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Mining synthetic hexaploids for multiple disease resistance to improve bread wheat

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Abstract. A collection of 253 synthetic hexaploid wheats (SHWs) produced from 192 *Aegilops tauschii* accessions and 39 elite durum varieties were studied to identify, characterise, and evaluate potentially untapped diversity of disease resistance in wheat. The diseases for which resistance was sought included cereal cyst nematode (CCN), root lesion nematode (RLN), *Stagonospora nodorum* blotch (SNB), *Septoria tritici* blotch (STB), and the 3 rusts, leaf rust, stem rust, and stripe rust, all important diseases of bread wheat worldwide, which can severely reduce wheat yield and quality. The SHWs exhibited a wide spectrum of resistance to the 8 pathogens. The frequency of disease-resistant SHWs ranged from 1% for one species of RLN (*Pratylenchus neglectus*), 3% and 10% for *Septoria nodorum* leaf and glume blotch, 10% for seedling resistance to yellow leaf spot, 16% for CCN, 21% for the second species of RLN (*Pratylenchus thornei*), 73% for *Septoria tritici* blotch, and 15%, 40%, and 24% for leaf rust, stem rust, and stripe rust, respectively. Five SHWs, Aus26860, Aus30258, Aus30294, Aus30301, and Aus30304, exhibited high levels of resistance to CCN, YLP, STB, LR, and SR, while 56 SHWs showed resistance to either 3 or 4 diseases. The genetics of resistance to CCN in some of the SHWs revealed that some of the accessions carry the same CCN gene(s) against pathotype *Ha13*, while others may carry different resistance gene(s). Additional studies were carried out to understand the relationship between the resistances identified in SHWs and the ones already present in common wheat, in particular the resistance genes *Cre1* and *Cre3* against CCN. The use of perfect markers associated with *Cre1* and *Cre3* suggested that some SHWs may carry a new CCN resistance gene(s), which could be deployed in breeding programs to increase the diversity of available resistance. The identification of SHWs with resistance to a range of diseases provides an opportunity to generate genetic knowledge and resistant germplasm to be used in future variety development.

Additional keywords: genetic diversity, synthetic hexaploid wheat, *Aegilops tauschii*, durum, *Triticum aestivum*.

Introduction

The effective control of diseases of wheat is critical to maintain stability in global food supplies. Ideally, control is achieved through the cultivation of varieties with genetic resistance to the range of important diseases present within a production region. Genetic resistance is the preferred means of disease control for many diseases because it avoids the use of pesticides, is cost effective, and a low-technological management option. Resistance is particularly important to farmers in developing countries where agriculture is less advanced and much of the world's wheat is produced.

In many cases the availability of genetic resistances is often limited because of the lack of genetic diversity within cultivated bread wheat. Bread wheat evolved from a few chance crossings between *Triticum turgidum* (or its predecessor) and *Aegilops tauschii* ~8000 years ago in the Fertile Crescent (Feldman 2001). From its inception, a genetic bottle-neck was imposed on common wheat's history, because only a limited number of individuals within the progenitor species were involved in the kindling of hexaploid wheat (van Ginkel and Ogbonnaya 2007). Second, modern sedentary agriculture and its associated practices also foster the erosion of genetic diversity as only genetic variants considered favourable are maintained for subsequent planting (Yong-Bi *et al*. 2005). Also the availability of effective disease resistance genes has been further reduced over time as the pathogens, particularly rusts, have evolved to acquire virulence on previously effective resistances.

To overcome the problems associated with the limited genetic diversity present within modern common wheats there has been increased emphasis on the production of synthetic hexaploid

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wheats (AABBDD) by recreating the original cross of the diploid *A. tauschii* (DD) and tetraploid *T. turgidum* (AABB), using these progenitor species collected from the centres of origin of wheat, to obtain the widest genetic diversity possible. Evaluation of synthetic hexaploid wheats has identified new sources of genetic resistance for the cereal cyst nematode (CCN) causal agent, *Heterodera avenae* (Eastwood *et al*. 1991), yellow leaf spot (YLS; *Pyrenophora tritici-repentis*) (Ogbonnaya *et al*. 2004), root lesion nematodes (RLN; *Pratylenchus* spp.) (Thompson and Haak 1997), leaf rust [LR; *Puccinia triticina* (formerly *Puccinia recondita* f. sp. *tritici*)], stem rust (SR; *Puccinia graminis* f. sp. *tritici*), and stripe rust (YR; *Puccinia striiformis* f. sp. *tritici*) (Bariana *et al*. 2004). Typically, in each of these studies the synthetic wheats were only evaluated against one or two diseases. To enable plant breeders to select synthetic hexaploid wheats (SHWs) with multiple disease resistance it is ideal that they are screened for a range of resistances.

The objectives of this study were (*i*) to assess the diversity of disease resistance in synthetic hexaploid wheats (SHWs) to 8 major wheat diseases in Australia, (*ii*) to identify SHWs with multiple disease resistance for high-priority use in wheat improvement, and (*iii*) in the case of CCN to determine whether some SHWs contain yet uncharacterised genes for CCN resistance.

Materials and methods

Plant materials

The number of SHWs evaluated ranged from 201 screened for resistance to *Stagonospora nodorum* blotch (SNB), *Septoria tritici* blotch (STB), YLS, and RLN, 246 screened for CCN resistance between 2002 and 2005, 160 each for LR and YR, to 120 for SR. The SHWs were produced from 192 and 39 *Ae. tauschii* and elite durum parents, respectively, by scientists at the International Maize and Wheat Improvement Centre (CIMMYT), Mexico, and the Victorian Department of Primary Industries. During evaluation against each disease, resistant and susceptible check varieties were used.

Disease assessment

Cereal cyst nematode

The CCN inoculum preparation and subsequent method of evaluation were described in Ogbonnaya *et al*. (2001). The nematode reproduction values obtained were then used to classify plant resistance relative to the control varieties Aus10894 (resistant), Frame (moderately resistant), and Meering (susceptible). Mean values of less than 5 and more than 30 cysts (range per plant root) were classified as resistant and susceptible, respectively, and those with 15–30 cysts were considered moderately resistant.

Two approaches were used to determine how many resistant SHWs carried the same gene(s). First, 5 of the SHWs that expressed high levels of resistance were inter-crossed in a diallel design to produce F_1 seeds which were selfed to produce F_2 populations that were subsequently evaluated for CCN resistance. The second approach was based on the use of functional molecular markers linked with known resistance genes *Cre1* and *Cre3*, first to identify SHWs carrying these genes and second to identify SHWs carrying yet uncharacterised CCN resistance genes.

Root lesion nematode

The RLN test for *P. neglectus* was conducted according to Williams *et al*. (2002). At least 10 seeds were divided into 2 tests of 5 single plant replicates. The susceptible check varieties were Pugsley and Machete, and the resistant check was the triticale cv. Abacus. Raw data from 5 replicates in each test were used to perform ANOVA. Experimental results were compared with the check varieties, using the least significant difference (l.s.d.). Lines were classified as follows:

R, resistant (not significantly different in severity from the resistant check);
S, susceptible (not significantly different in severity from the susceptible check);
M, moderate or intermediate (significantly lower in severity than the susceptible check and significantly higher than the resistant check;
MR, moderately Resistant (not significantly different in severity from the resistant check);
MS, moderately Susceptible (not significantly different in severity from the susceptible check).

The RLN test for *P. thornei* resistance was carried out using the procedure described by Thompson and Haak (1997).

Yellow leaf spot

Four seeds of each of the 208 lines, including resistant (H45), moderately susceptible (Silverstar), and susceptible (Chara, Annuello, and Yitpi), were sown into 4-cm pots filled with potting mix supplemented with nutrients. Each line was replicated 4 times and sown in a randomised complete block design. Seedlings were grown to the 2–3 leaf stage in a glasshouse with a temperature range of 15–25°C.

Three isolates of *Pyrenophora tritici repentis* (isolate identification number 03–0148, 03–0152, 03–0053) from geographically disperse locations across Victoria were cultured on potato dextrose agar (PDA). Subcultures were made by transferring 3-mm^2 plugs onto vegetable juice agar (V8 agar) and grown for 10 days at 20 ± 1°C with a 12-h photoperiod under white fluorescent and Growlux lights. A suspension was obtained from cultures by adding ~4 mL of sterile distilled water to each plate and scraping the mycelium and spores through a 500-µm sieve. Infective unit concentration was adjusted to 2×10^5/mL and a drop of Tween 20 added.

Seedlings were placed in a humidity tent set to 95–100% and inoculated to leaf wetness using a hand-held sprayer. Seedlings remained in the humidity tent for 3 days with a 12-h photoperiod and temperature of 21°C during the day and 16°C during the night. The symptoms were then allowed to develop for a further 9 days at the same temperatures and photoperiod.

Inoculated plants were assessed for lesion severity based on a variation of the scale developed for resistance screening to *P. teres* f. sp. *maculata* (Tekauz 1985) in barley. A scale of 1–9 was used in which 1 was resistant and 9 was susceptible.

Septoria tritici *blotch*

Plants were grown as described above for YLS. Eight seeds per line were grown per pot. Plants were grown for ~10–14 days after sowing in pots until the second leaf was fully expanded. Inoculation was then carried out with the single pycnidia isolate 79.2.1A collected in southern New South Wales, Australia, at a concentration of 4 million conidia/mL of water. The protocol for media and inoculum preparation was followed as previously described (Ballantyne 1983). Inoculated pots were placed into a misting chamber for 48 h at 95–100% relative humidity. The pots were then placed onto glasshouse benches and assessed for disease development after 21 days. Each genotype was assessed in 2 glasshouse experiments and disease severity scored using a quasi-quantitative modified Rosielle scale (0, immune; 1, resistant; 2, moderately resistant; 3, moderately susceptible; 4, susceptible; 5, very susceptible; 6, mixed susceptible reactions; 7, mixed resistant reactions) (Eyal *et al*. 1987).

Septoria nodorum blotch

Plants were grown under 22/18°C day/night temperature conditions and natural lighting in 150-mm-diameter pots containing a sand–loam mix with 1 g of Osmocote (slow-release fertiliser). Three seeds per genotype were planted in each pot. The experiment was conducted with 3 replicate pots per genotype, arranged in a randomised block design on the glasshouse bench.

In 2002 and 2003, plants were tested for seedling resistance and flag-leaf resistance. Plants were spray-inoculated to run-off at the 2 1/2-leaf stage with a conidial suspension (10^6 conidia/mL with 0.1 mL/L of Tween 80) of *S. nodorum*, produced from a mixture of grain cultures (Fried 1989) of the isolates WAC 4302, WAC 4305, WAC 4306, and WAC 4309, obtained from the Department of Agriculture and Food Western Australia. Inoculated plants were placed in a misting chamber for 48 h and then returned to the glasshouse bench. Disease was assessed 8 days after inoculation on the 2 lowest leaves that were fully emerged at inoculation, using a 0–5 scale (0, no infection; 5, severe infection). Plants were maintained and flag leaves inoculated at full head emergence (Feekes stage 10.3). Inoculated plants were placed in the misting chamber and rated 9 days after inoculation for percent necrosis on the flag leaf and the leaf below. Rating scale for leaf infection was based on a percentage scale from 0% (highly resistant) to 100% (highly susceptible) as described by James (1971).

In 2004 and 2005, plants were tested for flag-leaf resistance and glume blotch resistance. Plants were grown as above and both glumes and flag leaves were inoculated at full head emergence. Flag leaves were rated 9 days after inoculation and glumes 16 days after inoculation. Rating scales for glume and leaf infection were based on the percentage scale described by James (1971). Results were analysed by one-way ANOVA using GENSTAT6.

Rust diseases

SHWs were screened against LR, SR, and YR under field conditions at the University of Sydney Plant Breeding Institute, Cobbitty. Fifteen seeds of each genotype and the susceptible control Avocet S were planted as short 60-cm rows. A block of 50 rows was surrounded by a 60-cm susceptible infector row, consisting of a mix of several susceptible genotypes, to facilitate the development of epidemics of the 3 rusts. The following pathotypes were used: *Puccinia graminis* f. sp. *tritici* (Pgt), 98–1,2,3,5,6 (University of Sydney Plant Breeding Institute accession number 781219); *Puccinia triticina* (Pt), 104–1,2,3,(6), (7), 11, 13 (accession number 200347) and 76–1,3,5,10,12 (accession number 990423); and *Puccinia striiformis* f. sp. *tritici* (Pst), 134 E16A+ (021510). These pathotypes were chosen because of their predominance in commercial fields. The pathotype descriptions are provided in McIntosh *et al*. (1995). Infector rows were inoculated with uredioniospores of the 3 rust pathogens, immersed in light mineral oil (Shellsol T), using an ultra-low-volume applicator. The experiments were sown in mid May for YR and mid June for LR and SR. Disease assessments were made on a scale of 1–9 according to Bariana *et al*. (2004), where a host response score of 1 was considered very resistant, 2 resistant response, 3 resistant to moderately resistant, 4 moderately resistant, 5 moderately resistant to moderately susceptible, 6 moderately susceptible, 7 moderately susceptible to susceptible, 8 susceptible, and 9 very susceptible. Rust responses were noted at the time of anthesis when the susceptible control Avocent S exhibited an overall score of 9.

Results

Reactions of the resistant and susceptible controls to the various diseases in each test were consistent with previously published results and disease-response classification. The reactions of SHWs against different pathogens are summarised in Fig. 1. Disease reactions of the SHWs to all diseases are shown in Appendix 1.

Cereal cyst nematode

In total, 242 SHWs including 2 check wheat genotypes, the CCN resistant landrace AUS10894 and the CCN susceptible bread wheat Meering, were screened against the Australian nematode pathotype *Ha13*. The mean CCN count for AUS10894 was 6 cysts/plant, whereas the susceptible control Meering had a high CCN count of 50 cysts/plant. The response of SHWs ranged from 1 cyst to 15 for the resistant class, 16 to 30 for the moderately resistant, and 31 to more than 50 cysts for the susceptible class. Based on the reaction of the controls, 16% of the SHWs were resistant, 15% moderately resistant, and 69% were susceptible. The crosses involving Aus29682/Aus29644, Aus29670/Aus29644, and Aus29644/Aus29639 produced resistant progeny with less than 15 cysts/plant. The lack of susceptible segregants among these crosses indicates that these accessions had a resistance gene (s) in common (Table 1). However, the cross Aus29670/Aus29682 segregated for susceptible phenotypes, suggesting that the 2 SHWs involved carried different CCN resistance gene(s).

The *Cre3* marker amplified a diagnostic PCR product in 18 resistant and 11 of the moderately resistant SHWs and the *Cre3* control VP1620 (Fig. 2). In total, 40% of the CCN resistant SHWs carried *Cre3*. The remaining 44 SHWs that exhibited resistance against *Ha13* were tested using the *Cre1* diagnostic marker. While, as expected, amplification with the *Cre1* marker

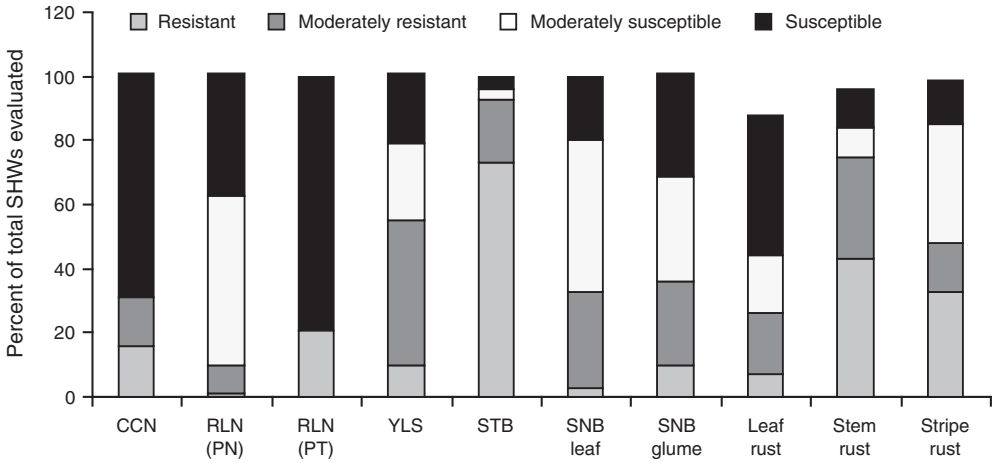

Fig. 1. Genetic variation in synthetic wheats for various diseases expressed as a percentage of the total number evaluated (total numbers varied: $n = 92–242$). CCN, Cereal cyst nematode; RLN, root lesion nematode; PN, *Pratylenchus neglectus*; PT, *P. thornei*; YLS, yellow leaf spot; STB, *Septoria tritici* blotch; SNB, *Septoria nodorum* blotch.

Table 1. Reaction of synthetic hexaploid wheat intercrosses, including standard checks to CCN infection

SHWs	Mean	Range	Classification
Aus29682/Aus29644	4	4–6	R
Aus29670/Aus29644	8	0–8	R
Aus29644/Aus29639	6	1–19	R
Aus29670/Aus29682	35	16– >50	S
Meering (Susceptible check)	46	23– >50	S
Aus10894 (Resistant check)	6	1–11	R

occurred in the landrace source of *Cre1*, Aus10894, no similar-sized PCR product was amplified in any of the 44 SHWs. These results suggested that these SHWs carry yet uncharacterised CCN resistance, which is different from that conditioned by *Cre1* and *Cre3*.

Root lesion nematode

For *P. neglectus*, one SHW displayed a very high level of resistance to RLN, equivalent to the triticale check Abacus, and significantly superior to the bread wheat resistant check Krichauff. A further 9% of the SHWs were not significantly different from Krichauff. Of the SHWs, 53% and 38% were classified as moderately susceptible and susceptible, respectively. On the other hand, for *P. thornei*, 1% of the SHWs expressed significantly higher levels of resistance than the resistant control GS50a. A further 20% displayed levels of resistance equivalent to GS50a, and 79% of the SHWs proved susceptible.

Yellow leaf spot

Of the 208 SHWs evaluated, 55% showed resistance levels equal to or better than the resistant control H45, with 10% of these lines showing significantly better resistance than H45. Twenty-four percent of the SHWs were moderately susceptible, similar in response to the Australian wheat cv. Chara, while 24% were susceptible, similar to Yitpi.

Septoria tritici *blotch*

A large proportion of SHWs expressed high levels of resistance to *M. graminicola* isolate 79.2.1A in the glasshouse. Of the 202 SHWs evaluated, 167 (72%) were scored as highly resistant, 20% as moderately resistant, 4% as moderately susceptible, and 4% as susceptible (Fig. 1).

Septoria nodorum *blotch*

There was a high incidence of resistance to SNB in the SHWs evaluated. Considerable differences in seedling and flag-leaf resistance among the SHWs were observed. Of the 102 SHWs evaluated for seedling resistance, 11% were rated as resistant (R), 33% as moderately resistant (MR), and 56% as moderately susceptible (MS) or susceptible (S) (Fig. 1). On the other hand, of the 190 SHWs evaluated for flag-leaf resistance, 16% were R, 32% MR, and 52% MS or S. Only one of the SHWs was R for both seedling and flag-leaf resistance and 19% were MR for both traits. Of the 88 SHWs evaluated for glume blotch, 3% were R, 9% MR, and 88% MS or S. None of the SHWs was R for both seedling and flag-leaf resistance. Six SHWs, which were either R or MR for seedling resistance, were also MR for flag-leaf resistance.

Rust diseases

SHWs exhibited a wide range of responses to LR, SR, and YR under field conditions. Rust response distribution of SHWs is presented in Fig. 1. A high proportion of SHWs showed moderately resistant to very resistant responses against all 3 diseases (34% for LR, 75% for SR, and 48% for YR). In addition, at least 9% of SHWs exhibited moderately resistant to moderately susceptible (MR-MS) responses against LR, SR, and YR. The MR-MS response is acceptable as a minimum disease standard for the release of wheat varieties in all wheat-growing regions of Australia. Four SHWs (Aus30268, Aus30301, Aus30625, and Aus30656) displayed very high levels of resistance to all 3 rusts diseases. A low proportion (~4%) of the SHWs exhibited heterogeneous rust responses.

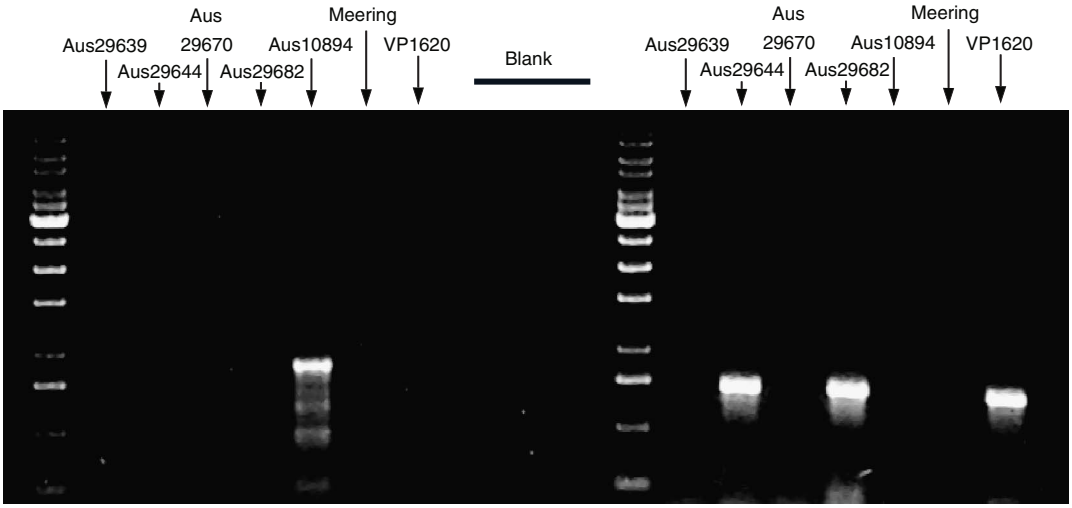

Fig. 2. Some SHWs have diagnostic fragments that co-segregate with resistance to *Cre1* and *Cre3* CCN resistance genes. The absence of the co-segregating fragment suggests a potentially new source of CCN resistance against the *Ha13* pathotype. Aus10894 is the land race source of *Cre1*, VP1620 is an elite wheat line carrying the *Cre3* gene derived from *Ae. tauschii*, and Meering is a common wheat cultivar and a susceptible check for CCN.

Genotypes showing MR-MS rust responses may carry useful and even yet uncharacterised adult plant resistance.

Discussion

One of the main objectives of this study was to identify SHWs that have high levels of resistance to several diseases that affect wheat productivity in Australia. Such SHWs could then be crossed with adapted breeding lines to simultaneously transfer multiple disease resistance into locally adapted wheat varieties. Previous studies have reported SHWs to be a reservoir for disease resistance in wheat (Siedler *et al*. 1994; Mujeeb-Kazi *et al*. 2001*a*, 2001*b*; Xu *et al*. 2004; Ogbonnaya *et al*. 2005). This study confirmed and extended previous studies by assessing up to 8 diseases relevant to the wheat industry in Australia and worldwide, and documenting additional uncharacterised genetic variation in SHWs. Five SHWs (Aus26860, Aus30258, Aus30294, Aus30301, and Aus30304) tested in this study had resistance to 6 diseases (CCN, YLS, STB, SNB, LR, and SR), whereas a further 20 and 32 genotypes, respectively, displayed resistance to 5 (CCN, YLS, STB, SNB, and RLN) and 4 (YLS, STB, LR, and SR) diseases, respectively. Many accessions carrying resistance to 2 or 3 diseases were identified.

Xu *et al*. (2004) reported that several SHWs displayed high levels of resistance to 2 foliar diseases, YLS and SNB, in wheat. The highest frequency of disease resistance found among SHWs in this study was against STB, where ~73% of the SHWs exhibited complete resistance against a single isolate used. However, screening of SHWs against a broad range of isolates from geographically diverse environments would be needed to obtain a more comprehensive picture of the genetic diversity of resistance to STB in SHWs. Little is known about the genetics of *Septoria tritici* resistance of SHWs and isolate specificity. It would also be important to understand the genetic relationship of resistance carried by SHWs with genetically characterised and named *Stb* genes. Arraiano and Brown (2006) reported the identification of isolate-specific complete resistance and isolate-nonspecific partial resistance among 238 European wheat varieties and breeding lines. Given the preponderance of STB resistance in SHWs, it is pertinent to determine the genetic relationship of these sources with the previously identified *Stb* genes. This information would allow a better understanding of genetic diversity among resistance sources.

The lowest frequency of resistance was found for RLN (*P. neglectus*), with only 9% of SHWs expressing moderate resistance, which was not significantly different from the most resistant cultivated bread wheat. The fact that only one RLN gene, *Rlnn1*, has been formally named (Williams *et al*. 2002) and was reported to be present in the South Australian germplasm, may reflect the narrow genetic basis of RLN resistance in bread wheat. Recently, Zwart *et al*. (2005) reported the identification of quantitative trait loci associated with resistance to 2 species of RLN (*Pratylenchus thornei* and *P. neglectus*), on chromosome 6D in wheat. Earlier, Thompson and Haak (1997) identified a range of *Ae. tauschii* accessions with resistance to RLN (*P. thornei*). However, further genetic characterisation of RLN resistance (for both *P. neglectus* and *P. thornei*) would be necessary to appreciate the extent of genetic diversity for resistance in SHWs.

In contrast to RLN, the availability of diagnostic DNA markers for known CCN resistance genes *Cre1* and *Cre3* facilitated the identification of a high proportion of SHWs carrying moderate levels of CCN resistance. To date, 9 resistance genes (*Cre1-8* and *CreR*) have been described in the Triticeae (Ogbonnaya *et al*. 2001). Of these, only 2 (*Cre1* and *Cre8*) are directly derived from bread wheat, whereas *Cre3* and *Cre4* are derived from *Ae. tauschii*. The others are from *Ae. ventricosa* (*Cre2*, *Cre5*, and *Cre6*), *Ae. triuncialis* (*Cre7*), and *Secale cereale* (*CreR*). It is likely that some SHW may carry uncharacterised gene(s).

The adoption of new farm-management practices such as minimum or zero tillage, stubble retention and, increasingly, intensive wheat cultivation has accelerated the spread of

YLS. To date, only a few sources of YLS resistance genes have been identified in wheat (Faris *et al*. 1996, 1997; Cheong *et al*. 2004; Tadesse *et al*. 2006*a*). Recently, Tadesse *et al*. (2006*b*) identified additional resistance in 3 SHWs. Eight major races of YLS have been classified on the basis of their virulence and ability to produce symptoms of tan necrosis and/or chlorosis (Hean *et al*. 2004). In the current study, based on 3 isolates commonly found in the state of Victoria, Australia, 10% of the SHWs displayed a higher degree of resistance than the current Australian common wheat YLS resistant standard, H45, whereas 45% expressed disease responses equivalent to H45. Xu *et al*. (2004) found that 34% of the SHWs sampled showed highly resistant reactions while 42% displayed moderate levels of resistance to the YLS race 1 in North America. In a recent study, Singh *et al*. (2006) reported that 17 SHW accessions were consistently resistant to all races of YLS (1, 2, 3, 5, 10, and 11) tested in North America. Results from the current study identified additional sources of resistance to YLS. However, genetic analyses of resistance would be necessary for characterisation of these sources.

A large proportion of SHWs exhibited high levels of resistance to all 3 rust diseases. Rust resistance genes *Lr21*, *Lr22A*, *Sr33*, *Sr45*, and *Yr28* have been identified in *Ae. tauschii* (McIntosh *et al*. 2003) and durum wheat varieties have been reported to carry *Sr9e*, *Sr13*, *Sr8b*, and *Yr24*, plus other uncharacterised genes (H. S. Bariana, unpublished). It is likely that some of the SHWs may carry a suite of these genes. Intermediate rust responses of some SHWs against LR and YR indicated the transfer of adult plant resistance from either durum wheat and/or Ae. *tauschii* parents. Triple-rust-resistant SHWs have been crossed with susceptible wheat genotypes to study the inheritance of resistance to the 3 rusts.

In conclusion, many SHWs carrying resistance to more than one disease have been identified and would serve as valuable sources of resistance in breeding programs. Several crosses have already been made between SHWs and elite Australian bread wheat varieties to simultaneously identify, characterise, and pyramid resistance loci into adapted germplasm.

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Appendix 1. Summary of disease ratings

SHWs ID	CCN	YLS	STB	SNB seedling	SNB adult	SNB glume	RLN *P. neglectus*	RLN *P. thornei*	Leaf rust	Stem rust	Stripe rust
Aus26860	R	MR	R	MS	R	–	–	S	VR	VR	MS-S
Aus26905	–	MR-MS	–	–	–	–	–	–	–	–	–
Aus29020	–	MR	–	MR	R	–	–	S	VR	VR	MS-S
Aus29636	S	MR	R	MR	R	–	MR	S	–	–	–
Aus29637	S	MR-MS	MS	MS	R	–	M	S	–	–	–
Aus29638	–	MR-R	R	MS	MS	–	MS	S	–	–	–
Aus29639	R	MR-R	R	MS	S	–	M	S	–	–	–
Aus29640	S	S-VS	–	S	MR	–	M	–	–	–	–
Aus29641	S	MR-MS	–	MS	S	–	S	S	–	–	–
Aus29642	S	MS-S	–	S	R	–	S	S	–	–	–
Aus29643	S	MR	–	MS	S	–	S	S	–	–	–
Aus29644	R	MR	–	S	S	–	MS	S	–	–	–
Aus29645	S	MR-R	–	R	R	–	MS	S	–	–	–
Aus29646	S	S-VS	R	MS	R	–	MS	S	–	–	–
Aus29647	S	S-VS	MR	MS	S	–	–	S	–	–	–
Aus29648	MR	MR-R	R	MS	MS	–	MS	S	–	–	–
Aus29649	S	MR	–	S	S	–	–	S	–	–	–
Aus29650	S	S-VS	–	MS	MR	–	–	S	–	–	–
Aus29651	S	MR	R	MS	MR	–	–	S	–	–	–
Aus29652	S	MR-MS	MR	S	R	–	S	S	–	–	–
Aus29653	MR	MR-R	R	MS	MS	–	S	S	–	–	–
Aus29654	S	MR-R	MR	MR	S	–	S	S	–	–	–
Aus29655	S	MR-MS	MS	S	R	–	M	S	–	–	–
Aus29656	S	MR	–	S	R	–	–	S	–	–	–
Aus29657	S	MR-R	–	MS	MR	–	S	S	–	–	–
Aus29658	S	VS	–	S	S	–	MS	S	–	–	–
Aus29659	S	MR-R	MR	S	R	–	S	–	–	–	–
Aus29660	MR	MR-MS	MS	R	R	–	M	–	–	–	–
Aus29661	S	MR-R	MR	S	S	–	S	–	–	–	–
Aus29662	S	MR	S	MS	MS	–	–	S	–	–	–
Aus29663	S	R-MR	MR	MR	MR	–	S	S	–	–	–
Aus29664	S	MR	S	MR	MR	–	S	S	–	–	–
Aus29665	–	–	–	–	–	–	–	S	–	–	–
Aus29666	–	MS-S	MR	MS	R	–	S	S	–	–	–
Aus29667	–	MR-R	–	MR	MR	–	–	S	–	–	–
Aus29668	–	MR-MS	–	MR	R	–	–	S	–	–	–
Aus29669	MR	MR-MS	–	MS	R	–	–	S	–	–	–
Aus29670	R	S-VS	R	MR	MR	–	S	S	–	–	–
Aus29671	S	MR	R	MS	S	–	M	S	–	–	–
Aus29672	S	MR	R	S	S	–	M	S	–	–	–
Aus29673	S	MR-MS	–	S	S	–	S	R	–	–	–
Aus29674	S	S-VS	–	MS	S	–	S	S	–	–	–
Aus29675	S	MR	S	S	MR	–	–	S	–	–	–
Aus29676	–	MR-R	–	S	R	–	S	S	–	–	–
Aus29677	S	MS	R	MS	S	–	M	S	–	–	–
Aus29678	S	MR-R	–	S	MS	–	S	S	–	–	–
Aus29679	S	MS-MR	MR	S	MR	–	MS	S	–	–	–
Aus29680	MR	S	R	MR	R	–	MS	–	–	–	–
Aus29681	S	MR-R	R	S	MS	–	S	S	–	–	–
Aus29682	R	MR	R	MS	R	–	S	R	–	–	–
Aus29683	S	MR	R	MS	R	–	M	S	–	–	–
Aus29684	S	MS-S	R	MS	R	–	M	R	–	–	–
Aus29685	S	S-VS	R	S	R	–	S	S	–	–	–
Aus30255	S	MR-R	–	R	R	–	S	S	R-MR	VR	MS-S
Aus30256	–	MR-MS	–	MR	MR	–	S	S	–	–	–
Aus30257	R	MR-MS	R	MR	MR	–	M	R	–	–	–
Aus30258	MR	MR-MS	R	MR	MR	–	MR	S	R-MR	VR	MS
Aus30259	S	MR-R	–	MR	R	–	M	S	–	–	–
Aus30260	S	MR	R	MS	MR	–	S	S	–	–	–
Aus30261	MR	S-VS	R	MS	MR	–	MS	S	S	VR	VR

Appendix 1. (*continued*)

SHWs ID	CCN	YLS	STB	SNB seedling	SNB adult	SNB glume	RLN *P. neglectus*	RLN *P. thornei*	Leaf rust	Stem rust	Stripe rust
Aus30262	MR	S	R	R	MR	–	M	S	R, MR	MS-S	VR
Aus30263	R	MR-MS	R	MS	R	–	S	S	MR-MS	VR	MS
Aus30264	S	MR-R	R	R	MR	–	M	R	R, MS-S	VR	R-MR
Aus30265	R	MS-MR	R	MR	MR	–	S	S	S	VR	VR
Aus30266	S	S-VS	R	MS	R	–	S	R	S	VR	VR
Aus30267	S	MS-MR	R	MR	MS	–	MS	S	R-MR	VR	MS-S
Aus30268	S	MR	R	MS	S	–	MS	S	VR	VR	VR
Aus30269	S	S	–	MS	MR	–	MS	S	VS	VR	R
Aus30270	S	S-VS	R	MR	MS	–	MS	S	MR-MS	VR	MR-MS, MS-S
Aus30271	S	MR-R	R	MS	MS	–	S	S	VS	R-MR	R-MR
Aus30272	S	MS-S	R	MR	MR	–	M	R	MS-S	VR	R
Aus30273	S	MR-MS	–	MS	MR	–	M	S	S	R-MR	R
Aus30274	MR	S-VS	R	MR	S	–	MS	S	S	MR-MS	R
Aus30275	–	MR	–	MS	MR	–	–	R	–	VR	S, MS
Aus30276	S	MS	R	R	MR	–	M	S	–	VR	MS-S
Aus30277	S	MS-S	R	MR	MR	–	S	S	MR-MS	VR	MR-MS
Aus30278	MR	MS-S	MR	MR	MS	–	MS	S	–	MR-MS	S, MR
Aus30279	S	S-VS	S	S	MS	–	S	S	R, MR-MS	VR	R
Aus30280	S	MS-S	MS	MS	MR	–	S	S	–	–	–
Aus30281	S	S-VS	–	MS	MR	–	–	S	S	VR	R
Aus30282	S	S-VS	R	MR	MR	–	S	S	R-MR	MS-S	R
Aus30283	MR	MS-S	R	MR	R	–	MS	S	MR	VR	MR-MS
Aus30284	–	MR-R	–	MR	MR	–	–	R	MR	MS-S	R-MR
Aus30285	S	MR	R	MR	MR	–	S	S	MR-MS	MR-MS	MR-MS
Aus30286	S	MR-R	R	MR	MR	–	S	S	MS-S	MS	MR-MS
Aus30287	S	MR	S	MS	MS	–	MS	S	MR-MS	MR-MS	MS-S
Aus30288		MR	–	MS	MR	–	–	S	MR-MS	VR	MS-S
Aus30289	S	MR	–	MS	MS	–	–	S	VR	MS, R-MR	MS-S
Aus30290	MR	MR	–	R	MR	–	MS	R	R-MR	VR	MS-S
Aus30291	–	MR	–	R	MS	–	–	S	MS	VR	MS
Aus30292	–	MS-MR	–	R	MR	–	–	S	VR	VR	MS-S
Aus30293	S	MR-R	R	R	R	–	MR	S	MS-S	VR	MS-S
Aus30294	R	MR	R	MR	MR	–	MR	S	MS-S	VR	MS-S
Aus30295	R	MR	R	MR	R	–	MS	S	S	VR	VR, S
Aus30296	S	MR-R	R	MR	R	–	MS	S	S	VR	VR
Aus30297	MR	MR	R	MR	MR	–	S	S	VR	MS-S	VR
Aus30298	MR	MS-S	R	MR	R	–	S	S	VR	R-MR, MS	MS
Aus30299	MR	MR	R	MR	MR	–	MS	S	VR	R-MR, MS-S	MS-S
Aus30300	R	MS-MR	R	MR	MS	–	MS	R	MR	VR	VR
Aus30301	MR	MR-MS	R	MR	MR	–	S	S	VR	VR	VR
Aus30302	R	MS-S	S	MR	MR	–	S	S	R-MR	VR	R-MR
Aus30303	S	MR	MR	R	MR	–	S	S	MR-MS	MR-MS, R-MR	MR-MS
Aus30304	MR	MR	R	MR	R	–	MR	S	S	MR-MS	R-MR
Aus30305	MR	MR	R	MS	MS	–	MR	S	S	MS-S, R-MR	MR-MS
Aus30625	S	R-MR	R	–	MS	MS	M	S	R	R	R-MR
Aus30626	MR	MR	R	–	MR	R	S	S	MS-S	MS-S	R
Aus30627	R	MR-MS	R	–	S	S	S	S	MS	R-MR	R
Aus30628	S	MR	R	–	MS	S	S	S	S	R	MR-MS
Aus30629	S	MR	R	–	MS	S	M	S	S	R	MS
Aus30630	S	MR	R	–	MS	S	S	S	S	R	MR-MS
Aus30631	MR	–	R	–	S	S	MS	S	R	R	MS
Aus30632	S	S	R	–	S	S	M	S	R	R-MR	MS
Aus30633	S	MR	R	–	MS	S	M	S	MS	R	MR-MS
Aus30634	R	–	–	–	–	–	–	–	–	–	–
Aus30635	S	R-MR	R	–	S	S	M	S	S	R	VR
Aus30636	MR	MR	R	–	S	S	M	S	R-MR	R	MR-MS
Aus30637	MR	MR	R	–	MS	MS	S	S	S	R-MR	MS
Aus30638	S	MR	R	–	MS	S	M	S	S	MR	R

(*Continued to next page*)

Appendix 1. (*continued*)

SHWs ID	CCN	YLS	STB	SNB seedling	SNB adult	SNB glume	RLN *P. neglectus*	RLN *P. thornei*	Leaf rust	Stem rust	Stripe rust
Aus30639	R	MR	R	–	S	MS	M	S	MS	R-MR	R-MR
Aus30640	S	MR	R	–	MS	S	M	S	MS-S	R-MR	MS
Aus30641	S	MR-MS	R	–	S	S	MR	S	–	R-MR	MS
Aus30642	S	MR-MS	R	–	MR	S	S	S	R	MS-S	VR
Aus30643	S	MS	R	–	S	S	M	S	R	MR	MS
Aus30644	R	MR-MS	R	–	MS	MS	M	S	MS-S	MS-S	VR
Aus30645	S	MR	R	–	MS	MR	MS	S	MS-S	R-MR	MS-S
Aus30646	R	–	R	–	MS	MS	M	S	S	–	–
Aus30647	MR	MR	R	–	MS	S	M	S	MR-MS	MR	MR-MS
Aus30648	S	R-MR	R	–	MS	S	S	S	R-MR	MR-MS	MS-S
Aus30649	R	MR	R	–	MS	S	S	S	S	MS-S	MR-MS
Aus30650	S	–	R	–	MS	S	M	S	MS-S	R-MR	MR-MS
Aus30651	MR	MR	R	–	MS	MS	S	S	S	MR	MR-MS
Aus30652	MR	R	R	–	MS	MS	MS	S	S	R-MR	MR-MS
Aus30653	S	MR	R	–	MS	S	MS	S	R-MR	MS-S	MR-MS
Aus30654	R	MR-MS	–	–	S	MS	S	R	–	–	–
Aus30655	S	MR	R	–	MS	MR	MR	S	MR-MS	R-MR	MR-MS
Aus30656	S	MR-MS	R	–	MR	R	S	S	R	R-MR	R
Aus30657	S	MR	R	–	S	S	S	S	S	R-MR	MR-MS
Aus30658	S	MR	R	–	MS	MS	S	S	R-MR	MR	MS-S
Aus30659	MR	MR-MS	R	–	S	MS	MS	S	S	MR	MS-S
Aus30660	S	MR	R	–	S	MS	M	S	S	–	VS
Aus30661	S	MR	MS	–	S	MS	MS	S	R	MR	MR-MS
Aus30662	S	MR	R	–	MS	MS	S	S	R	MR-MS	MR-MS
Aus30663	R	MR	R	–	MS	S	M	S	S	MR	MR
Aus30664	S	MR	R	–	MS	MS	MR	S	S	R-MR	R-MR
Aus30665	MR	MR	R	–	S	MS	M	S	S	MR	R-MR
Aus30666	MR	MR-MS	R	–	S	MS	M	S	S	MR-MS	MS-S
Aus30667	MR	MR	R	–	S	MS	M	R	S	MR	MS
Aus30668	S	MR-MS	R	–	MS	MS	M	S	S	MR	MS
Aus30669	S	MR-MS	R	–	S	MS	MS	S	S	MR	MS
Aus30670	S	MR	R	–	MR	MS	S	S	MR-MS	R-MR	MS
Aus30671	S	MS	R	–	MS	S	S	S	–	–	–
Aus30672	R	MR-MS	R	–	MS	MS	MS	S	R-MR	MR	MS
Aus30673	R	MR-MS	R	–	MS	MS	M	S	S	R-MR	MR
Aus33376	S	MR	MR	–	MR	MS	S	S	MR, VS	–	R-MR
Aus33377	S	MR	MR	–	MS	MS	MS	S	MS	–	MR
Aus33378	S	MR-MS	R	–	MS	MS	MS	S	MS	–	R
Aus33379	S	MR-MS	R	–	S	MS	MR	R	MS-S	–	R
Aus33380	S	MR-S	–	–	MS	S	M	R	MS	–	R
Aus33381	S	MR	R	–	MR	MS	MS	R	MS-S	–	MR-MS
Aus33382	S	MR	MR	–	–	–	MR	S	R	–	R
Aus33383	S	MR	MR	–	S	S	MS	S	R-MR	–	R
Aus33384	S	MR	R	–	MR	R	MS	R	MR	–	R
Aus33385	S	MR	MR	–	MR	MS	MS	R	VS	–	R
Aus33386	S	MR	MR	–	MS	MS	MS	R	VS	–	MR
Aus33387	S	MR-S	MR	–	MR	MR	S	S	MS-S	–	MR, VS
Aus33388	S	MR	R	–	MS	S	S	R	S	–	R
Aus33389	MR	MR	R	–	S	MS	M	S	R-MR	–	MR-MS
Aus33390	S	MR	MR	–	MR	MS	M	R	MS	–	MR
Aus33391	S	S	MR	–	MR	MS	M	R	R-MR, VS	–	R
Aus33392	S	MR	MR	–	MS	MS	MS	R	MR	–	R
Aus33393	S	S	MR	–	MS	MS	MS	R	–	–	–
Aus33394	S	S	MR	–	–	–	MS	R	VS	–	R
Aus33395	S	S	R	–	MR	MS	S	R	VS	–	MR
Aus33396	S	MR-MS	R	–	MS	MS	S	S	S	–	MR, S
Aus33397	S	MR	MR	–	–	–	MR	–	VS	–	MR-MS
Aus33398	S	MR	MR	–	MR	MS	MR	S	MR-MS	–	R-MR, S
Aus33399	S	MR	MR	–	–	–	MS	R	MR-MS	–	MR-MS
Aus33400	S	MR	R	–	MS	MS	M	S	–	–	–

Appendix 1. (*continued*)

SHWs ID	CCN	YLS	STB	SNB seedling	SNB adult	SNB glume	RLN *P. neglectus*	RLN *P. thornei*	Leaf rust	Stem rust	Stripe rust
Aus33401	–	–	–	–	–	–	S	–	MR-MS	–	R
Aus33402	S	MR-MS	MR	–	MR	MR	M	R	R-MR	–	R
Aus33403	R	MR-MS	MR	–	MS	MR	S	R	R-MR	–	R
Aus33404	S	MR-S	MR	–	MR	MR	M	R	VS	–	MS
Aus33405	S	R	MR	–	S	MS	R	R	S	–	R
Aus33406	S	MR	R	–	MS	MS	MS	S	VS	–	R
Aus33407	S	MR-MS	R	–	MS	MR	S	S	R-MR, MS-S	–	R
Aus33408	S	MR	R	–	MR	S	M	R	R-MR	–	R-MR
Aus33409	S	MR-MS	R	–	MS	MS	S	S	–	–	MS
Aus33410	S	R-MR	R	–	MS	MS	MR	R	MS	–	R-MR
Aus33411	MR	MR	R	–	MR	MS	M	R	–	–	MS-S
Aus33412	S	MR-MS	R	–	MS	MR	MS	R	MS-S	–	R
Aus33413	S	MR	R	–	MR	MS	MS	S	R-MR	–	R
Aus33414	S	S	MS	–	–	MS	M	S	MR	–	MS-S
Aus33415	S	MR	R	–	S	MS	MR	R	–	–	–
Aus33416	S	MR	R	–	–	–	MR	–	MR, VS	–	R
Aus33417	S	MR	MR	–	MS	S	S	R	–	–	–
Aus33418	S	MR-MS	MR	–	–	–	S	–	MR-MS	–	MS-S
Aus33419	S	S	R	–	MR	MS	M	S	S	–	MR
Aus33420	S	S	R	–	MS	MS	M	R	–	–	–
Aus33421	S	MR-S	R	–	–	–	S	–	MR	–	R-MR
Aus33422	S	MR-MS	MR	–	MR	MS	S	S	–	–	–
Aus33423	S	MR	R	–	–	–	S	–	R	–	R
Aus33424	S	MR-MS	MR	–	MR	MS	MS	R	MR	–	R-MR

VR, Very resistant; R, resistant; MR, moderately resistant; LM, light moderate; M, moderate; HM, high moderate; MS, moderately susceptible; S, susceptible; VS, very susceptible.