil. RIL populations segregated in a continuous variation indicating that resistance in GS50a was quantitatively inherited. The heritability estimate for the resistance in GS50a was 0.68. The frequency distribution of F_2 and F_3 progeny suggested that resistance in GS50a is controlled by more than one gene and is additive in gene action. F_3 RILs of GS50a x Alpowa showing levels of resistance or susceptibility higher than their parents will be used in bulk segregant analysis for molecular marker development. The highly resistant lines obtained in this study will provide a source of resistance for breeding wheat varieties with improved genetic resistance.

Keywords: Alpowa, gene, genetic analysis, GS50a, Louise, Otis, *Pratylenchus thornei*, resistance, wheat

Introduction

The root-lesion nematodes *Pratylenchus neglectus* and *P. thornei* are economically important pathogens of wheat and other crops in the PNW states of Idaho, Oregon and Washington (Smiley et al. 2005a,b). These nematodes occurred in high numbers in many wheat fields (Smiley et al. 2004). *P. neglectus* is more widely distributed than *P. thornei* and mixtures of these species have been found in the same field (Smiley et al. 2004). It was estimated that lesion nematodes reduced region-wide yield as much as 5% annually, valued at $51 million (Smiley 2009). Yields of spring and winter cereals have been reduced from 9 to 40% and from 6-17%, respectively, in Oregon (Smiley 2009). Wheat plants usually have little or no visual symptoms expressed in the crop canopy except a generally unthrifty appearance. Plants damaged by these parasites are unable to extract all available soil water and nutrients, leading to premature onset of plant stress at times when non-parasitized plants would continue to grow and mature normally (Smiley and Machado 2009; Smiley and Nicol 2009).

There are no registered chemical or biological controls that are effective for reducing populations of these nematodes in wheat fields. Crop rotations are not likely to be economically feasible for dryland wheat because they do not allow sufficiently intensive wheat production to maintain farm profitability. The development of resistant and tolerant varieties is the most

efficient and cost-effective option for protecting crops against root-lesion nematodes (Smiley and Nicol 2009). When roots are invaded, nematodes multiply rapidly in susceptible varieties but not in resistant varieties. Levels of plant resistance therefore affect growth and yield of current crops and also nematode populations in soil that are capable of posing risk to subsequent plantings of intolerant crops or varieties.

All of the PNW wheat varieties examined were found to be susceptible to both species during past years of testing (Sheedy et al. 2007, 2008; Smiley et al. 2008). Spring wheat varieties, Alpowa (one of the most widely grown spring wheat varieties in the western United States), and Louise and Otis (WSU varieties widely used as parents in PNW wheat breeding programs), have poor levels of resistance to these nematodes. Crosses have been made to incorporate resistance into these locally adapted varieties from Persia 20 (syn. CItr11283, AUS5205), an Iranian landrace wheat expressing a high level of resistance to *P. neglectus*, and from GS50a, an Australian bread wheat line exhibiting a useful level of resistance to *P. thornei*. GS50a was a single plant selection from a severely affected field of the susceptible wheat variety Gatcher (Zwart et al. 2004) and is the most widely used source of *P. thornei* resistance (Thompson et al. 1999). F_4 recombinant inbred lines (RILs) have been obtained for the crosses between Persia 20 and Alpowa and between GS50a and Alpowa. Selecting and breeding wheat varieties for resistance is very likely to improve productivity and economic efficiency in annually cropped fields where non-host rotation crops such as safflower and flax are not profitable, and where production of successive susceptible crops, such as wheat, canola, mustard, pea or chickpea can be improved by using a resistant wheat variety.

Selecting wheat lines resistant to lesion nematodes in a conventional breeding program relies on the time-consuming and laborious procedure of inoculating plants in a greenhouse, growing plants under optimum temperature and moisture conditions for 16 weeks, and then extracting and counting the nematodes that have multiplied in the plant roots using microscopy. Applying molecular marker-assisted selection for root-lesion nematode resistance could greatly accelerate breeding for high levels of resistance. Use of molecular markers to test DNA extracted from leaves of young wheat seedlings will potentially eliminate the need for large-scale nematode resistance tests and allow rapid, efficient identification of resistance genes, facilitating development of new resistant varieties.

The primary limitation to develop wheat varieties or lines with resistance to lesion nematodes is the limited information available about the genetic basis of resistance (Toktay et al. 2006). Identification of new sources of resistance and understanding of the genetics and molecular basis of different sources of resistance genes are very important for producing wheat varieties with durable and superior resistance. The objectives of this research were to 1) introduce resistance from GS50a into PNW wheat varieties, 2) evaluate nematode resistance reactions of progeny lines derived from the crosses, 3) understand the genetic basis of resistance in GS50a, and 4) advance progeny lines for use in breeding programs to develop varieties with improved resistance and for identifying molecular markers to efficiently track the resistance genes.

Materials and Methods

Plant materials

Crosses were made in the greenhouse between susceptible parents (Alpowa, Louise, Otis) and the resistant parent (GS50a). For the cross of GS50a x Alpowa, a total of 194 seeds originating from F_3 generation were advanced to the F_4 generation in the greenhouse by using the single seed descent breeding method. F_4 lines from GS50a x Louise and F_3 lines from GS50a x Otis were also obtained. As the project is progressing, more advanced generations will be obtained. F_1 seed from each crossed head and seed from each plant of the F_2 to F_4 generations was kept separately. Six reference wheat varieties or lines (AUS28451R, CPI133872, Excalibur, Iraq43, Morocco 426, and Persia 20) were collected from collaborators in Australia (Table 1).

Nematode resistance evaluation

Four greenhouse experiments were conducted to evaluate levels of resistance to *P. thornei* for the progeny lines. Parental lines, unplanted and inoculated pots or tubes, and six standard varieties replicated 5-6 times in a randomized block design were included as controls in all experiments. F_2 (experiment 1, in 2008) and F_3 (experiment 2, in 2009) lines of GS50a x Alpowa were grown in the greenhouse with temperature maintained at approximately 72 ºF. Plants were grown in small plastic pots containing 1 lb of partially sterilized Walla Walla silt loam. Each pot was inoculated at planting with either 675 (experiment 1) or 900 (experiment 2) *P. thornei* from pure cultures raised on wheat roots in the greenhouse. The nematode species identity was confirmed using the procedure described by Yan et al. (2008). Each pot was fertilized with 0.002 lb of slow-release fertilizer (Multicote® All Purpose, 17-17-17 NPK). All pots were hand-watered to maintain an optimum soil water content at 25 percent.

F_2 lines of GS50a x Otis (experiment 3, in 2008) and GS50a x Louise (experiment 4, in 2007) were assessed for resistance reactions in a controlled-environment growth room. The tests were identical to those described above, with the following exceptions. Tubes were filled with 0.3 lb of soil, inoculated with 300 *P. thornei* (experiments 3 and 4), and bottom-watered through capillary action from a water reservoir, which maintained the soil at 30 percent water content.

Nematodes from all four experiments were extracted from the roots and soil in each pot or tube after 16 weeks of growth using a 48-hour Whitehead tray method (Whitehead and Hemming 1965). Numbers of nematodes in 1 ml of extracted sample were counted on a nematode counting slide under a microscope. Results were expressed as number of nematodes per kg of oven-dry soil.

Data analysis

The number of nematodes per kg of oven-dry soil after the growth of plants in each experiment was subjected to analysis of variance (ANOVA), with the designated wheat lines or other entries as treatments and the replicates as blocks. The raw nematode counts were skewed and were therefore normalized through logarithmic transformation before statistical analysis, using $\text{Log}_e(x+1)$. The least significant difference (LSD) ($P = 0.05$) was calculated and indicated in Table 2 using transformed means for statistical comparisons where required. The predicted means were back-transformed (BTM) after analysis and expressed in units of number of nematodes/kg of oven-dry soil. The nematode multiplication rate (MR), which is an index of

resistance, was calculated by dividing the number of nematodes extracted from roots and soil after 16 weeks of plant growth by the number of nematodes initially inoculated into the soil. A MR less than that of the resistant parent and also less than one was considered as highly resistant and a MR higher than that of the susceptible parent was considered as highly susceptible in this study.

Inheritance of resistance was estimated using classical genetic methods (Yan and Chen 2008). The heritability of resistance in GS50a was calculated using logarithmically transformed data of the F_3 population of GS50a x Alpowa based on the equation $H = [VF_3 - (VP_1 + VP_2)/2] /VF_3$, where H denotes the heritability, VF_3 denotes the total variance of the F_3 lines, VP_1 denotes the variance of the resistant parent (GS50a), and VP_2 denotes the variance of the susceptible parent (Alpowa). Chi-square analysis was used to test significance of parental means and population means for the cross of GS50a x Alpowa.

Results and Discussion

Resistance reaction of check varieties to root-lesion nematodes

Ten varieties used in previous studies were selected as references for resistance comparison. The known reactions of resistance and susceptibility of these varieties were shown in Table 1. The PNW spring wheat varieties Alpowa, Louise and Otis were reported to be susceptible to *P. thornei* (Sheedy et al. 2007, 2008). GS50a was reported to be partially resistant to *P. thornei* (Thompson 2008). Persia 20, AUS28451R, and CPI133872 were reported to have resistance to both *P. neglectus* and *P. thornei* (Sheedy et al. 2007, 2008; Zwart et al. 2005). Iraq43 (AUS4926) and Morocco 426 (AUS13124) were reported to have resistance levels equivalent to or better than that of GS50a (Schmidt et al. 2005, Zwart et al. 2006). Excalibur with the resistance gene *Rlnn1* had a high level of resistance to *P. neglectus* (Williams et al. 2002). To our knowledge, the reaction of Excalibur to *P. thornei* was not available.

The resistant reactions of these varieties to *P. thornei* from our tests are shown in Figure 1. Generally, most of the varieties showed the similar trend as reported in previous studies. AUS28451R and CPI133872 demonstrated a high level of resistance to *P. thornei*. GS50a (mean = 7.11) used as the parent resistant to *P. thornei* allowed less nematode multiplication than that of Alpowa (mean = 8.28), Louise (8.37) and Otis (8.08) used as the susceptible parents (Figure 1). Persia 20 produced a lower population of *P. thornei* than GS50a, the current standard for *P. thornei* resistance, confirming that Persia 20 confers dual resistance to both *Pratylenchus* species. It was noted that the same variety showed variations in response to these nematodes for individual experiments probably due to minor differences in temperature, water, light, and nematode virulence. Our experiments revealed that Excalibur was susceptible to *P. thornei*.

Table 1. Resistance reaction of parental and control varieties to *Pratylenchus* species in previous studies.

Variety	Class[a]	Source	Reaction[b]		Reference
			P. neglectus	*P. thornei*	
Alpowa	SWS	Pacific Northwest	S	S	Sheedy et al. 2007, 2008
Louise	SWS	Pacific Northwest	S	S	Sheedy et al. 2007, 2008
Otis	HWS	Pacific Northwest	S	S	Sheedy et al. 2007, 2008
GS50a	HWS	Australia	S	R	Thompson. 2008
Persia 20	HWF	Iranian landrace	R	R	Sheedy et al. 2007, 2008
AUS28451R	HWS	Iranian landrace	R	R	Sheedy et al. 2007, 2008
CPI133872	HWS	Australia	R	R	Zwart et al. 2005
Excalibur	-	Australia	R	-	Williams et al. 2002
Iraq43	-	Middle-Eastern landrace	-	R	Schmidt et al. 2005; Zwart et al. 2006
Morocco 426	-	Middle-Eastern landrace	-	R	Schmidt et al. 2005; Zwart et al. 2006

[a] - = Not available

[b] S = Susceptible, R = Resistant

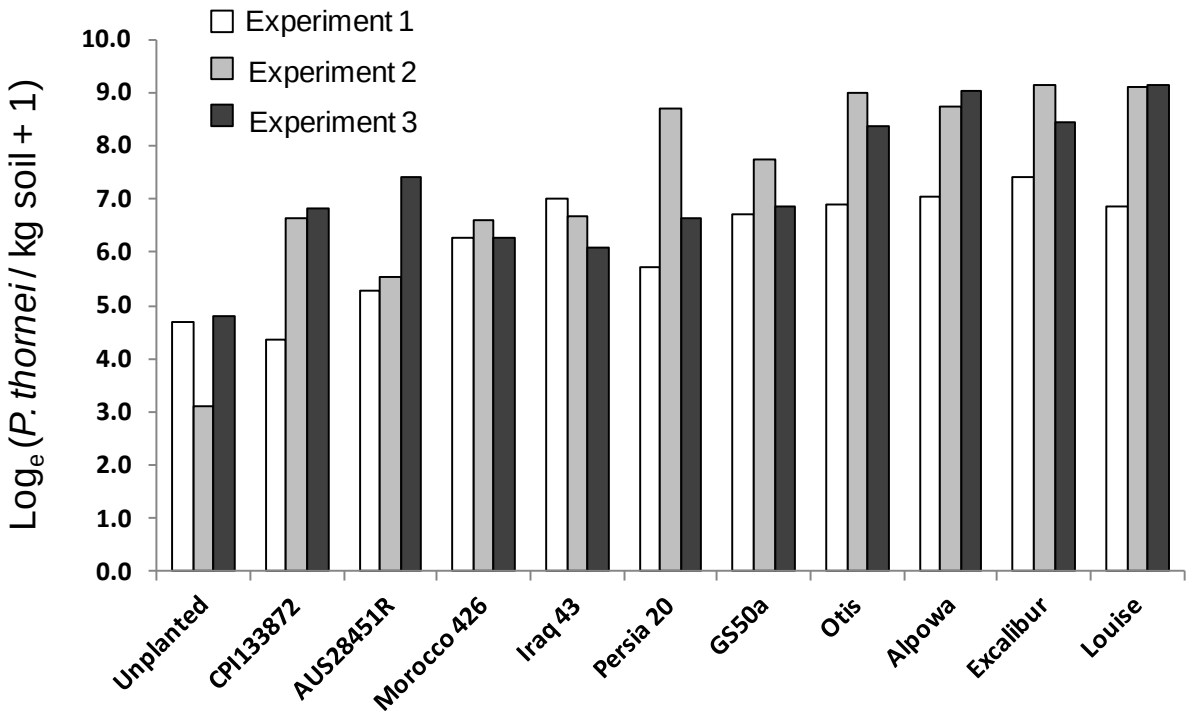

Figure 1. Root lesion nematode (*Pratylenchus thornei*) counts for parental and control varieties in the tests of F_2 (experiment 1) and F_3 (experiment 2) lines of GS50a x Alpowa and in the test of F_2 generation (experiment 3) of GS50a x Otis. Numbers are from 6 replicates in each test and are expressed as transformed values Log$_e$ (*P. thornei*/kg soil + 1).

Genetic analysis of resistance in GS50a

The distributions of nematode counts in populations of GS50a x Alpowa, GS50a x Otis and GS50a x Louise are illustrated in Figures 2, 3 and 4. Nematode counts of the F_2 and F_3 RILs of GS50a x Alpowa ranged from 39 to 8,216 *P. thornei*/kg of soil and from 86 to 67,195, respectively. Nematode counts of the F_2 RILs of GS50a x Otis ranged from 84 to 16,857 *P. thornei* /kg of soil and the F_2 RILs of GS50a x Louise ranged from 86 to 29,142. The level of resistance in GS50a is considered to be partial. The logarithmically transformed data for the F_2 and F_3 populations of GS50a x Alpowa and F_2 populations of GS50a x Otis and GS50a x Louise showed a continuous variation. The frequency distributions of nematode counts indicated that the resistance in GS50a was quantitatively inherited and controlled by more than one gene. There was no significant difference between the population mean and the parental means in the tests of GS50a x Alpowa F_2 ($P = 0.98$) and F_3 ($P = 0.92$) lines, indicating that additive effects were the predominant mode of inheritance for *P. thornei* resistance. These results were consistent with previous international research (Zwart et al. 2004). The heritability estimate for *P. thornei* resistance in GS50a was 0.68 based on the F_3 population of GS50a x Alpowa.

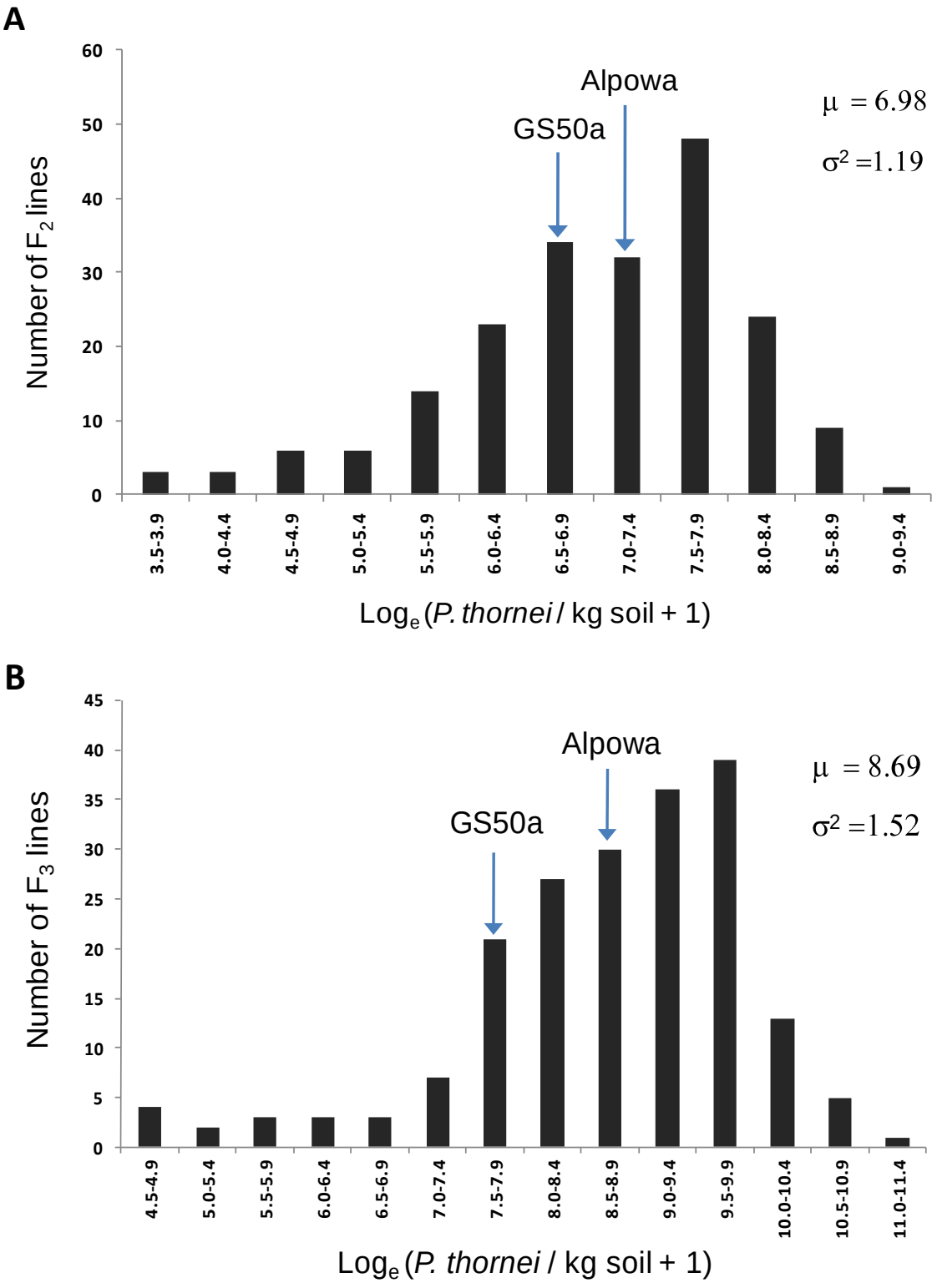

Figure 2. Frequency distribution of recombinant inbred lines (RILs) from GS50a x Alpowa populations for resistance to *P. thornei*. **A:** 203 F_2 RILs in the 2008 greenhouse experiment. **B:** 194 F_3 RILs in the 2009 greenhouse experiment. Nematode counts are expressed as transformed values Log_e (*P. thornei*/kg soil + 1).

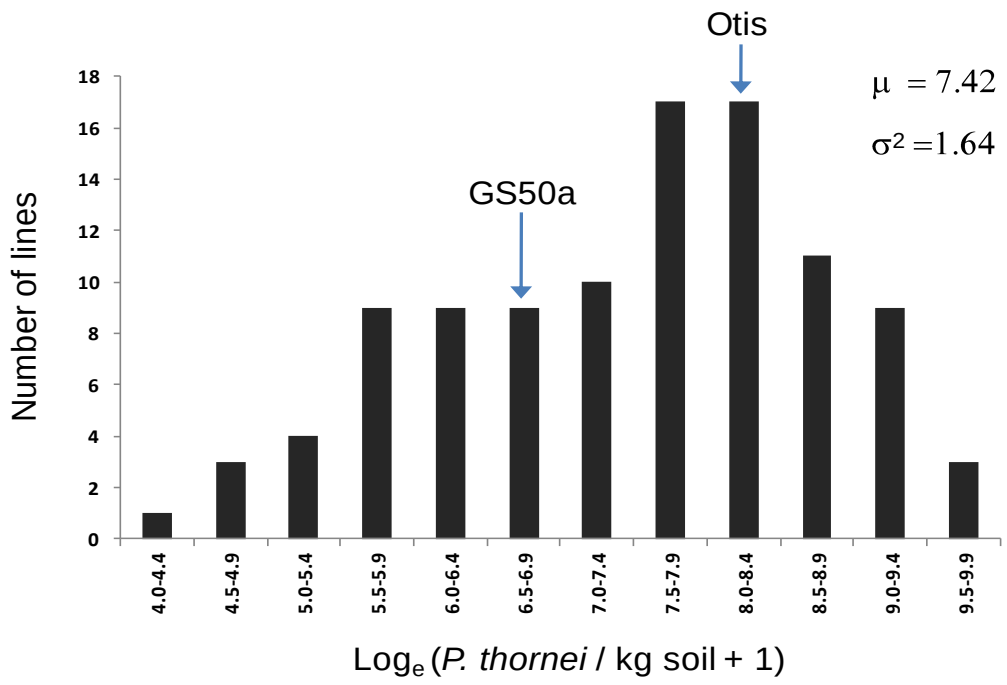

Figure 3. Frequency distribution of F_2 recombinant inbred lines (RILs) from GS50a x Otis population (102 lines) for resistance to *P. thornei* in the 2008 greenhouse experiment. Nematode counts are expressed as transformed values Log$_e$ (*P. thornei*/kg soil + 1).

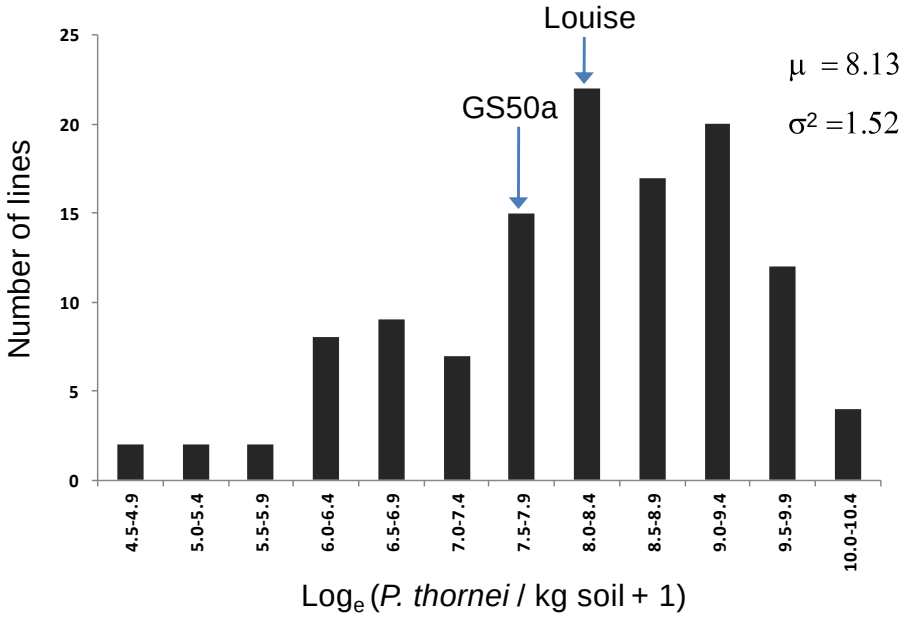

Figure 4. Frequency distribution of F_2 recombinant inbred lines (RILs) from GS50a x Louise population (120 lines) for resistance to *P. thornei* in the 2007 greenhouse experiment. Nematode counts are expressed as transformed values Log$_e$ (*P. thornei*/kg soil + 1).

Table 2. The parents and selected F_3 lines from the cross of GS50a x Alpowa differed significantly in resistance to *P. thornei*.

Parent or line	$Log_e(x+1)$[a]	BTM[b]	MR[c]	Parent or line	$Log_e(x+1)$[a]	BTM[b]	MR[c]
AG155	4.47	86	0.04	AG152	9.93	20,480	10.24
AG138	4.53	92	0.05	AG045	9.95	20,917	10.46
AG015	4.72	112	0.06	AG087	9.96	21,220	10.61
AG080	4.88	130	0.07	AG110	10.07	23,661	11.83
AG037	5.04	153	0.08	AG179	10.08	23,818	11.91
AG173	5.24	187	0.09	AG162	10.11	24,623	12.31
AG031	5.76	316	0.16	AG153	10.14	25,354	12.68
AG158	5.76	316	0.16	AG084	10.18	26,416	13.21
AG204	5.93	373	0.19	AG039	10.23	27,784	13.89
AG078	5.98	394	0.20	AG017	10.24	27,947	13.97
AG064	6.14	462	0.23	AG123	10.32	30,322	15.16
AG067	6.30	541	0.27	AG116	10.33	30,744	15.37
AG091	6.65	773	0.39	AG210	10.38	32,162	16.08
AG013	6.78	883	0.44	AG043	10.39	32,438	16.22
AG130	6.85	942	0.47	AG114	10.45	34,527	17.26
AG185	7.21	1,356	0.68	AG004	10.51	36,504	18.25
AG093	7.24	1,390	0.70	AG108	10.56	38,649	19.33
AG127	7.31	1,495	0.75	AG068	10.83	50,499	25.25
AG194	7.34	1,547	0.77	AG183	10.87	52,384	26.19
AG016	7.39	1,619	0.81	AG095	10.91	54,751	27.38
AG134	7.43	1,676	0.84	AG012	10.12	67,195	33.60
AG140	7.45	1,712	0.86	Unplanted	3.10	21	0.16
AG003	7.45	1,719	0.86	GS50a	7.76	2,340	2.20
AG006	9.91	20,095	10.05	Alpowa	8.75	6,296	4.54
AG079	9.92	20,387	10.19	LSD $_{(P=0.05)}$	1.63		

[a] Nematode counts (x) after 16 weeks of growth in the greenhouse were transformed by $log_e(x+1)$ before analysis.

[b] BTM represents back-transformations of $log_e(x+1)$ means after analysis.

[c] MR represents multiplication rate that was calculated by dividing the final population in soil plus roots by the initial population (900/lb of soil) for each entry.

Selection of resistant lines for use in wheat breeding programs

In the F_3 population of GS50a x Alpowa, 35 RILs showed a resistance level higher than or equivalent to the resistant parent GS50a, and 105 RILs showed a susceptible level higher than or equivalent to the susceptible parent Alpowa. Of these F_3 lines, 50 percent showed a resistant or susceptible level consistently higher than or equivalent to their parents in the testing of the F_2 population. The reaction of 46 F_3 RILs of GS50a x Alpowa to *P. thornei* is shown in Table 2. These resistant and susceptible lines will be used in bulk segregant analysis for molecular marker development. The highly resistant lines (with multiplication rate lower than one) obtained in this study will provide a better source of resistance for developing wheat varieties with improved genetic resistance to the root-lesion nematode *P. thornei*.

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