

Chapter 21

Germplasm Enhancement for Resistance to *Pyrenophora tritici-repentis* in Wheat

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Abstract Yellow spot (syn. tan spot), caused by *Pyrenophora tritici-repentis*, is an important foliar disease of wheat in Australia that causes losses exceeding 50 % when conditions are favourable for disease development. Although good progress has been made internationally to understand yellow spot resistance, relatively few resistance genes have been identified and mapped in Australian germplasm and only one (*tsn1* on chromosome 5BL) is in general and known use in Australian breeding programs. Although *tsn1* is an important yellow spot resistance gene, it doesn't explain the full spectrum of resistance and there is a significant opportunity to enhance expression of yellow spot resistance through identification of resistance factors other than *tsn1*. Six doubled haploid (DH) mapping populations (five of which were fixed for *tsn1*) were screened for yellow spot resistance at the seedling/ tillering and adult plant stages at the Department of Agriculture and Food, Western Australia (DAFWA) and the Department of Environment and Primary Industries Victoria (DEPIVic) from 2009 to 2012. Four of the above populations were screened at the Department of Agriculture, Fisheries and Forestry Queensland (DAFFQ). Frequency distribution of individuals within each population for various levels of yellow spot resistance was continuous indicating that resistance is conditioned by several genes with partial effects. A few lines within each population consistently showed high levels of resistance probably resulting from a combination of several genes with additive effects. Nine new loci for yellow spot resistance were mapped

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by the Australian Wheat and Barley Molecular Marker Program (AWBMMP) at the University of Adelaide on chromosomes 1AS, 2AS, 5AS, 5AL, 4B, 6BS, 7BL, 2D and 7D in five of the six populations phenotyped so far. High LOD scores of 9–11 have been obtained for some of the QTL with a percentage disease reduction of 24–42 %. Efforts are now focused on identifying additional yellow spot resistance genes using newly developed populations and rapid phenotyping methods and developing a series of fixed lines, each carrying yellow spot resistance genes from various sources, in elite Australian backgrounds. These materials will provide proof-of-concept for achieving better resistance by pyramiding resistance genes, and they should be directly useful as parents for wheat breeding.

Keywords Phenotyping • Quantitative trait loci • Resistance • Tan spot • Yellow leaf spot

Pyrenophora tritici-repentis is a necrotrophic fungus that causes yellow spot of wheat. The disease is also known as yellow leaf spot or tan spot. There has been an increased prominence of this disease in recent years due to implementation of new farm practices such as minimum tillage, stubble retention, increased wheat-after-wheat cropping and use of susceptible varieties. In Australia, Shipton (1968), Rees et al. (1982), Rees and Platz (1983), and Bhathal et al. (2003) measured 20–50% losses in grain yield of bread wheat from this disease. In Western Australia this disease often occurs as a complex with *Stagonospora nodorum* blotch.

P. tritici-repentis is known to produce at least three host specific toxins (HSTs) that interact with specific host sensitivity genes to cause necrosis and/or extensive chlorosis (Ali et al. 1999; Lamari et al. 1995, 2005; Strelkov et al. 2002). These are Ptr ToxA, for which sensitivity is conditioned by the gene *Tsn1* on chromosome arm 5BL (Anderson et al. 1999; Faris et al. 1996); Ptr ToxB, for which sensitivity is conditioned by the gene *Tsc2* on chromosome arm 2BS (Friesen and Faris 2004), and Ptr ToxC, for which sensitivity is conditioned by the gene *Tsc1* on chromosome arm 1AS (Effertz et al. 2002). Isolates of the pathogen can be classified into eight theoretical races based on the presence or absence of each of the three toxins. All eight of these combinations have been found among North American isolates. Recently, however, ToxA and ToxB gene-deficient isolates from Arkansas have been found not to fit this race classification system (Ali et al. 2010). These isolates may contain different toxin compounds that produce ToxA-like symptoms on some cultivars of wheat.

Improved resistance to the disease is the most economical and effective method of control and is therefore a key target for Australian wheat breeding programs. Resistance can be both qualitative (Gamba and Lamari 1998; Lamari and Bernier 1989) and quantitative (Elias et al. 1989; Friesen and Faris 2004). Genes such as *tsn1*, *tsn2*, *tsn3*, *tsn4*, *tsn5*, *tsn6*, and *tsn-syn1* and *Tsn-syn2*, are known to affect toxin sensitivity and to confer race-specific resistance to necrosis (Anderson et al. 1999; Singh et al. 2006, 2008; Tadesse et al. 2006a, b, 2010) and the recessive genes *tsc1* and *tsc2* condition resistance to chlorosis (Abeysekara et al. 2010; Effertz et al.

2002; Friesen and Faris 2004). Non-race-specific quantitative trait loci have been mapped on almost all chromosomes of wheat (Chu et al. 2008, 2010; Faris and Friesen 2005; Faris et al. 2012; Li et al. 2011; Singh et al. 2012; Sun et al. 2010; Patel et al. 2013). Incorporating both qualitative and quantitative resistance is essential to develop cultivars with high levels of durable resistance.

Although good progress has been made internationally to understand yellow spot resistance, only one gene (*tsn1* on chromosome 5BL) is in general and known use in Australian breeding programs. While *tsn1*, is an important YS resistance gene, it doesn't explain the full spectrum of resistance in the Australian germplasm. Furthermore, Faris et al. (2012) have shown that the amount of variation explained by *tsn1* can vary considerably (5–30 %) with different isolates and suggest possible variability in ToxA gene regulation amongst isolates.

The Department of Agriculture and Food Western Australia (DAFWA) has been conducting research for the past 3 years on genetic enhancement of resistance to the disease and improved germplasm development under a national project involving several collaborating organisations and co-funded by the Grains Research and Development Corporation. The project aims to provide improved genetic solutions to yellow spot management by: (i) development of improved phenotyping methods, (ii) identification of new resistance genes, (iii) improved germplasm and associated genetic knowledge that enhance resistance expression and diversity and (iv) improved parental stocks which can be readily utilised within breeding programs.

Phenotyping methods have been improved in three areas. Firstly, enhanced spore production techniques have been developed based on a modified method of Evans et al. (1993) which allow the production and storage of large amounts of yellow spot inoculum. This has greatly improved inoculation of a large number of plots both in the field and glasshouse. Secondly improved field phenotyping methods have been developed based on Shankar et al. (2008) which overcome the effect of maturity and height on disease expression allowing the assessment of true adult plant resistance. This basically involves inoculating individual plots at heading and rating at a specific thermal time after inoculation. Thirdly, a rapid assay for evaluating adult plant resistance has been developed under controlled environmental conditions under which the same plant can be assessed at both seedling and adult stages. Under this method plants head within 5–8 weeks under controlled environment as compared to 11–16 weeks in the field. Moderate to good correlations ($r=0.7-0.8$) are obtained between assessments made under controlled environmental conditions and field conditions.

Twelve doubled haploid mapping populations have been developed or made available to the project. Of these, six populations (five of which were fixed for *tsn1*) were phenotyped for yellow spot resistance from 2009 to 2012 at the seedling/tillering and adult plant stages at DAFWA and the Department of Environment and Primary Industries Victoria (DEPIVic). Four of the above populations were phenotyped at the seedling/tillering and adult plant stages at the Department of Agriculture, Fisheries and Forestry Queensland (DAFFQ). Frequency distribution of individuals within each population for various levels of yellow spot resistance was continuous indicating that resistance is conditioned by several genes with partial effects. A few

lines within each population consistently showed high levels of resistance probably resulting from a combination of several genes with additive effects.

Genetic maps were developed by the Australian Wheat and Barley Molecular Marker Program (AWBMMP) at the University of Adelaide using DArT, SSR and SNP markers. Nine major QTL other than *tsn1* have been mapped on chromosomes 1AS, 2AS, 5AS, 5AL, 4B, 6BS, 7BL, 2D and 7D in five of the six populations phenotyped so far. Of these, QTL on 2A and 6B appear commonly between two populations but it is yet to be determined if they are at the same chromosomal positions. Statistical analyses have been completed on two of the populations and good correlations ($r=0.6-0.8$) and high heritability estimates ($H^2=0.7-0.9$) were obtained for yellow spot severity at various growth stages, environments and sites. High LOD scores of 9–11 have been obtained for the resistance QTL detected in these populations with a percentage disease reduction of 24–42 %.

Germplasm development is being carried out collaboratively by DAFWA and AWBMMP. The strategy involves pyramiding at least three resistance loci from different sources into elite Australian backgrounds. In a first set of crosses QTL on 2A and 5BL detected in one population are being pyramided with QTL on 1A detected in another population. Resistant parents were selected using both phenotypic and genotypic data and a cross was made in the spring of 2011. Although all the 100 F₁s produced turned out to be grass clumps 194 F₂ seeds were produced off these grass clumps by growing them at a constant 26 °C temperature and gibberellic acid treatment. The F₂s were then grown out in single seed trays and leaf material sent to AWBMMP for marker-assisted selection. F₂ and F₃ segregation indicated that the grass clump trait was controlled by two to three complementary genes.

Currently 16 F₄ lines, homozygous at the three resistance loci as well as fixed at the *Vrn-A1* locus have been selected. Fourteen of these are mid to late maturing while two are early maturing. In addition 28 other F₄ lines, also homozygous at the three resistance loci but segregating for either grass clumps or at the *Vrn-A1* locus, have been selected. These are being further selected against the grass clump trait and are being fixed for the *Vrn-A1* locus. All F₄/F₅ selections will be grown out for a bulk F₅/F₆ harvest and phenotyped for proof of concept. These are potential parental stocks enriched for resistance in wheat breeding.

This year crosses are being made to pyramid up to five resistance loci in both short season and long season backgrounds by crossing lines with three stacked genes with parental selections involving two additional genes from other available populations.

A spin off research activity under gene pyramiding for yellow spot resistance is developing sister lines with different resistance QTL combinations. For this, individuals which are heterozygous at the three resistance loci are being selected in each generation while allowing the rest of the genome to become homozygous. The aim is to obtain different QTL combinations within sister lines so that effects of individual loci and loci in various combinations can be compared within a similar background. Currently 25 F₄ lines heterozygous at the three resistance loci have been selected and will be further selected in F₅ for various QTL combinations.

In another activity within the project, genetic variability within the Australian germplasm is being combined by developing a large multiparent advanced generation intercross (MAGIC) population from a complex cross involving different sources of yellow spot resistance. The four parents include GBA Ruby, H45, King Rock and Magenta. The four-way cross was completed last spring and 583 F₁s produced. Two F₂ seeds from each of these F₁s are being progressed through SSD to F₅. Four way crossing is continuing this year with the aim to produce a total of around 2,000 F₅ lines. This population will provide a unique opportunity to maximise genetic recombination, generate potential parental stocks enriched for yellow spot resistance and create opportunities for fine mapping resistance loci.

Another major activity within this project is identification of elite yellow spot resistant lines amongst material acquired from CIMMYT and ICARDA and amongst various mapping populations. Of over 1,000 lines screened so far 42 lines representing targeted yellow spot resistant material were tested under a ring test at various growth stages across Australia and 19 of these have been identified as having good levels of broad spectrum yellow spot resistance.

New resistance genes, germplasm and screening methodologies developed under this project are important resources that can be used by breeders for rapid development of varieties with high levels of resistance at both seedling and adult plant stages that is effective in various environments and across various national sites. Newly developed resistant varieties will greatly reduce the estimated annual losses of \$212 M per annum caused by this disease in Australia (Murray and Brennan 2009).

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