Synthetic hexaploid wheats for resistance to root-lesion nematodes

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INTRODUCTION

Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*), attack wheat roots, causing inefficient uptake of soil nutrients and water, resulting in chlorosis, stunting, and reduced tillering, biomass, and grain yield[1]. These two species occur widely and losses to the Australian grains industry are estimated at $260m/annum[2,3]. Wheat lines with tolerance to *P. thornei*, the dominant species in northern Australia, have been developed[1] but they still allow nematodes to multiply in their roots. Because *P. thornei* completes a life cycle in 4-6 weeks under favourable conditions, populations can build rapidly on susceptible wheat. One tolerant selection (GS50a) from the intolerant variety Gatcher, has been shown to have resistance with the nematodes in its roots exhibiting a lower rate of reproduction. It is highly desirable to obtain more effective and diverse sources of resistance for effective long-term control. Resistance to cereal cyst nematode (CCN, *Heterodera avenae*), which has been a problem in southern Australia has been found in *Aegilops tauschii*[4] the D-genome donor to bread wheat. This paper reviews initial research[5] to identify resistance to *P. thornei* in *Ae. tauschii,* then subsequent research[6,7,8,9,10] to identify and characterise resistance in synthetic hexaploid wheats (AABBDD) produced from the hybridisation[11] of *Ae. tauschii* with durum wheats (AABB).

MATERIALS AND METHODS

Nematode Resistance Testing

All nematode resistance testing[1] was undertaken in replicated glasshouse experiments using methods that have been calibrated so as to rank varieties similar to field results obtained in the deep black soils of the northern region of Australia. Pots of partially sterilised vertisolic soil were inoculated with a pure culture of *P. thornei* or *P. neglectus* and a single plant per pot was grown for 16 weeks at 22°C. In earlier experiments[5,6] pots were top watered to return the soil to field capacity periodically, while in later experiments[6,7] water tension was kept at a constant 2 cm by bottom capillary matting. After 16 weeks nematodes were extracted from the soil containing roots and were counted under a compound microscope. The number of nematodes/kg soil plus roots was used as a measure of resistance/susceptibility. These counts were transformed by ln (x+C) to normalise residuals for ANOVA and were compared with standard wheats of established resistance/susceptibility levels.

Plant material

The following material was tested.

(a) 244 Accessions of *Ae. tauschii* from Central Asia, part of the Kihara collection held at the Australian Winter Cereals Collection Tamworth[5].

(b) 164 synthetic hexaploid wheats from CIMMYT and 22 CCN-resistant synthetic hexaploids from R. F. Eastwood, formerly DPI Victoria[6].

(c) 32 durum and 53 *Ae. tauschii* parents of the above synthetic hexaploids[6]

(d) 11 F1 hybrids between 11 resistant synthetic hexaploids and Janz, a *P. thornei*-susceptible Australian variety[6].

(e) 10 BC1F2:6 derivatives of two CCN-resistant synthetic hexaploids backcrossed into the Australian wheat varietiy Meering and 12 F6 derivatives of CCN-resistant *Ae. tauschii* and Condor/Meering[6]. All derivatives had been selected for CCN resistance conferred by *Cre3*[4].

(f) F1s and F2s from a half diallel of crosses between 5 nematode-resistant synthetic hexaploids, GS50a and Janz[7].

(g) A doubled haploid mapping population derived from an F1 cross between the nematode-resistant synthetic hexaploid CPI 133872 and Janz[8].

(h) The International Triticeae Mapping Initiative (ITMI) population of recombinant inbred lines from a cross between the synthetic hexaploid W-7984 and the bread wheat Opata[9].

RESULTS AND DISCUSSION

Aegilops tauschii

Out of the 244 accessions of *Ae. tauschii* 39 were found to be more resistant to *P. thornei* than the bread wheat GS50a in two experiments[5]. Resistance was relatively more common in accessions of *Ae. tauschii* spp. *strangulata* (50%) than in *Ae. tauschii* spp. *tauschii* var. *meyeri* (14%), or in *Ae. tauschii* spp. *tauschii* var. *typica* (9%).

Synthetic Hexaploid Wheats and their Durum and *Ae. tauschii* parents

Out of the 186 accessions of synthetic hexaploid wheats 59 (32%) were found to be more resistant to *P. thornei* than the bread wheat GS50a in a first experiment[6]. In this experiment a higher frequency of resistance was evident in the durum parents (72% of 39 lines with lower counts than GS50a) and in the *Ae. tauschii* parents

(55 % of 53 lines). Examination of the results indicated that, in general, synthetic hexaploids with only one resistant parent were not as resistant as those with both parents resistant. Thus resistance genes from both the D genome and the A and/or B genome were contributing to resistance in the synthetic hexaploid.

Derivatives of Synthetic Hexaploids and Bread Wheats

F1 Hybrids of Synthetic Hexaploids with Janz

The F1 hybrids between resistant synthetic hexaploids and the susceptible bread wheat Janz had intermediate levels of resistance to *P. thornei* compared with the two parents.

CCN-Resistant Derivatives

When the accessions of *Ae. tauschii* were tested[5] a number with the *Cre3* gene for resistance to CCN[4] were also found to be resistant to *P. thornei.* Consequently, a number of bread wheat derivatives that had been positively selected through several generations for CCN resistance conferred by *Cre3* were tested for *P. thornei* resistance. These derivatives were found to be susceptible to *P. thornei* indicating the resistance to *P. thornei* in the original *Ae. tauschii* accessions was from genes other than *Cre3.*

Half diallel study of *P. thornei* resistance

From results of the above experiments five synthetic hexaploid wheats were selected for further study based on (a) the synthetic hexaploid and both of its durum and *Ae. tauschii* parents were consistently more resistant than GS50a in repeated experiments, (b) the *Ae. tauschii* parents represented the various subspecies and varieties of the species, and (c) the durum parents represented both CIMMYT and Australian material. A half diallel of crosses was made with these five resistant synthetic hexaploids, GS50a and Janz.

In tests of F1s and F2s of the half diallel for *P. thornei* resistance, general combining ability (GCA) was shown to be more important than specific combining ability[7]. All five synthetic hexaploids had better GCA than GS50a, with synthetic hexploid CPI133872 having the best GCA. It was concluded that inheritance of *P. thornei* resistance was polygenic and additive in gene action.

Resistance to *P. neglectus*

The five synthetic hexaploids with superior resistance to *P. thornei* were found to have excellent resistance to *P. neglectus* (Figure 1). This contrasted with GS50a and derivatives, which although resistant to *P. thornei* were found to be quite susceptible to *P. neglectus.* Likewise, some land-race wheats from WANA (West Asia and North Africa) countries that had been found to be resistant to *P. thornei* were susceptible to *P. neglectus.*

QTL Analyses

A doubled haploid mapping population from a cross between CPI133872 and Janz was created and phenotyped for resistance to *P. thornei* and *P. neglectus*[8]. A map with 148 wheat microsatellite markers and 21 amplified fragment length polymorphism markers was created. A codominant microsatellite marker *Xbarc183* on the distal end of chromosome 6DS was allelic for resistance to both *P. thornei* (from CPI133872) and *P. neglectus* (from Janz). By composite interval mapping a second major QTL for *P. thornei* resistance was identified on chromosome 6DL and another for *P. neglectus* resistance on 4DS.

In parallel work, the ITMI population was characterised for *P. thornei* resistance in two experiments. QTLs for resistance to *P. thornei* coming from the synthetic parent were located on chromosomes 2BS and 6DS[1,9].

Recent addition of 242 DArT markers to the CPI133872/Janz map has confirmed stable QTLs for *P. thornei* resistance on chromosomes 2BS, 6DS and 6DL and for *P. neglectus* resistance on 2BS and 6DS[10]. The markers for *P. thornei* and *P. neglectus* were in separate locations on 2BS but were coincident on 6DS.

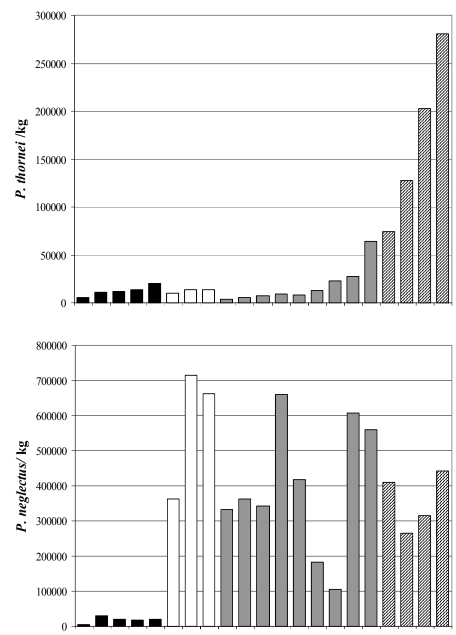

Figure 1. Comparison of resistance level to both *P. thornei* and *P. neglectus* of synthetic hexaploid wheats (black), GS50a and derivatives (white), bread wheats collected from West-Asia North-Africa (grey) and commercial wheat cultivars (striped).

CONCLUSIONS

These studies have shown that synthetic hexaploid wheats are a rich source of resistance genes to root-lesion nematodes, both *P. thornei* and *P. neglectus*, that could be profitably used in the Australian wheat industry. In studies with CPI 133872/Janz and the ITMI populations, chromosomes 2B and 6D have consistently been found to bear QTLs for resistance to both nematode species, but additional minor QTLs are indicated. The resistance appears to be additive and greater when resistances from both the B and D genomes are present in the synthetic hexaploid.

The five synthetic hexaploid wheats identified in these studies offer a chance to effectively solve problems with root-lesion nematodes in the Australian wheat industry. They have multiple genes for resistance to both *P. thornei* and *P. neglectus* which will expedite resistance breeding to obtain varieties with resistance to both species. This is a great advantage as there are many field locations in which both species occur.

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