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RESEARCH

Identification of Resistance to *Pratylenchus neglectus* and *Pratylenchus thornei* in Iranian Landrace Accessions of Wheat

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ABSTRACT

The pathogenic nematodes *Pratylenchus neglectus* (Rensch, 1924) Filipjev and Schuurmans Stekhoven, 1941 and *Pratylenchus thornei* Sher and Allen, 1953 cause severe yield losses in wheat (*Triticum aestivum* L.). The objectives in this study were to assay a collection of Iranian landrace accessions collected from 12 provinces in Iran to identify novel sources of resistance to both species and to characterize agronomic traits critical for consideration in wheat breeding. Seventy-eight accessions were assayed for dual resistance to parasitic nematodes *P. neglectus* and *P. thornei* in controlled environment assays. Field trials conducted in Pullman, WA, and Pendleton, OR, evaluated stripe rust (*Puccinia striiformis* f. sp. *tritici*) resistance, days to heading, grain volume weight, plant height, seed protein content, seed kernel characterization, glume tenacity, and pubescence. The accessions were assayed with simple-sequence repeat (SSR), single-nucleotide polymorphism (SNP), and known vernalization markers for hierarchical cluster analysis to identify relatedness among accessions. Thirty-two accessions were identified as resistant or moderately resistant to both *Pratylenchus* species. Six were identified with moderate adult plant resistance to stripe rust in the field. The range of mean agronomic traits over locations was 53 to 105 cm for plant height, 46 to 84 d for post planting days to heading, and 151 to 728 kg m^{-3} for grain volume weight. The genetic cluster analysis identified three clusters based on the number of rare polymorphisms in the subset. The nematode resistance was distributed over the three clusters. The diversity within this subset could be useful for wheat breeders to integrate genetic variation and resistance to both *Pratylenchus* spp.

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Abbreviations: GLiM, generalized linear model; IT, infection type; lsmeans, least squared means; PCR, polymerase chain reaction; PNW, Pacific Northwest; SKCS, single-kernel characterization system; SNP, single-nucleotide polymorphism; SSR, simple-sequence repeat.

In 1935, the University of Tehran in Iran began collecting wheat accessions from all over the country and maintained them at the university. A total of 11,000 landrace accessions were collected and the province of origin recorded. The collection was transferred to the University of California at Davis through Calvin Qualset between 1986 and 1990, and 7000 accessions were rescued through greenhouse multiplication (C. Qualset, personal communication, 2015). Seed increases for each accession were later shared with curators Bent Skovmand at the International Maize and Wheat Improvement Center (CIMMYT), Mexico, and Harold Bockleman at the USDA National Small Grains Collection, Aberdeen Idaho (Dworkin, 2009).

Subsets of the Iranian collection have been phenotyped for resistance or tolerance to biotic and abiotic stresses. Accessions from these subsets were found to have increased root biomass (Waines and Ehdaie, 2007), tolerance to drought and heat stress (Denčić et al., 2000; Ehdaie et al., 1988), and increased tolerance to saline soils

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(Jafari-Shaberstari et al., 1995). Resistance to wheat diseases common bunt (*Tilletia tritici* and *T. laevis*), dwarf bunt (*Tilletia controversa*; Bonman et al., 2006), Russian wheat aphid (*Diuraphis noxia*; Bockelman and Haley, 2004; Ehdaie and Baker, 1999), and to root-lesion nematodes (*Pratylenchus thornei*; Sheedy and Thompson, 2009) have also been found in this collection. Landrace accessions originating from the Middle East curated by CIMMYT have also been found to have increased genetic variation that would be useful for increasing variation in breeding programs (Dreisigacker et al., 2005; Hoisington et al., 1999).

Root-lesion nematodes *P. neglectus* and *P. thornei* are soilborne pathogens that feed on wheat roots (Sheedy and Thompson, 2009; Thompson et al., 1999; Vanstone et al., 2008). These nematodes use a stylet to mechanically pierce cell walls, penetrate the root cortex, secrete enzymes, and extract cell contents (Townshend et al., 1989; Zunke, 1990). The damage to the root system limits water and nutrient uptake causing drought-like systems in the above ground biomass and will reduce grain yields and quality (Smiley et al., 2005a; Thompson et al., 1999, 2008b; Vanstone et al., 2008). The two *Pratylenchus* species are often found together in soil samples. In the Middle East, 56 to 61% of sampled fields found damaging populations of *P. neglectus* and *P. thornei* (Ghaderi et al., 2010; Greco et al., 1988; Mani and Al Hinai, 1996) while 95 to 96% of sampled fields in the Pacific Northwest (PNW) were found to have both species (Smiley et al., 2004; Strausbaugh et al., 2004).

In Australia and Mexico, yield reduction by *P. neglectus* has been reported as high as 35 and 70% for *P. thornei* (Nicol and Ortiz-Monasterio, 2004; Thompson et al., 2008b). Yield reduction in the PNW region of the United States has been reported as high as 35% by *P. neglectus* and 60% by *P. thornei* (Smiley and Machado, 2009; Smiley et al., 2005a,b). Yield loss to *Pratylenchus* infection can be reduced by tolerant and resistant wheat cultivars. Tolerance is the ability of a host to yield well despite nematode damage (Van Gundy et al., 1974), while resistance is the ability of a host to inhibit nematode reproduction (Smiley, 2009; Thompson et al., 2008b; Vanstone et al., 2008). Genetic resistance to one species of *Pratylenchus* is often not effective against the other species, (Farsi et al., 1995; Hollaway et al., 2000; Taylor et al., 2000; Zwart et al., 2005). Resistance to *P. neglectus* and *P. thornei* has been reported in IWA8608077, one of the accessions from the Iranian collection (Sheedy et al., 2007; Sheedy and Thompson, 2009; Thompson et al., 2008a). More sources of resistance to both species would be useful in breeding programs incorporating *Pratylenchus* resistance. More than 80 landrace accessions from the Iranian collection have been reported as resistant or moderately resistant to *P. thornei* but have not been tested for resistance to *P. neglectus* (Sheedy and Thompson, 2009).

The objectives in this paper were to (i) identify accessions from the Iranian landrace collection that are resistant to both *Pratylenchus* species by confirming *P. thornei* resistance and evaluating resistance to *P. neglectus*, (ii) evaluate agronomic and seed traits and multiple disease resistance traits of accessions to facilitate their potential use in wheat breeding programs, and (iii) assess the genetic variation in an Iranian landrace subset.

MATERIALS AND METHODS

Landrace Accessions and Controls

Although 92 wheat accessions were identified by Sheedy and Thompson (2009) as resistant or moderately resistant to *P. thornei*, 78 of these accessions were tested in this study because they were identified as *T. aestivum* and had been tested in multiple years by Sheedy and Thompson. The resistant accessions originated from 12 provinces in Iran (Table 1). The provinces listed in Table 1 are the names given at the original time of collection as received by the USDA National Small Grains Collection and do not reflect any recent changes.

Screening for nematode resistance is time consuming and variable (Schmidt et al., 2005; Sharma et al., 2011); therefore, all greenhouse experiments included a series of susceptible and resistant spring wheat cultivars, landrace accessions, and a synthetic hexaploid as controls with an unplanted check (13 total) (Table 2).

Nematode Resistance Assays

The nematode resistance assays were designed so that responses to the two nematode species were evaluated in a total of four experiments each. Separate populations of *P. neglectus* and *P. thornei* were increased and maintained on monoxenic carrot cultures. The carrot disks were prepared using a modified protocol described by Castillo et al. (1995). Cultures were kept in a growth cabinet (Percival Scientific) in the dark at 22°C.

Nematode Resistance Assay Experiments

Each experiment included the wheat accessions being evaluated (see below for details), the 12 controls (Table 2), and unplanted check. The accessions and controls were planted in D40H Deepots (Stuewe and Sons) containing 150 g of 3:1 pasteurized Palouse silt loam soil (fine-silty, mixed, superactive, mesic Pachic Ultic Haploxerolls) to sand plus four granules of Osmocote 14-14-14 slow release fertilizer (Scotts Co. LLC). All nematode experiments were conducted in a Conviron GR48 (Controlled Environments, LTD) controlled environmental chamber at 24°C with a 14-h day length. Two weeks after planting, each Deepot was inoculated with ~100 nematodes (*P. neglectus* or *P. thornei*) in water on the soil surface and then covered with 1 g of the 3:1 soil mix. Deepots were top-watered as needed. The unplanted check Deepot was filled with soil, not planted, and inoculated to determine if nematode populations were increasing in the absence of a host. This unplanted check was assayed for the presence of nematodes but the data were not included in the statistical analysis of results.

Experiment 1 for both species was designed as a completely random design of the 78 Iranian accessions, 12 controls, and unplanted check (91 treatments) with four replications. The randomization and plot numbers were the same for each species. The Deepots occupied four D98T (Stuewe and Sons) support racks per species. The eight center holes in the support

Table 1. A list of the Iranian landrace accessions used in the greenhouse and field trials. Accessions are listed in order with the corresponding identification numbers for the Australian Winter Cereals Collection (AWCC), International Maize and Wheat Improvement Center (CIMMYT), and National Small Grains Collection (NSGC). Provinces of origin are included as indicated by the NSGC.

Accession	AWCC	CIMMYT	NSGC	Origin
IWA8604094	AUS28290	CWI55599	PI627880	Markazi
IWA8604259	AUS28291	CWI55629	PI627947	Kordestan
IWA8604272	AUS28295	CWI55636	PI623425	Markazi
IWA8604394	AUS28297	CWI55665	PI623428	Eastern Azerbaijan
IWA8604409	AUS28298	CWI55668	PI623443	Markazi
IWA8604568	AUS28302	CWI55698	PI628045	Esfahan
IWA8604571	AUS28303	CWI55700	PI628047	Bakhtaran
IWA8604686	AUS28304	CWI55733	PI628100	Khorasan
IWA8604710	AUS28307	CWI55748	PI628116	Esfahan
IWA8604716	AUS28309	CWI55752	PI628120	Esfahan
IWA8604740	AUS28315	CWI55758	PI628132	Kerman
IWA8604765	AUS28321	CWI55769	PI628144	Khorasan
IWA8604782	AUS28323	CWI55776	PI628150	Zanjan
IWA8604794	AUS28325	CWI55781	PI628158	Khorasan
IWA8604807	AUS28326	CWI55787	PI628167	Yazd
IWA8604895	AUS28329	CWI55814	PI623452	Khorasan
IWA8606031	AUS28332	CWI55873	PI623459	Hamadan
IWA8606074	AUS28334	CWI55889	PI623467	Bakhtaran
IWA8606081	AUS28336	CWI55891	PI623473	Ilam
IWA8606083	AUS28338	CWI55893	PI623475	Ilam
IWA8606091	AUS28339	CWI55895	PI623481	Bakhtaran
IWA8606134	AUS28342	CWI55909	PI623501	Ilam
IWA8606188	AUS28349	CWI55939	PI623514	Western Azerbaijan
IWA8606229	AUS28355	CWI55953	PI623522	Western Azerbaijan
IWA8606267	AUS28369	CWI55979	PI623538	Western Azerbaijan
IWA8606270	AUS28372	CWI55982	PI623541	Western Azerbaijan
IWA8607438	AUS28375	CWI56750	PI623946	Bakhtaran
IWA8607542	AUS28387	CWI56829	PI624007	Bakhtaran
IWA8607547	AUS28389	CWI56831	PI624009	Bakhtaran
IWA8607575	AUS28391	CWI56853	PI624026	Bakhtaran
IWA8607576	AUS28392	CWI56854	PI624027	Bakhtaran
IWA8607766	AUS28399	CWI56946	PI624144	Hamadan
IWA8607776	AUS28400	CWI56952	PI624145	Hamadan
IWA8607818	AUS28401	CWI56969	PI624162	Bakhtaran
IWA8607820	AUS28402	CWI56970	PI624163	Kordestan
IWA8607866	AUS28407	CWI56991	PI624194	Bakhtaran
IWA8607871	AUS28408	CWI56996	PI624198	Bakhtaran
IWA8607923	AUS28409	CWI57039	PI624229	Bakhtaran
IWA8607958	AUS28410	CWI57066	PI624249	Ilam
IWA8607960	AUS28412	CWI57068	PI624251	Ilam
IWA8607961	AUS28413	CWI57069	PI624252	Hamadan
IWA8607962	AUS28414	CWI57070	PI624253	Hamadan
IWA8607963	AUS28415	CWI57071	PI624254	Hamadan
IWA8607995	AUS28426	CWI57091	PI624274	Bakhtaran
IWA8608010	AUS28430	CWI57099	PI624282	Bakhtaran
IWA8608014	AUS28433	CWI57102	PI624286	Bakhtaran
IWA8608064	AUS28442	CWI57123	PI624300	Eastern Azerbaijan
IWA8608074	AUS28448	CWI57131	PI624305	Eastern Azerbaijan
IWA8608077	AUS28451	CWI57134	PI621458	Eastern Azerbaijan
IWA8608080	AUS28452	CWI57136	PI624307	Eastern Azerbaijan
IWA8608082	AUS28453	CWI57137	PI624308	Eastern Azerbaijan
IWA8608147	AUS28469	CWI57174	PI624325	Kordestan
IWA8608152	AUS28470	CWI57176	PI624327	Kordestan
IWA8608177	AUS28475	CWI57190	PI624336	Kordestan
IWA8608767	AUS28631	CWI57547	PI624663	Kordestan
IWA8608802	AUS28638	CWI57573	PI624686	Bakhtaran
IWA8608819	AUS28642	CWI57586	PI624699	Bakhtaran
IWA8608830	AUS28644	CWI57596	PI624708	Bakhtaran
IWA8608846	AUS28649	CWI57607	PI624718	Bakhtaran
IWA8608909	AUS28666	CWI57653	PI624763	Bakhtaran
IWA8608911	AUS28667	CWI57655	PI624765	Bakhtaran
IWA8608915	AUS28668	CWI57657	PI624767	Bakhtaran
IWA8608928	AUS28674	CWI57666	PI624776	Bakhtaran
IWA8608938	AUS28677	CWI57672	PI624782	Bakhtaran
IWA8608982	AUS28685	CWI57701	PI624815	Ilam
IWA8608983	AUS28686	CWI57702	PI624816	Ilam
IWA8608990	AUS28687	CWI57706	PI624821	Ilam
IWA8608992	AUS28689	CWI57708	PI624823	Ilam
IWA8609012	AUS28693	CWI57722	PI624838	Ilam
IWA8609023	AUS28701	CWI57733	PI624849	Ilam
IWA8609031	AUS28703	CWI57741	PI624857	Ilam
IWA8609035	AUS28706	CWI57744	PI624860	Ilam
IWA8609036	AUS28707	CWI57745	PI624861	Ilam
IWA8609045	AUS28712	CWI57751	PI624867	Ilam
IWA8609049	AUS28714	CWI57755	PI624871	Kordestan
IWA8609061	AUS28723	CWI57764	PI624879	Kordestan
IWA8609064	AUS28725	CWI57766	PI624882	Kordestan
IWA8609076	AUS28728	CWI57773	PI624890	Kordestan

racks were left empty to prevent overcrowding. The four support racks were placed in one large flow tray (Stuewe and Sons). Experiment 1 for both species (Pn1, Pt1) was planted on 13 May 2011. Although both species were evaluated in the same growth chamber, they were isolated from each other because each flow tray caught all exudates from the Deepots within it. For Exp. 1 only, because our objective was to evaluate resistance to both species and because the phenotyping was costly, we combined the samples for the two species, by plot, before enumeration.

Soil and root samples were collected from each Deepot for all experiments. First, the plant tops were cut at the soil surface, then the entire 151 g of soil plus roots was removed from each Deepot, placed in individual plastic bags, stored overnight at 4°C and then sent to Western Laboratories Inc. (Parma, ID; http://www.westernlaboratories.com) for nematode extraction and enumeration. Western Labs uses a modified Oosterbrink elutriator extraction procedure described by Smiley et al. (2011). The protocol followed by Western Laboratories concentrates

Table 2. The list of controls for the greenhouse nematode and stripe rust assays. Each entry is identified with the corresponding response to both *Pratylenchus* sp. and strip rust, the origin of the entry, and the identification or registration number for each. The citations are given for each entry where the response was identified.

Entry name	Control response†			Origin	Germplasm type	Identification number	Citation
	Pratylenchus neglectus	*Pratylenchus thornei*	*Puccinia striiformis*				
Louise	S	S	HTAP§	PNW‡	Cultivar	PI634865	Kidwell et al., 2006a; Sheedy et al., 2007; Carter et al., 2009
Otis	S	S		PNW	Cultivar	PI634866	Kidwell et al., 2006b; Sheedy et al., 2007
Alpowa	S	S		PNW	Cultivar	PI566596	Sheedy et al., 2007
Iraq 43		R		Middle East	Landrace	AUS4926	Schmidt et al., 2005
Morocco 426		R		Middle East	Landrace	AUS13124	Schmidt et al., 2005
Persia 20	R			Middle East	Landrace	CI 11283	Das et al., 2004
CPI133872	R	R		CIMMYT	Synthetic	CIGM89.576	Zwart et al., 2005
GS50a		R		Australia	Breeding line	n/a	Thompson et al., 1999
Gatcher		S		Australia	Cultivar	W3720-W	Thompson et al., 1999
Seri (M82)		S		CIMMYT	Cultivar	CM33027	Sheedy et al., 2007
Excalibur	R			Australia	Cultivar	AUS99161	Williams et al., 2002
Janz	S	S		Australia	Cultivar	PI591910	Zwart et al., 2005
AUS28451	R	R		Middle East	Landrace	PI623470	Sheedy et al., 2007; Thompson et al., 2008a
Avocet			S	Australia	Cultivar	AUS20601	Yan et al., 2003
Hyak			R	PNW	Cultivar	PI511674	Chen, 2005
Lemhi			S	PNW	Cultivar	CI 11415	Chen and Line, 1992
Tyee			R	PNW	Cultivar	CI 17773	Chen and Line, 1992
Hank§			S	PNW	Cultivar	BZ 992-322	Lin and Chen, 2009

† S, susceptible control; *R*, resistant control; HTAP, high temperature adult plant resistance.

‡ PNW, Pacific Northwest, United States.

§ Control used in the field.

the final extracted volume of nematodes to ~10 mL of water and a single 1-mL aliquot is taken for nematode enumeration.

Experiment 2 was designed as a completely random design of 57 accessions that had been selected from Exp. 1 results based on lack of difference from the resistant check as defined with a Dunnett's test (see below for details). The 12 controls and unplanted check were also included for a total of 70 treatments with three replicates. The Deepots occupied three D98T support racks, again leaving the eight center holes empty. Any remaining spaces in the racks were filled with Deepots containing soil only to maintain even temperature and spacing within the support racks. These extra Deepots were not inoculated. As for Exp. 1, the support racks were placed in one large flow tray for each species. The *P. neglectus* Exp. 2 (Pn2) was planted on 3 Oct. 2011, and the *P. thornei* Exp. 2 (Pt2) was planted on 4 Oct. 2011. Again, although both species were evaluated in the same growth chamber, they were isolated from each other within each flow tray. Root and soil were kept separate for each species and plot in Exp. 2 through 4.

Experiments 3 and 4 were conducted using 46 accessions selected from Exp. 1 and 2 based on a Dunnett's test. The 12 controls and unplanted check were also included for a total of 59 treatments with one replication, planted as above. The 59 Deepots were placed randomly into one D98T support rack, leaving the eight center holes open and filling the remaining space with soil-only Deepots. A single rack was placed into a flow tray for each species. Experiments 3 and 4 for *P. neglectus* were planted on 9 July 2012 (Pn3) and 3 Aug. 2012 (Pn4), respectively. Experiments 3 and 4 for *P. thornei* were planted on 30 May 2012 (Pt3) and 7 June 2012 (Pt4), respectively.

Nematode Damage Ratings

For Exp. 3 and 4 only, roots were visually rated for nematode damage at harvest before extraction at 12 wk after inoculation. The rating scale was 1 to 5 where 1 = little to no root browning throughout the root system; 2 = approximately one-fourth of the total root system had browning; 3 = approximately one-half of the total root system had browning; 4 = approximately three-fourths of the total root system has browning; and 5 = entire root system had browning.

Statistical Analysis of Nematode Resistance

Because the nematode enumeration from Western Labs was count data and not distributed normally, the data were analyzed using a generalized linear model (GLiM) with a Poisson distribution and a log link function (Thall and Vail, 1990) with the SAS GENMOD procedure (version 9.4; SAS Institute, 2012). Experiments Pn1, Pt1, Pn2, and Pt2 were analyzed with the model as follows: $f(y) = \beta_0 + \beta_1 x_i + \beta_2 x_i + \varepsilon_i$, where $f(y)$ is the log transformation of y, the count data for each Deepot; β_1 is the entry (accessions under evaluation) as a fixed effect; β_2 is the replicate as a random effect, and ε_i is the error term. The least squared means (lsmeans) for each accession and controls were compared with the dual-resistant accession IWA8608077 (PI 621458) using a Dunnett's calculation (Dunnett, 1955). Accessions that were not significantly different from IWA8608077 at probability $P \geq 0.05$ were tested again in the next experiment. This statistical method was used because it is the more common way to analyze nematode

results across experiments (Sheedy and Thompson, 2009; Thompson et al., 2009). The models were tested for statistical assumptions of normality and heteroscedasticity.

The final 46 accessions and the 12 controls that were tested in experiments Pn1 through Pn4 and Pt1 through Pt4 were then analyzed using a GLiM model with a normal distribution link function. Because the number of replicates in each experiment varied, the replicates were nested within experiment with the model as follows: $f(y) = \beta_0 + \beta_1 x_i + \beta_2 x_{k(j)} + \varepsilon_{ijk}$, where $f(y)$ is the natural log transformation of y, the count data for each Deepot; β_1 is the entry (accession under evaluation) as a fixed effect; β_2 is the replicate nested within experiment as a random effect; and ε_{ijk} is the error term. The lsmeans for each accession and controls were compared with the dual-susceptible control cultivar Louise using a Dunnett's calculation. Accessions that were significantly different from Louise at probability $P \leq 0.05$ were considered to be resistant. The models were tested for statistical assumptions of normality and heteroscedasticity. The lsmeans were back transformed to better interpret the results below.

Broad-sense heritability (h^2 = Var(G)/Var(P)) on a plot basis was calculated with the asymptotic variance–covariance matrix (Self and Liang, 1987) for the final 46 accessions minus the controls from all four experiments for each nematode species using SAS code provided by Holland et al. (2003). The model was as follows: $f(y) = \beta_0 + \beta_1 x_i + \beta_2 x_{k(j)} + \varepsilon_{ijk}$, where $f(y)$ is the natural log transformation of y, the count data for each Deepot; β_1 is the entry (accession under evaluation) as a random effect; β_2 is the replicate nested in experiment as a random effect, and ε_{ijk} is the error term.

The root damage ratings for the final 46 accessions and controls from Pn3 and Pn4 and Pt3 and Pt4 were associated with nematode counts using a simple analysis of variance with the root rating as the independent effect and the count data as the dependent effect in the SAS GLM procedure. The model was as follows: $f(y) = \beta_0 + \beta_1 x_i + \varepsilon_i$, where $f(y)$ is the natural log transformation of y, the count data for each Deepot; β_1 is the root rating as a fixed effect; and ε_i is the error term. The transformed lsmeans were plotted against the root ratings for Pn3 and Pn4 and Pt3 and Pt4.

Agronomic Traits

Agronomic traits for each of the 78 accessions were recorded in two field experiments conducted at the Washington State University Spillman Agronomy Farm, Pullman, WA, in 2012 and 2014 and the Columbia Basin Agricultural Research Center at Pendleton, OR, in 2014. The experiments were planted on 9 May 2012 and 10 Apr. 2014 for Pullman and 3 Apr. 2014 for Pendleton. Each plot was a single head row, 0.76 m long. Plots were spaced 0.36 m apart, four plots across, with 0.5 m between sets of four plots. All 78 accessions plus spring wheat cultivars Zak (PI 607839), Eden (PI 630983), Calorwa (PI 566594), and Scarlet (PI 601814) as controls (82 treatments) were planted in a randomized complete block design with two replicates in 2012 and a single replicate in 2014 for each location. The cultivar Hank was included as the border for the Pullman location and Louise for the Pendleton location. Days to heading were recorded based on spike emergence from the boot for 50% of the plot; plant height was measured with a meter stick from the ground to the base of the tallest inflorescence postheading; presence of awns and pubescence were visually determined after heading. Each plot was hand harvested on 20 Sept. 2012 and 3 Sept. 2014 for Pullman and 25 Aug. 2014 for Pendleton and threshed using a Vogel thresher (custom made at Bill's Welding and Machine Shop, Pullman, WA).

Threshing ability was evaluated by deriving the difference between dirty- and clean-grain volume weights for the Pullman 2012 plots only. Dirty-grain volume weights were obtained directly after threshing using a Seedborough drop-funnel test-weight machine (Seedburough Equipment). Clean weights were obtained from the same samples after running the grain through a wire mesh with 0.5-mm spacing over a 60 hz Owosso continuous air blower (Owosso Corp.) using a custom-made gravity-based cleaner (Bill's Welding and Machine Shop, Pullman, WA). Samples with an increased difference between the two values were considered to have moderate or reduced threshing ability

Seed Traits

Seed traits were determined on the field-grown samples from the Pullman location only. Kernel color was visually evaluated postharvest for red or white seed coat. Kernel hardness was determined with a single-kernel characterization system (SKCS) 4100 single kernel characterization system set for 50-kernel averages (Perten Instruments). Grain protein, starch, and moisture content analyses were conducted with 20-g subsamples from each plot with an near-infrared grain analyzer (Perten Instruments). The instrument was set for a calibration specific to whole wheat that was developed by the USDA Western Wheat Quality Laboratory, with a specific weight module to determine whole seed content.

Statistical Analysis of Agronomic and Seed Traits

The agronomic traits days to heading, grain volume weight, and plant height were analyzed to obtain lsmeans across year, location, and replicates using SAS PROC MIXED with the model $Y = \beta_0 + \beta_1 x_i + \beta_2 x_i + \beta_3 x_{k(j)} + \varepsilon_{ijk}$, where Y is the trait value, β_1 is the entry as a fixed effect, β_2 is the location as a fixed effect, β_3 is the year nested in location as a fixed effect, and ε_{ijk} is the error term. The lsmeans were then plotted as histograms to visually show trait distributions within each location. The grain traits were analyzed as described above minus the location effect to obtain lsmeans and plotted as histograms.

Threshing ability for each entry from the Pullman 2012 location only was calculated as the difference between the dirty and clean test weight; the lsmeans for each entry were compared with the accession IWA8606229 using a Dunnett's comparison, with entries significantly different from IWA8606229 at probability $P \leq 0.10$ considered to have reduced threshing ability. Accession IWA8606229 was chosen because it was consistent between replicates and had less than 1 kg m^{-3} difference between dirty- and clean-grain volume weights. The adapted cultivars intended as controls matured much more quickly than the accessions and so were susceptible to bird damage and were not harvested.

Stripe Rust Resistance Assays

Stripe Rust Resistance in a Controlled Environment

Because stripe rust is a yield-limiting disease in several environments where these accessions might be used as sources of nematode resistance, a growth chamber experiment was

conducted to evaluate seedling resistance to stripe rust race PST 100 plus control cultivars; the stripe-rust-susceptible spring wheat cultivars Avocet (AUS20601) and Lemhi (CIltr 11415; Heyne, 1959) and the stripe rust resistant differential cultivars Tyee (YrTye; Cltr17773; Allan et al., 1980; Chen and Line, 1992) and Hyak (Yr17; PI511674; Allan et al., 1990; Chen, 2005; Table 2). The first three experiments included five replications of 50 entries (46 accessions from nematode Exp. 3 and 4 plus controls) planted in a completely random design. The entries were planted in Sunmix potting soil (SunGro Horticulture) in T-12-08H cell packs in holeless trays (East Jordan Plastics Inc.). Experiment 1 was planted on 8 May 2012, Exp. 2 on 22 May 2012, and Exp. 3 on 2 July 2012. Seedlings were inoculated 9 d after planting as described by Wu et al. (2009). Plants were bottom watered as needed and grown in a GR48 Conviron controlled environment chamber at 22°C with a 14-h day length. Each experiment was scored for stripe rust infection 2 wk after inoculation using the expanded infection type (IT) scale (1–9; Chen and Line, 1992). All 78 accessions were examined in Exp. 4 and 5, plus controls, designed and grown as above with five replicates each line and planted on 24 Oct. 2014 and 24 Nov. 2014, respectively.

Stripe Rust Resistance in a Field Environment

Stripe rust was also evaluated in the field under natural infection in the replicated trials at Spillman in 2012 and 2014. The response to stripe rust was recorded using the IT scale (1–9) and a percentage plot severity on 6 July 2012 and 13 Aug. 2014 and compared with the susceptible spring wheat cultivar Hank (PVP200000191).

Statistical Analysis of Stripe Rust Resistance

Resistance to stripe rust was analyzed with a GLiM using the SAS GLIMMIX procedure with a normal distribution link function (Golub and Welsch, 1969). The GLIMMIX model was as follows: $f(\gamma) = \beta_0 + \beta_1 x_i + \beta_2 x_{k(j)} + \varepsilon_{ijk}$, where $f(\gamma)$ is the IT score for each entry, β_1 is the entry as a fixed effect, β_2 is the replicate nested within experiment as a random effect for greenhouse experiments or replicate nested within year for field experiments, and ε_{ijk} is the error term. The lsmeans for each entry were compared with susceptible Avocet for greenhouse experiments and Hank for field experiments using a Dunnett's calculation. Entries significantly different from Avocet or Hank at probability $P \leq 0.05$ were considered to be resistant. Broad-sense heritability on a plot basis for the 46 accessions evaluated for stripe rust resistance in all trials was calculated as above.

Genotype Analysis

Accessions were genotyped with markers linked to the major developmental loci (*Vrn-1* and *Ppd*-1), 37 SSR, and 26 SNP markers from the wheat 9K Illumina SNP chip (Cavanagh et al., 2013) to determine relationships among accessions (Supplemental Table S1). A single plant of each accession was grown in the greenhouse with similar day length, temperature, and fertility to that described above for the nematode trials. Leaf tissue was harvested at Zadoks stage 31 (first node detected) ~1 mo after planting. DNA was extracted using an in-house Sarkosyl lysis buffer based protocol (5 M Tris-Cl [pH 7.5], 1 M EDTA [pH 8.5], 0.25 M NaCl, 20% SDS).

Polymerase chain reactions (PCRs) for the SSR markers totaled 15 μL and contained 1 μL of DNA template (75 ng μL^{-1}), 1 unit of Taq polymerase (in-house), 250 μM dNTPs, 0.45 μM combine primer, 0.05 μM M13 dye tag, 1× PCR buffer with 1.5 mM $MgCl_2$, 1.5 mM extra $MgCl_2$, and 5 μL of mineral oil (Amresco). Amplification was performed in a GeneAmp PCR system (Life Technologies) or a T100 thermocycler (Bio-Rad) as follows: 94°C for 5 min then 42 cycles of 94°C for 1 min, designated annealing temperature for 1 min, 72°C for 1 min, then a final extension at 72°C for 10 min. The protocols for *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* primers were as described by Fu et al. (2005) and Yan et al. (2004) and those for the *Ppd-D1* primers were as in Beales et al. (2007; Supplemental Table S2).

Products were visualized using 2% agarose gels for the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Ppd-D1* and a LiCOR IR^2 DNA analyzer (LiCor Biosciences) for the SSR products. The SNPs were analyzed following the iPLEX Gold protocol (Agena BioScience) using SNPs identified by Aaron Carter and Deven See (Washington State University and USDA–ARS, Pullman, WA). The SNP data was acquired using a Sequenom MassARRAY Analyzer 4 (Sequenom Inc.).

Genotypic Cluster Analysis

Visualized bands from the SSR markers were scored as different polymorphisms when there were at least five base pair differences between bands or different banding patterns. The assessed polymorphisms for each SSR marker were assigned numbers to designate the polymorphisms for the cluster analysis. For each marker, rare polymorphisms with a frequency of <10% within the population were assigned the largest values, while common polymorphisms were given smaller values. Single-nucleotide polymorphisms were called as either AT, CG, or heterozygous for each marker then assigned a number to designate the SNPs for the cluster analysis. The Ward's minimum variance method (Ward, 1963) was used to construct hierarchical clusters among the 78 accessions using the CLUSTER procedure in SAS. A dendogram was constructed using the TREE procedure in SAS. The optimal number of clusters was determined using the semipartial R^2 value, R^2 value, pseudo-F, and pseudo-T^2 statistic criteria.

RESULTS

Nematode Resistance Assays

At the completion of the first experiment, 20 accessions had increased lsmeans for combined counts of *P. neglectus* and *P. thornei* when compared with the dual-resistant accession IWA8608077 (Table 3) and were not tested in Exp. 2. The accessions IWA8604765 and IWA8608982 were also removed from the subset because the majority of the plants died during the experiment. At the completion of Exp. 2, another 19 accessions were identified with significantly increased lsmeans for counts of *P. neglectus* or *P. thornei* when compared with IWA8608077 (Table 4) and were dropped for Exp. 3 and 4. Accessions IWA8608938, IWA8608819, IWA8607576, IIWA8608802, and IWA8608983 either failed to germinate or died in Exp. 2 and were also dropped for Exp. 3 and 4. The accessions IWA8604686, IWA8604710, IWA8606229, IWA8608911,

Table 3. The 78 accessions screened for resistance to *Pratylenchus neglectus* and *P. thornei* in Exp. 1 with controls. The least squared means (lsmeans) were determined across all four replicates, combined over species, per accession for the count data with the corresponding back-transformed mean (BTM) of the natural log. Accessions were compared using Dunnett's method to the dual resistant accession IWA8608077.

Entry	lsmeans	BTM (ln*x* + 1)	*P*-value	Entry	lsmeans	BTM (ln*x* + 1)	*P*-value
Louise	3.80	121.28	0.000	IWA8607776	0.01	2.76	0.005
Otis	2.73	41.54	0.050	IWA8607818	2.10	22.11	1.000
Alpowa	0.01	2.76	0.005	IWA8607820	3.02	55.82	0.001
Iraq 43	3.12	61.65	0.000	IWA8607866	0.01	2.76	0.005
Morocco 426	0.01	2.76	0.005	IWA8607871	0.01	2.76	0.005
Persia 20	2.10	22.11	1.000	IWA8607923	0.07	2.92	0.016
CPI133872	0.01	2.76	0.005	IWA8607958	2.10	22.11	1.000
GS50a	0.01	2.76	0.005	IWA8607960	0.01	2.76	0.005
Gatcher	0.01	2.76	0.005	IWA8607961	0.01	2.76	0.005
Seri	0.01	2.76	0.005	IWA8607962	0.01	2.75	0.012
Excalibur	0.01	2.76	0.005	IWA8607963	2.73	41.54	0.050
Janz	1.74	15.41	0.871	IWA8607995	0.01	2.76	0.005
IWA8608077	2.10	22.11	†	IWA8608010	2.10	22.11	1.000
IWA8604094	0.01	2.76	0.005	IWA8608014	2.10	22.11	1.000
IWA8604259	0.01	2.76	0.005	IWA8608064	0.01	2.76	0.005
IWA8604272	2.74	42.21	0.044	IWA8608074	0.01	2.76	0.005
IWA8604394	0.07	2.92	0.008	IWA8608080	2.10	22.11	1.000
IWA8604409	0.01	2.76	0.005	IWA8608082	2.73	41.54	0.050
IWA8604568	2.74	42.21	0.044	IWA8608147	0.01	2.76	0.005
IWA8604571	3.96	142.05	0.000	IWA8608152	2.74	42.21	0.044
IWA8604686	2.10	22.11	1.000	IWA8608177	3.12	61.65	0.000
IWA8604710	0.01	2.76	0.005	IWA8608767	3.41	82.41	0.000
IWA8604716	3.96	142.05	0.000	IWA8608802	2.10	22.11	1.000
IWA8604740	2.74	42.21	0.044	IWA8608819	0.01	2.76	0.005
IWA8604765	2.10	22.11	1.000	IWA8608830	0.01	2.76	0.005
IWA8604782	3.12	61.65	0.000	IWA8608846	0.01	2.76	0.005
IWA8604794	0.01	2.76	0.005	IWA8608909	0.06	2.88	0.009
IWA8604807	0.01	2.76	0.005	IWA8608911	0.01	2.76	0.005
IWA8604895	0.01	2.76	0.005	IWA8608915	2.10	22.11	1.000
IWA8606031	0.01	2.76	0.005	IWA8608928	0.01	2.76	0.005
IWA8606074	0.01	2.76	0.005	IWA8608938	0.01	2.76	0.005
IWA8606081	3.04	56.74	0.001	IWA8608982	2.38	29.28	0.951
IWA8606083	2.10	22.11	1.000	IWA8608983	0.07	2.92	0.008
IWA8606091	2.10	22.11	1.000	IWA8608990	0.01	2.76	0.005
IWA8606134	2.10	22.11	1.000	IWA8608992	2.97	53.02	0.004
IWA8606188	0.01	2.76	0.005	IWA8609012	2.74	42.21	0.044
IWA8606229	2.10	22.11	1.000	IWA8609023	2.73	41.54	0.050
IWA8606267	0.01	2.76	0.005	IWA8609031	2.73	41.54	0.050
IWA8606270	2.10	22.11	1.000	IWA8609035	0.01	2.76	0.005
IWA8607438	0.01	2.76	0.005	IWA8609036	3.62	101.17	0.000
IWA8607542	0.01	2.76	0.005	IWA8609045	3.11	60.97	0.000
IWA8607547	0.01	2.76	0.005	IWA8609049	0.01	2.76	0.005
IWA8607575	0.01	2.76	0.005	IWA8609061	0.01	2.76	0.005
IWA8607576	0.01	2.76	0.005	IWA8609064	0.01	2.76	0.005
IWA8607766	2.90	49.31	0.026	IWA8609076	2.10	22.11	1.000

† Control for Dunnett's comparison.

IWA8609061, and IWA8609064 were considered to be more susceptible to *P. neglectus* than IWA8608077 in Exp. 2 but not in Exp. 1. These conflicting results were reevaluated in Exp. 3 and 4.

The smallest range between low and high counts occurred in Exp. 1 (2.75–142.05 on a back-transformed scale; Table 3). The range for *P. neglectus* in Exp. 2 was 2.40 to 1233.61 and 1.86 to 130.73 for *P. thornei* on a

Table 4. The 57 accessions screened in Exp. 2 and controls. The least squared means (lsmeans) were determined across all three replicates with the corresponding back-transformed mean (BTM) of the natural log. Accessions were compared using Dunnett's method to the dual resistant accession IWA8608077.

Entry	*Pratylenchus neglectus*			*Pratylenchus thornei*		
	lsmeans	BTM (ln*x* + 1)	*P*-value	lsmeans	BTM (ln*x* + 1)	*P*-value
Louise	0.02	2.66	1.000	2.16	23.53	0.061
Otis	0.06	2.55	1.000	0.16	2.33	0.744
Alpowa	4.02	151.05	0.003	0.19	3.28	0.963
Iraq 43	4.50	244.40	0.000	2.16	23.53	0.061
Morocco 426	2.81	45.16	0.114	0.19	3.28	0.963
Persia 20	0.02	2.66	1.000	2.16	23.53	0.061
CPI133872	0.02	2.66	1.000	0.19	3.28	0.963
GS50a	2.83	46.05	0.109	0.12	2.42	0.768
Gatcher	2.81	45.16	0.114	0.04	2.61	0.806
Seri	3.22	68.18	0.040	3.87	130.73	0.000
Excalibur	2.17	23.91	0.407	0.38	1.86	0.659
Janz	3.86	128.70	0.005	n/a†	n/a	n/a
IWA8608077	0.22	3.39	‡	0.25	3.47	‡
IWA8604094	5.23	509.18	0.000	0.16	2.33	0.744
IWA8604259	0.14	3.13	1.000	0.04	2.61	0.806
IWA8604394	5.22	500.25	0.000	0.19	3.28	0.963
IWA8604409	4.64	280.34	0.000	0.04	2.61	0.806
IWA8604686	3.14	62.87	0.049	2.16	23.53	0.061
IWA8604710	4.07	159.40	0.002	0.16	2.33	0.744
IWA8604794	0.22	3.39	1.000	0.04	2.61	0.806
IWA8604807	2.68	39.50	0.159	0.19	3.28	0.963
IWA8604895	0.13	2.40	1.000	0.25	3.47	1.000
IWA8606031	0.06	2.55	1.000	0.19	3.28	0.963
IWA8606074	2.99	54.02	0.074	0.04	2.61	0.806
IWA8606083	5.59	725.60	0.000	n/a	n/a	n/a
IWA8606091	2.17	23.91	0.407	0.19	3.28	0.963
IWA8606134	2.17	23.91	0.407	0.19	3.28	0.963
IWA8606188	0.02	2.66	1.000	0.04	2.61	0.806
IWA8606229	3.21	67.30	0.041	0.16	2.33	0.744
IWA8606267	0.13	2.40	1.000	0.12	2.42	0.768
IWA8606270	0.06	2.55	1.000	0.19	3.28	0.963
IWA8607438	6.12	1233.61	0.000	2.16	23.53	0.061
IWA8607542	0.22	3.39	1.000	0.16	2.33	0.744
IWA8607547	3.22	68.18	0.040	0.19	3.28	0.963
IWA8607575	0.13	2.40	1.000	0.19	3.28	0.963
IWA8607576	n/a	n/a	n/a	2.16	23.53	0.061
IWA8607766	3.90	133.71	0.005	0.25	3.47	1.000
IWA8607776	2.83	46.04	0.109	0.25	3.47	1.000
IWA8607818	4.32	203.67	0.001	0.25	3.47	1.000
IWA8607866	0.07	2.90	1.000	0.25	3.47	1.000
IWA8607871	2.88	48.46	0.101	3.52	91.99	0.001
IWA8607923	1.64	14.02	0.828	0.19	3.28	0.963
IWA8607958	2.34	28.34	0.303	0.19	3.28	0.963
IWA8607960	3.29	72.61	0.033	n/a	n/a	n/a
IWA8607961	0.06	2.55	1.000	2.75	42.68	0.014
IWA8607962	2.34	28.34	0.303	0.19	3.28	0.963
IWA8607995	0.06	2.55	1.000	2.41	30.24	0.035
IWA8608010	0.07	2.90	1.000	0.04	2.61	0.806
IWA8608014	0.14	3.13	1.000	0.04	2.61	0.806
IWA8608064	0.14	3.13	1.000	0.19	3.28	0.963
IWA8608074	0.22	3.39	1.000	0.25	3.47	1.000
IWA8608080	0.13	2.40	1.000	0.04	2.61	0.806
IWA8608147	0.06	2.55	1.000	0.19	3.28	0.963
IWA8608802	n/a	n/a	n/a	n/a	n/a	n/a
IWA8608819	0.02	2.66	1.000	0.25	3.47	1.000
IWA8608830	0.02	2.66	1.000	0.25	3.47	1.000
IWA8608846	0.06	2.55	1.000	0.19	3.28	0.963
IWA8608909	0.07	2.90	0.259	0.04	2.61	0.806
IWA8608911	2.44	31.17	0.041	0.19	3.28	0.963
IWA8608915	3.21	67.30	0.101	n/a	n/a	n/a
IWA8608928	2.88	48.46	1.000	0.19	3.28	0.963
IWA8608938	0.02	2.66	1.000	0.19	3.28	0.963
IWA8608983	n/a	n/a	n/a	n/a	n/a	n/a
IWA8608990	0.02	2.66	1.000	0.04	2.61	0.806
IWA8609035	0.14	3.13	1.000	0.04	2.61	0.806
IWA8609049	2.62	37.16	0.181	0.19	3.28	0.963
IWA8609061	3.34	76.45	0.029	0.19	3.28	0.963
IWA8609064	3.44	85.11	0.021	0.04	2.61	0.806
IWA8609076	5.64	765.86	0.000	0.19	3.28	0.963

† n/a, not available.

‡ Control for Dunnett's comparison.

back-transformed scale (Table 4). The largest range of counts occurred in Exp. 3 for both species at 1 to 10,164 for *P. neglectus* and 1 to 7,200 for *P. thornei* (data not shown). The count range for *P. neglectus* in Exp. 4 was 1 to 5,220 and 1 to 1,240 for *P. thornei* (these are true counts, as only one replicate was evaluated in Exp. 3 and 4). The nematode counts for the unplanted check Deepots ranged from 0 to 30 for Exp. 1, 2, and 4 for both species. In Exp. 3 the counts in the unplanted checks were 180 for *P. neglectus* and 120 for *P. thornei* but were not a significant increase from initial inoculation (data not shown).

Over all experiments, nine accessions had reduced lsmeans for counts of *P. neglectus*, while 29 accessions had reduced lsmeans for *P. thornei* compared with dual-susceptible Louise and were considered resistant. Seven of these accessions had significantly reduced lsmeans for counts of *P. neglectus* and *P. thornei* and were scored as dual resistant (Table 5). The three accessions with the most consistent resistance to *P. neglectus* were IWA8607575, IWA8608010, and IWA8607547 and IWA8608830, IWA8608846, and IWA8609064 for *P. thornei*. Only one accession, IWA8607766, had increased lsmeans for both species and designated as dual susceptible (Table 5). The heritability estimates were 68.7 and 41.6% for *P. neglectus* and *P. thornei*, respectively (Table 6).

Table 5. The 46 accessions, in order, screened in all four experiments plus controls. The least squared means (lsmeans) were determined across all experiments with the corresponding back-transformed mean (BTM) of the natural log. The average root ratings were calculated from Exp. 3 and 4. Accessions were compared with susceptible control 'Louise' using Dunnett's method. The score is a reference assigned by the authors to interpret the *P*-value and the corresponding BTM data.

	Pratylenchus neglectus					*Pratylenchus thornei*				
Entry	lsmeans	BTM (ln*x* + 1)	*P*-value	Average rate	Score†	lsmeans	BTM (ln*x* + 1)	*P*-value	Average rate	Score†
Louise	3.73	113.2	‡	3	S	3.39	80.6	‡	3	S
Alpowa	3.51	90.7	0.756	5	S	3.32	74.9	0.916	3	S
Otis	2.99	53.9	0.318	4	MS	1.73	15.3	0.017	4	R
Iraq43	4.62	274.5	0.215	3	S	3.31	74.5	0.908	3	S
Morocco 426	3.03	56.1	0.312	3	MS	1.82	16.7	0.024	3	R
Persia20	2.74	42.2	0.155	3	MR	2.89	49.1	0.462	3	MS
CPI133872	2.06	21.4	0.017	2	R	2.19	24.3	0.085	3	MR
GS50a	3.09	60.0	0.360	4	MS	1.53	12.5	0.008	2	R
Gatcher	3.03	56.4	0.315	4	MS	2.47	32.2	0.174	4	MR
Seri	2.91	49.9	0.238	3	MR	3.67	106.9	0.674	3	S
Excalibur	2.73	41.9	0.152	3	MR	2.33	28.1	0.144	3	MR
Janz	3.68	108.0	0.948	4	S	1.90	18.1	0.049	2	R
IWA8604259	2.51	33.6	0.090	4	MR	2.24	25.6	0.089	3	MR
IWA8604686	3.73	112.9	0.997	2	S	2.58	35.9	0.245	1	MR
IWA8604710	2.91	49.8	0.251	4	MS	2.14	23.1	0.073	2	MR
IWA8604794	2.58	36.0	0.123	5	MR	1.75	15.7	0.019	3	R
IWA8604807	2.67	39.1	0.152	2	MR	1.63	13.9	0.012	2	R
IWA8604895	2.53	34.2	0.107	2	MR	1.93	18.7	0.054	3	R
IWA8606031	2.64	37.9	0.141	3	MR	1.88	17.8	0.037	1	R
IWA8606074	2.97	53.2	0.276	4	MS	2.29	26.8	0.094	2	MR
IWA8606091	3.33	75.9	0.565	4	S	1.87	17.6	0.029	2	R
IWA8606134	3.32	75.5	0.559	4	S	2.26	26.2	0.119	2	MR
IWA8606188	2.39	29.5	0.053	3	R	1.45	11.6	0.004	2	R
IWA8606229	3.63	102.9	0.898	3	S	2.35	28.6	0.152	2	MR
IWA8606267	2.18	24.1	0.047	4	R	2.30	27.0	0.117	2	MR
IWA8606270	2.85	47.0	0.220	4	MR	2.30	27.0	0.130	2	MR
IWA8607542	2.53	34.0	0.122	3	MR	1.49	12.1	0.007	2	R
IWA8607547	2.06	21.3	0.016	3	R	1.98	19.7	0.051	2	R
IWA8607575	1.33	10.2	0.001	3	R	1.30	10.0	0.003	1	R
IWA8607576	2.46	31.8	0.153	5	MR	1.36	10.6	0.003	1	R
IWA8607766	3.95	140.6	0.780	2	S	3.13	62.1	0.763	3	S
IWA8607776	2.62	37.4	0.136	3	MR	1.96	19.3	0.074	2	MR
IWA8607818	4.38	216.0	0.384	5	S	2.34	28.1	0.163	2	MR
IWA8607866	2.49	32.8	0.111	2	MR	2.03	20.7	0.073	4	MR
IWA8607871	2.58	36.0	0.123	2	MR	2.12	22.6	0.068	2	MR
IWA8607923	2.77	43.3	0.216	3	MR	1.65	14.2	0.031	5	R
IWA8607958	3.20	66.8	0.447	4	MS	1.35	10.5	0.004	1	R
IWA8607961	2.20	24.4	0.049	3	R	2.20	24.6	0.100	3	MR
IWA8607962	2.89	48.9	0.258	3	MS	1.95	19.2	0.047	2	R
IWA8607995	2.70	40.3	0.149	4	MR	1.96	19.3	0.040	2	R
IWA8608010	1.75	15.6	0.008	2	R	1.67	14.4	0.014	2	R
IWA8608014	2.78	43.8	0.185	3	MR	1.38	10.8	0.003	1	R
IWA8608064	2.61	37.0	0.133	2	MR	1.19	9.0	0.003	4	R
IWA8608074	2.47	32.1	0.090	4	MR	1.93	18.7	0.054	2	R
IWA8608077	2.77	43.3	0.216	2	MR	2.33	28.1	0.144	1	MR
IWA8608080	2.89	48.8	0.239	3	MR	2.18	24.0	0.082	2	MR
IWA8608147	2.34	28.2	0.053	3	R	1.88	17.8	0.037	3	R
IWA8608830	2.37	29.1	0.058	4	MR	0.84	6.3	0.000	1	R
IWA8608846	2.56	35.1	0.115	3	MR	1.28	9.8	0.004	2	R
IWA8608909	2.51	33.5	0.101	4	MR	1.75	15.7	0.019	4	R

(cont'd.)

Table 5. Continued.

Entry	*Pratylenchus neglectus* lsmeans	*Pratylenchus neglectus* BTM (ln*x* + 1)	*Pratylenchus neglectus* *P*-value	*Pratylenchus neglectus* Average rate	*Pratylenchus neglectus* Score†	*Pratylenchus thornei* lsmeans	*Pratylenchus thornei* BTM (ln*x* + 1)	*Pratylenchus thornei* *P*-value	*Pratylenchus thornei* Average rate	*Pratylenchus thornei* Score†
IWA8608911	2.94	51.5	0.271	2	MS	1.41	11.1	0.005	2	R
IWA8608928	2.13	22.9	0.032	3	R	1.48	11.9	0.006	2	R
IWA8608990	2.37	29.1	0.051	3	R	1.49	12.0	0.005	2	R
IWA8609035	2.48	32.6	0.082	3	MR	1.84	17.1	0.026	5	R
IWA8609049	2.67	39.4	0.156	3	MR	1.77	16.0	0.025	1	R
IWA8609061	3.01	54.9	0.312	3	MS	2.12	22.7	0.079	2	MR
IWA8609064	2.89	49.0	0.260	4	MS	1.00	7.4	0.000	1	R
IWA8609076	3.47	87.1	0.714	3	S	1.28	9.8	0.004	1	R

† R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

‡ Control for Dunnett's comparison.

Table 6. The broad-sense heritability (h^2) estimates from the final 46 accessions for the nematode and stripe rust assays. Estimates were made using the transformed count data for all four experiments for each nematode species and the infection-type scores from all four experiments for the stripe rust.

Assay†	h^2	SE
	%	
Pratylenchus neglectus	68.7	0.1105
Pratylenchus thornei	41.6	0.1279
Stripe rust_C	32.1	0.0886
Stripe rust_F	58.9	0.2487

† C, greenhouse environment; F, field environment.

Table 7. The analysis of variance (ANOVA) results for Exp. 3 and 4 of the nematode greenhouse assays. The model associates the natural log transformed nematode counts for each species with the root rating as a fixed effect.

Source	DF	Sum of squares	Mean square	*F*-value	*P*-value > *F*
Pratylenchus neglectus					
Model	1	17.97	17.97	3.49	0.065
Error	88	453.58	5.15		
Corrected Total	89	471.55			
Pratylenchus thornei					
Model	1	14.61	14.61	2.43	0.123
Error	88	529.43	6.02		
Corrected Total	89	544.03			

In Exp. 3 and 4, root ratings were assessed before nematode extraction and enumeration. In the *P. neglectus* experiments, no accessions averaged a root rating score of 1 (little to no root browning), while the majority (20 accessions) averaged a score of 3 (approximately one-half of the total root system had browning). In the *P. thornei* experiments, 11 accessions averaged a root rating score of 1, and the majority (24 accessions) averaged a score of 2. The seven accessions identified as dual resistant had average root scores ranging from 2 to 3 for *P. neglectus* and 1 to 3 for *P. thornei*. The single dual-susceptible accession had average root rating scores of 2 and 3 for *P. neglectus* and *P. thornei*, respectively (Table 6). The root rating categories were associated with the variation in the count data on the log scale at $P = 0.065$ for *P. neglectus* and $P = 0.123$ for *P. thornei*, indicating a portion of the variance in the count data could be predicted by the root rating (Table 7). The data, plotted as scatter or box plots, show a moderate negative linear relationship between the *P. neglectus* transformed lsmeans and root ratings and moderate positive linear relationship with *P. thornei* (Supplemental Fig. S1).

Agronomic and Seed Traits

The grain volume weights ranged from 164.6 to 728.2 kg m^{-3} for the Pullman location and 101.5 to 827.0 kg m^{-3} for the Pendleton location (Fig. 1a). Heading dates ranged from 50 to 85 d after planting for Pullman and 44 to 72 d for Pendleton (Fig. 1b). Plant height ranged from 70 to 105 cm for Pullman and 50 to 97 cm for Pendleton; some lodging occurred in the taller accessions (Fig. 1c). The data analysis showed that location had a significant effect (Supplemental Table S3), so histograms are split out by location. Nine accessions from the Pendleton location did not produce enough grain at harvest to get an accurate grain volume weight (data not shown). One accession, IWA8604807, failed to elongate at both locations and was classified as a winter type (Supplemental Table S4). Thirty-one accessions had pubescence of the leaves or heads and six had awnless head types (Supplemental Table S4).

The difference between dirty- and clean-grain volume weight was significantly different than the free threshing control accession for 12 accessions, which were considered to have reduced threshing ability (Supplemental Table S5).

Grain protein content ranged from 11.3 to 16.9% for the Pullman location across both years (Fig. 2b). The SKCS values ranged from 8.3 to 71.4, indicating there are both soft and hard seed classes in this subset (Fig. 2d). The SKCS values can be broken into market class categories as hard or mixed (SKCS $\geq$ 40), soft (SKCS $\leq$ 39), and super soft (SKCS < 13) according the Western Wheat Quality Laboratory standards at Washington State University. The

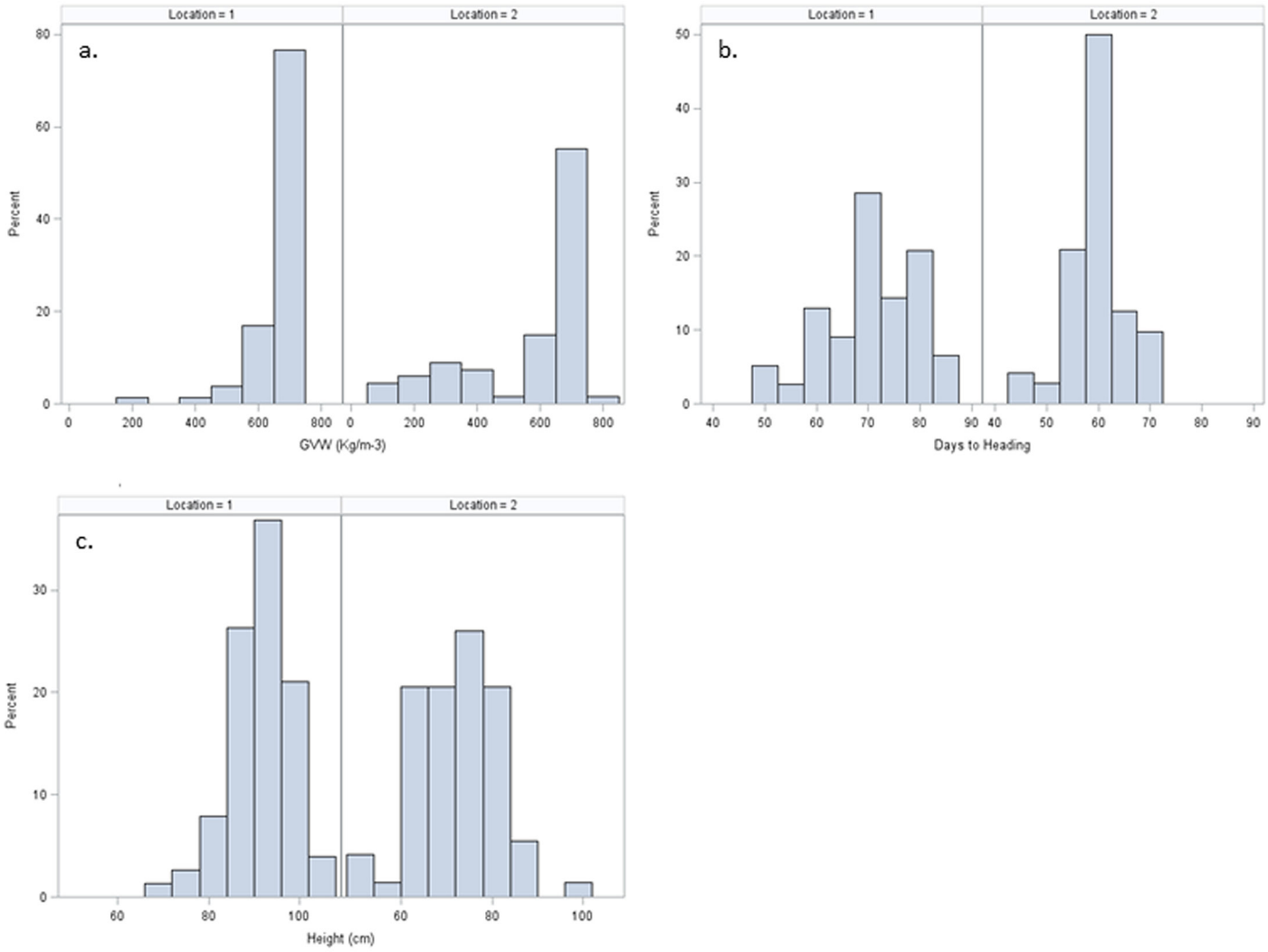

Fig. 1. Histograms showing the distribution of agronomic traits collected in 2012 and 2014 at the Pullman and Pendleton locations. Location 1 indicates the Pullman distribution and Location 2 indicates the Pendleton distribution for the (a) grain volume weight, (b) days to heading, and (c) plant height traits.

visual rating of kernel color found 41 red and 37 white kernelled accessions (Supplemental Table S4).

Stripe Rust Assays

Seedling emergence varied greatly throughout all five stripe rust experiments, making it difficult to obtain consistent rates of infection by the stripe rust. Therefore, the broad-sense heritability estimate for seedling stripe rust was low at 32.1% (Table 6). The IT scores for the resistant controls, Hyak and Tyee, were 2.04 and 3.23, respectively, while the IT scores for the susceptible controls, Avocet and Lemhi, were 5.73 and 5.19 respectively across all five experiments (Table 8). Although the moderate scores for the susceptible checks indicated some accessions may have been rated resistant as a result of escape in some of the experiments, the lsmeans of the IT scores ranged from 1.05 to 7.57 across all five seedling experiments. Three accessions had IT scores lower than 3, were significantly different from the susceptible control, and designated as resistant. Accessions with IT scores below 4, but not significantly different (19), were designated moderately resistant or susceptible.

The stripe rust races in the field under natural infection were determined as predominantly PSTv-4, PSTv-11, and PSTv-37 with PSTv-46 and PSTv-53 in lower frequencies (Anmin Wan, USDA–ARS Plant Pathology, Washington State University, personal communication, 2014). The lsmeans for the susceptible control Hank were 6.72 for the IT score and 81.92 for the percentage severity (Table 8). Only one accession, IWA8608928, had an IT score below 3 that was significantly different than the control and designated as resistant. Five accessions were designated as moderately resistant with significant *P*-values but with IT scores ranging from 3.91 to 4.91. The remaining accessions were scored as susceptible (Table 8). The broad-sense heritability estimate for stripe rust under field conditions was 58.9% (Table 6).

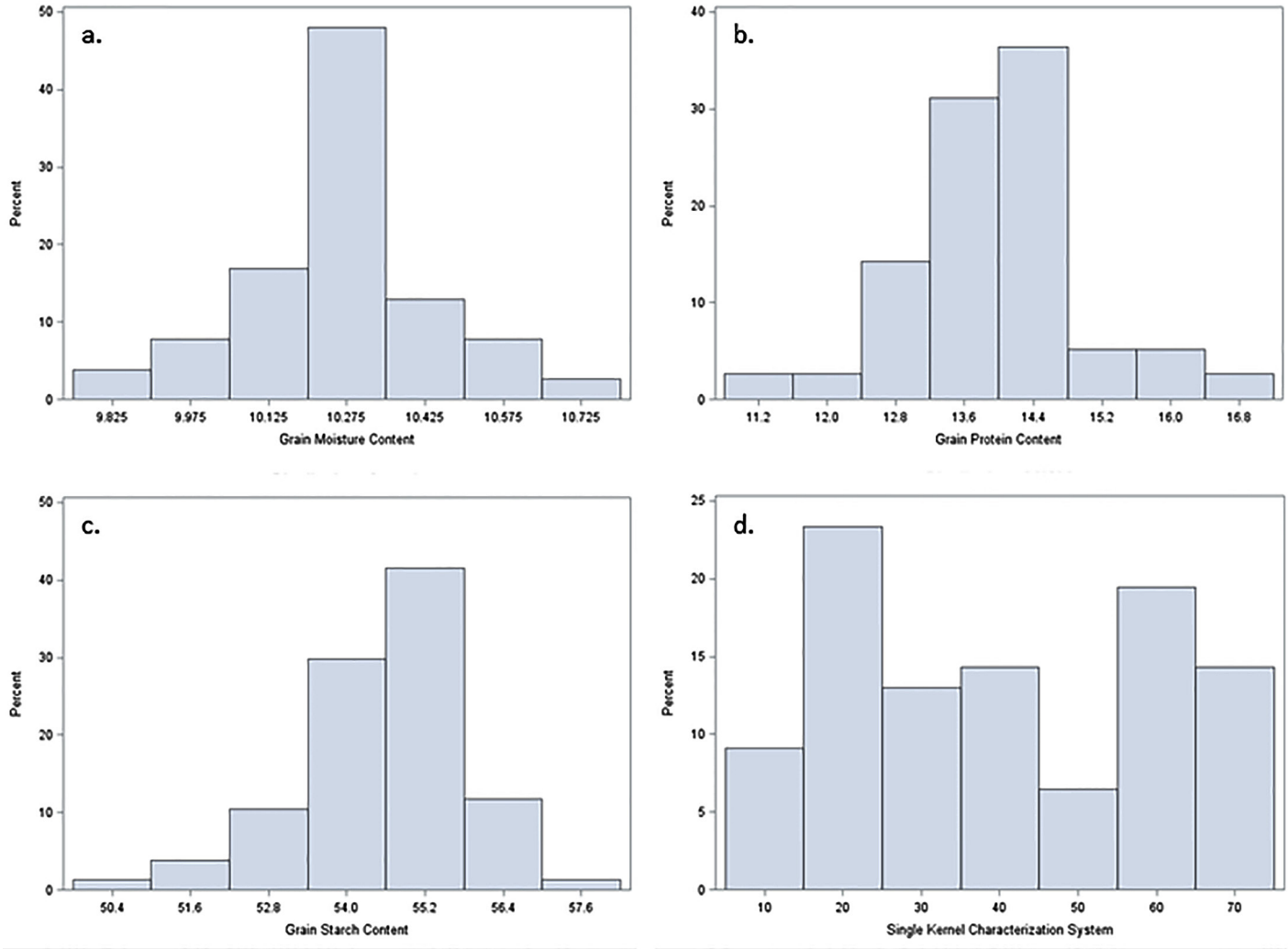

Fig. 2. Histograms showing the distribution of grain traits (a) moisture content, (b) protein content, (c) starch content, and (d) the single kernel characterization system from seed collected at the Pullman location.

Genotype and Cluster Analysis

Genotyping results for the *Vrn-1* locus showed 19 accessions had more than one spring habit Vrn allele for spring growth. The most common was *Vrn-B1a*, present in 44 accessions; *Vrn-A1a* present in 36 accessions; and *Vrn-D1a*, present in 16 accessions (Supplemental Table S6). The accession IWA8604807 did not contain any of the spring Vrn alleles confirming winter type. Four other accessions also did not contain any of the known spring Vrn genes after two PCR attempts but grew as spring types in the field. Genotyping results were obtained for the Ppd-D1, SSR, and SNP markers for these accessions, so it is unlikely the negative results are due to failed PCR, and therefore, it is possible that these accessions contain different Vrn alleles at these loci. All of the accessions genotyped with the *Ppd-D1b* allele, indicating they are photoperiod sensitive (data not shown).

A total of 37 SSR markers were assessed covering each of the three genomes. Genome B had the highest percentage of rare polymorphisms called, followed by D then A (Supplemental Table S7). Two of the 26 SNP markers, IWA1562 and IWA5068, were not polymorphic in this subset and were excluded from the cluster analysis (data not shown). The cluster analysis split the accessions into three significant clusters containing 35, 28, and 15 accessions for each cluster in order 1 to 3 (Fig. 3; Supplemental Table S6). The clusters are divided by the presence or absence of rare polymorphisms. The number of rare polymorphisms in the cluster increases with the cluster number, so Cluster 1 has the lowest amount of rare polymorphisms and Cluster 3 has the most. The Vrn loci are evenly distributed among the clusters (Supplemental Table S6).

DISCUSSION

Landrace accessions are a rich source of resistance and tolerance to abiotic and biotic stresses (Denčić et al., 2000; Ehdaie et al., 1988; Jafari-Shaberstari et al., 1995). Previous studies have identified *P. thornei* resistance in Iranian landrace accessions, and this report indicates *P. neglectus* resistance is also present in the collection. The accessions with *P. neglectus* resistance originated from different provinces in Iran

Table 8. All 78 accessions screened in controlled environment stripe rust assays and field assays. The least squared means (lsmeans) were calculated over five seedling experiments in the controlled environment and two adult plant experiments for the field at the Spillman Agricultural Farm, Washington State University. Accessions were compared with susceptible controls 'Avocet' for the controlled environment and 'Hank' for the field using Dunnett's method. The call is a reference assigned by the authors to interpret the Dunnett's comparison *P*-value and the corresponding lsmeans of the infection-type (IT) score.

	Controlled			Field			
				lsmeans			
Entry	Lsmeans IT score	*P*-value	Call†	IT score	Percentage severity of plot with infection	*P*-value	Call†
Avocet	5.73	‡					
Hank				6.72	81.92	‡	S
Hyak	2.04	<0.0001	R				
Lemhi	5.19	1.0000	S				
Tyee	3.23	0.0056	R				
IWA8604094	5.44	1.0000	S	7.57	68.97	0.9996	S
IWA8604259	4.72	0.9899	S	5.91	52.31	0.8294	S
IWA8604272	4.15	0.9998	S	5.91	62.31	0.9746	S
IWA8604394	4.91	1.0000	S	6.57	68.97	0.9923	S
IWA8604409	n/a	n/a	n/a	7.57	75.64	0.9996	S
IWA8604568	2.22	0.6689	MR/MS	7.57	70.64	0.9996	S
IWA8604571	5.78	1.0000	S	6.57	72.31	0.9970	S
IWA8604686	4.69	0.9860	S	7.57	57.31	0.9700	S
IWA8604710	4.18	0.7916	S	7.57	50.64	0.8341	S
IWA8604716	1.59	0.3453	MR/MS	6.57	37.31	0.2592	S
IWA8604740	5.16	1.0000	S	7.57	43.97	0.6278	S
IWA8604765	4.40	0.9998	S	7.57	60.64	0.9913	S
IWA8604782	4.62	1.0000	S	3.91	13.97	0.0058	MR/MS
IWA8604794	4.70	0.9889	S	7.57	65.64	0.9971	S
IWA8604807	3.78	0.2912	MR/MS	6.72	49.42	0.8075	S
IWA8604895	4.62	0.9896	S	6.57	68.97	0.9923	S
IWA8606031	5.35	1.0000	S	7.57	68.97	0.9996	S
IWA8606074	4.25	0.7561	S	6.72	71.92	0.9996	S
IWA8606081	6.54	1.0000	S	6.72	71.92	0.9996	S
IWA8606083	5.57	1.0000	S	6.57	65.64	0.9913	S
IWA8606091	3.76	0.2278	MR/MS	5.91	45.64	0.5257	S
IWA8606134	5.58	1.0000	S	6.57	60.64	0.9661	S
IWA8606188	4.46	0.8911	S	4.91	43.97	0.6278	S
IWA8606229	3.37	0.1152	MR/MS	4.91	45.64	0.6839	S
IWA8606267	4.74	0.9978	S	4.91	40.64	0.4939	S
IWA8606270	4.43	0.8911	S	5.91	53.97	0.8239	S
IWA8607438	6.00	1.0000	S	6.57	57.31	0.8239	S
IWA8607542	3.90	0.3704	MR/MS	5.91	72.31	0.9714	S
IWA8607547	3.60	0.1688	MR/MS	6.57	62.31	0.9385	S
IWA8607575	4.53	0.9768	S	7.57	67.31	0.9989	S
IWA8607576	5.43	1.0000	S	7.57	47.31	0.7438	S
IWA8607766	4.17	0.7578	S	6.57	43.97	0.3054	S
IWA8607776	4.68	0.9896	S	7.57	62.31	0.9927	S
IWA8607818	4.66	0.9896	S	7.57	72.31	0.9996	S
IWA8607820	1.05	0.7131	MR/MS	7.57	75.64	0.9996	S
IWA8607866	3.96	0.5051	MR/MS	7.57	70.64	0.9996	S
IWA8607871	4.69	0.9896	S	7.57	70.64	0.9996	S
IWA8607923	3.99	0.5816	MR/MS	5.91	48.97	0.4156	S
IWA8607958	4.42	0.9115	S	4.91	48.97	0.8033	S
IWA8607960	4.70	1.0000	S	6.57	72.31	0.9927	S
IWA8607961	5.79	1.0000	S	4.91	43.97	0.6278	S
IWA8607962	5.42	1.0000	S	5.91	58.97	0.8341	S
IWA8607963	5.50	1.0000	S	6.57	62.31	0.9385	S

(cont'd.)

Table 8. Continued.

	Controlled			Field			
				lsmeans			
Entry	Lsmeans IT score	*P*-value	Call†	IT score	Percentage severity of plot with infection	*P*-value	Call†
IWA8607995	5.01	0.9998	S	7.57	68.97	0.9996	S
IWA8608010	4.70	0.9911	S	6.57	70.64	0.9923	S
IWA8608014	3.73	0.3146	MR/MS	6.57	52.31	0.6560	S
IWA8608064	4.34	0.8624	S	6.57	52.31	0.6960	S
IWA8608074	1.94	<0.0001	R	4.91	25.64	0.0410	MR/MS
IWA8608077	3.79	0.3038	MR/MS	3.91	20.64	0.0118	MR/MS
IWA8608080	2.57	0.0009	R	3.91	27.31	0.0234	MR/MS
IWA8608082	3.55	0.7916	MR/MS	3.91	27.31	0.0234	MR/MS
IWA8608147	4.61	0.9781	S	5.91	52.31	0.8294	S
IWA8608152	1.95	0.9482	MR/MS	5.91	47.31	0.6715	S
IWA8608177	6.36	1.0000	S	5.91	50.64	0.7110	S
IWA8608767	7.45	1.0000	S	7.57	57.31	0.9700	S
IWA8608802	2.70	0.8698	MR/MS	6.57	57.31	0.8239	S
IWA8608819	3.41	0.9891	MR/MS	7.57	47.31	0.7438	S
IWA8608830	5.22	1.0000	S	7.57	53.97	0.9213	S
IWA8608846	4.02	0.6615	S	6.57	53.97	0.6715	S
IWA8608909	4.49	0.9024	S	6.57	57.31	0.7897	S
IWA8608911	4.99	0.9998	S	6.57	62.31	0.9385	S
IWA8608915	5.20	1.0000	S	7.57	48.97	0.8033	S
IWA8608928	2.40	0.0009	R	2.91	17.31	0.0096	R
IWA8608938	3.81	0.9894	MR/MS	6.57	50.64	0.6839	S
IWA8608982	6.12	1.0000	S	6.72	51.92	0.8588	S
IWA8608983	4.59	0.9998	S	6.72	66.92	0.9971	S
IWA8608990	5.41	1.0000	S	6.57	68.97	0.9927	S
IWA8608992	5.75	1.0000	S	6.57	58.97	0.8294	S
IWA8609012	2.54	0.2860	MR/MS	7.57	60.64	0.9913	S
IWA8609023	4.68	0.9999	S	7.57	58.97	0.9799	S
IWA8609031	6.01	1.0000	S	7.57	62.31	0.9927	S
IWA8609035	4.13	0.5841	S	6.57	60.64	0.9746	S
IWA8609036	7.57	0.9792	S	6.57	48.97	0.5257	S
IWA8609045	4.29	0.9933	S	4.91	52.31	0.8791	S
IWA8609049	4.23	0.7131	S	7.57	77.31	0.9996	S
IWA8609061	4.16	0.6468	S	6.57	60.64	0.9014	S
IWA8609064	4.93	0.9998	S	6.72	74.42	0.9996	S
IWA8609076	3.68	0.9804	MR/MS	5.22	69.42	0.9455	S

† R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

‡ Control for Dunnett's comparison.

similar to results for *P. thornei* resistance reported by Sheedy and Thompson (2009). The wide distribution of resistance indicates the larger 7000-accession collection from Iran may have additional accessions with *Praylenchus* resistance.

The method employed in this study to identify resistant accessions is somewhat different than previous approaches where a stepwise elimination over several experiments occurred. This method was employed for two primary reasons: first to help reduce the cost of the trials by screening fewer lines as the experiments progressed and, two, to be conservative in estimating resistant accessions with a two control approach given the variability known to *Pratylenchus* assays. The difference in approach most likely explains why only 29 accessions were designated as resistant to *P. thornei* in this study compared with 34 by Sheedy and Thompson (2009). The authors felt the conservative approach was necessary, as studies have shown even small differences in nematode populations in the field can lead to significant yield loss in intolerant cultivars (Smiley, 2009).

The *P. neglectus* resistance locus, Rlnn1, was previously identified by Williams et al. (2002) in the Australian cultivar Excalibur. The nine accessions identified as *P. neglectus* resistant in this study showed consistent increased control of nematode populations compared with Excalibur. Similarly, 14 of the 29 accessions identified as *P. thornei* resistant showed increased control compared with the resistant standard GS50a, an Australian breeding line. These results are similar to that of Sheedy and Thompson (2009) who found

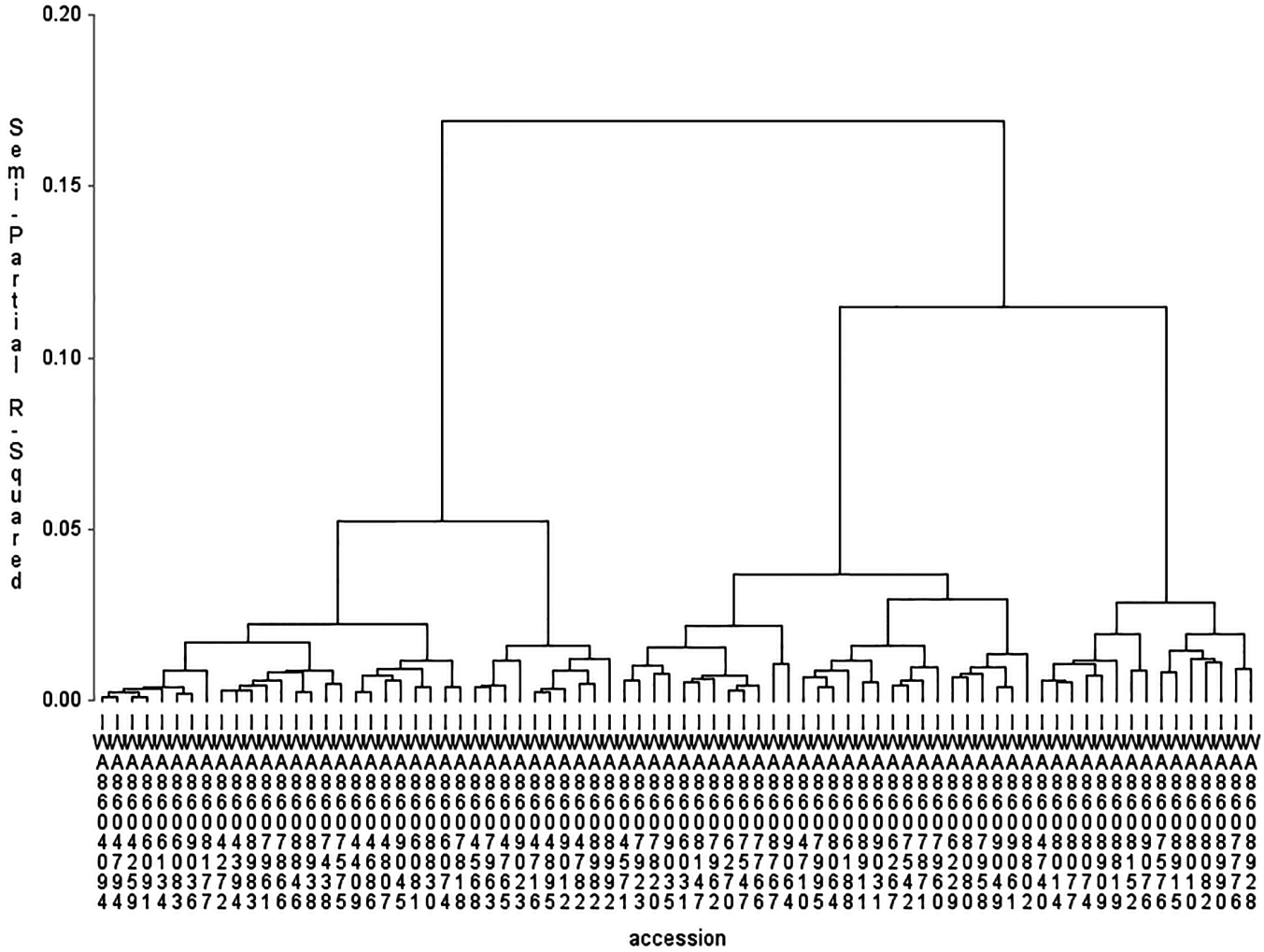

Fig. 3. Dendogram for the Ward cluster analysis of the genotype data. The data includes polymorphisms found in the 78 accession subset by 37 simple-sequence repeat and 26 single-nucleotide polymorphism markers as well as the Vrn1 locus.

25 accessions with increased control of *P. thornei* populations than the resistant standard GS50a as did Schmidt et al. (2005) with two accessions from another Middle-Eastern landrace collection. Incorporating the novel resistance found in these landraces with that found in Australian lines could greatly improve control of *Pratylenchus* populations.

The broad-sense heritability estimates reported in this study indicate resistance to *P. neglectus* and *P. thornei* are moderately heritable under the evaluation conditions used in this study. Previous estimates of broad-sense heritability have been as low as 25% for *P. neglectus* in barley (*Hordeum vulgare* L.; Sharma et al., 2011) and as high as 94% for *P. thornei* in wheat (Sheedy et al., 2012). Narrow-sense heritability estimates have been reported between 63 and 87% for *P. neglectus* and between 89 and 93% for *P. thornei* (Thompson et al., 2012).

The root rating method developed in this study shows potential as a low-cost means of identifying putative *Pratylenchus* resistance in wheat. The slightly negative linear relationship between the *P. neglectus* transformed counts and the root ratings likely indicates the damage to the roots was too severe to maintain the high nematode population to the end of the trial, when the soil samples were collected. An added benefit to a root rating approach could be reducing the time of the assays. A cost-effective strategy would be to screen large numbers of germplasm with the root rating method, perhaps at multiple time points, to reduce the number screened by the traditional nematode counting methods for confirmation of resistance.

Stripe rust resistance was assayed in this study because this disease is one of the most globally destructive diseases of wheat and frequently occurs in areas where nematode damage is prevalent. Seedling stripe rust resistance to Australian pathotypes has been identified in Iranian landrace accessions AUS28183 and AUS28187 from the Watkins collection (Bansal et al., 2011), indicating resistance might also be found in this subset. The stripe rust race PST 100 has been a predominant race in the United States since 2003 (Christopher et al., 2013). The accessions IWA8608928 (PI25776) and IWA8608074 (PI24305) showed resistance to stripe rust and also showed resistance or moderate resistance to both *Pratylenchus* species. Two other accessions,

IWA8608077 (PI621458) and IWA8608080 (PI624307), showed resistance or moderate resistance to *Pratylenchus* and resistance or moderate resistance to stripe rust in either the controlled or field experiments. The broad-sense heritability estimates reported in this study under field conditions fall within the range of those reported by Carter et al. (2009), indicating these accessions would be good candidates for molecular mapping studies. The lower heritability estimate from the greenhouse assays most likely are due to the inconsistent germination rates seen in these assays. The four accessions with resistance to *Pratylenchus* and stripe rust will be useful to breeding programs for incorporating resistance to multiple pathogens from a single source.

Agronomic traits have not been extensively examined in the Iranian landrace collection and are environment specific. Most accessions had later days to heading, were taller, and had increased straw biomass than commonly grown adapted spring wheat cultivars in field trials conducted in California (Moghaddam et al., 1997). Lower grain weight and grain yield have also been associated with Pakistani and Iranian landraces than cultivars grown in nonstress environments (Moghaddam et al., 1997; Masood et al., 2005). Although the field trial results in this study were influenced by natural stripe rust infection, the accessions also showed an increase in plant height and lower grain volume weights than spring wheat cultivars commonly grown in the states of Washington and Oregon. The accessions with reduced threshing ability and pubescence on the leaves or heads, which are not accepted in commercial processing of wheat, will have to be selected against in crosses with these accessions.

Also reported in this study were later heading dates than PNW-adapted cultivars, which may be partially attributed to the spring *Vrn1* loci. Vernalization and photoperiod requirements are important for yield potential through geographic adaptations (Santra et al., 2009). The *Vrn-A1* locus conferring spring growth habit on chromosome 5A eliminates vernalization requirements, but the *Vrn-B1* and *Vrn-D1*, homeoelogous loci, conferring spring habit on chromosomes 5B and 5D, require some vernalization to promote flowering (Santra et al., 2009). Spring habit conferred by the *Vrn-B1* loci were more commonly found in Turkish landraces (Andeden et al., 2011), indicating some vernalization was required for flowering. Spring wheat is typically planted in the fall in the Middle East, and vernalization and photoperiod developmental genes are critical for avoiding late frost damage to spikes and timing grain fill to avoid heat and drought stress or optimize late season rainfall (Mohammadi et al., 2012).

Increased genetic diversity has been found in many Middle Eastern landrace accessions compared with landraces from other parts of world (Dreisigacker et al., 2005; Hoisington et al., 1999). This increased diversity is expected in a species center of origin. Rare polymorphisms were detected in 19.2% of this subset with the highest number of polymorphisms detected in the B genome, which is similar to findings by Huang et al. (2002) in Middle Eastern accessions. Given that this is a very small subset of the Iranian collection, it is probable this is an underestimation of diversity.

Reluctance to use landraces in breeding programs stems from the likely transfer of additional undesirable traits linked with the desired trait (Able et al., 2006; Hoisington et al., 1999). The selection of the accessions that are incorporated into a breeding program will be crucial to the time of return investment (Smale, 1997). Other challenges associated with landrace incorporation have included changes in epistatic networks reducing trait heritability, breaking desired linkage blocks, and segregation distortion (Able et al., 2006; Remington et al., 2001; Warburton et al., 2006). Landraces have been successfully incorporated into cultivars for improved agronomic and resistance traits. The most well-known case was the incorporation of the *Rht*, height reducing, genes from the Japanese landrace Shiro Daruma resulting in the "Green Revolution". The use of marker-assisted selection for the desired traits may help to overcome some of the challenges associated with landrace incorporation and increase breeding efficiency as would breeding strategies that increase opportunities for recombination (Dreisigacker et al., 2005; Hoisington et al., 1999).

Thus far, landraces have been a consistent source of resistance to *P. thornei* and *P. neglectus* and will be instrumental in developing resistant cultivars for nematode management. This study has identified four accessions, IWA8608928 (PI624776), IWA8608077 (PI621458), IWA8608080 (PI624307), and IWA8608074 (PI624305), with resistance to multiple pathogens that have few undesirable traits and are good candidates for introgression.

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