Stacking yellow spot resistance genes into fixed lines results in genetic gain

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Key Messages

- The identification and combining of new yellow spot resistance genes will eventually provide farmers with varieties with improved resistance to this important foliar disease of wheat.
- Nine new genes for yellow spot resistance have been detected on chromosomes 1AS, 2AS, 2D, 4B, 5AL, 5AS, 6BS, 7BL and 7D in five of the nine mapping populations screened at various growth stages, environments and national sites.
- Resistance genes on chromosomes 1AS and 2AS have been successfully stacked along with known resistance gene *tsn1* in 32 fixed lines.
- Very resistant individuals identified amongst sixteen of the 32 lines screened for yellow spot resistance at various growth stages and environments indicate additive gene interactions and significant genetic gain.

Introduction

Pyrenophora tritici-repentis is a necrotophic fungus that causes yellow spot (YS) of wheat. The disease is also known as yellow leaf spot or tan spot. Although good progress has been made internationally to understand yellow spot resistance in wheat, 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. Under the current national project a major effort has been made to improve the understanding of genetics of YS resistance in present and future donors, identify novel quantitative trait loci (QTL) for resistance and develop a series of fixed lines, each carrying YS resistance genes from 2 or 3 resistance sources in elite Australian backgrounds that can be used as parents in resistance breeding.

Nine doubled haploid (DH) mapping populations were screened for YS resistance at various growth stages, environments and national sites from 2009 to 2013. Five of these populations (also fixed for *tsn 1*) were genotyped by the Australian Wheat and Barley Molecular Marker Program for marker trait associations and nine new resistance loci (genes) were mapped on chromosomes 1AS, 2AS, 2D, 4B, 5AL, 5AS, 6BS, 7BL and 7D (Fig. 1).

Methods

Resistance loci on 1AS detected in mapping population IGW2574/Annuello and 2AS detected in mapping population Calingiri/Wyalkatchem (Fig. 2) were stacked along with *tsn1* on 5BL (present in both parents of the Calingiri/Wyalkatchem population) using single seed descent (Fig. 1). Resistant parents were selected from the two populations using both phenotypic and genotypic data and a cross was made in the spring of 2011. F_1s turned out to be grass clumps and were treated with gibberellic acid to yield 194 F_2 seed. F_2s were progressed through to F_4 while being subjected to marker assisted selection (MAS). Thirty two F_5 lines have been developed which are triple homozygotes at the three YS resistance loci and are also fixed for the *vrn-1* locus. These fixed lines are both in short season and long season backgrounds. Concurrently isogenic sister lines with various YS resistance gene combinations are also being developed for validation purposes. Sixteen of the 32 F_5 lines carrying three YS resistance genes 1AS, 2AS and *tsn1* were phenotyped at various growth stages and environments at South Perth for proof of concept that YS resistance can be enhanced by combining *tsn1* with additional resistance genes.

Field assessment 2014. The trial was conducted in an irrigated field nursery in a randomised block design with three replications at South Perth. Lines were sown as paired 10 cm rows of up to 10 seeds per row sown 10 cm apart, separated by 30 cm centres from adjacent rows. Plots were fertilised with a mixture of superphosphate, urea and potash (6:4:1) at a dose rate of 100 kg/ha at planting and at 8 weeks after sowing.

Plots were protected from powdery mildew infection with 250 g/ha of Quinoxyfen and 125 g/ha Bupirinate applied at 4 weekly intervals for 12 weeks. Infection was established by inoculating individual plots at half head emergence (Feekes stage 10.3) by spraying flag leaves with the conidial suspension to run-off. High humidity was created by watering the site just before inoculation and covering individual plots with plastic bags secured over PVC rings (15 cm high, 30 cm diameter) for 48 h after inoculation (Shankar et al 2008). Before covering the plots, the plastic bags were misted internally with water. Inoculated plants were shaded from direct sunlight during moist incubation by further covering the plastic bags with shade cloth bags (84-90% cover factor). Percentage leaf area diseased (PLAD) was scored at 380°C and at 560°C thermal days (sum of average daily temperatures) after inoculation. Flag leaves of five individual plants were scored at both times and the average for each plot was calculated and used for analysis.

Controlled environment assessment 2014. Lines were grown under controlled environment with 24/22°C day/night temperatures in 120-mm-diameter pots containing a sand-loam mix with 1 g of Osmocote (slow release fertiliser). The experiment was conducted in a randomised block design with 3 replicate pots. Four seeds per line were planted in each pot. Seedlings were spray inoculated to run-off at the two-and-a-half-leaf stage with the conidial suspension. Inoculated seedlings were incubated for 24 h in a humidifier and disease assessed on a 0-5 scale, nine days after inoculation on the two lowest leaves that were fully emerged at inoculation. Immediately after rating the seedlings, plants were provided with a 22 h photoperiod consisting of 12 h of natural day light and 10 h of high pressure sodium light with an active radiation of 400-500 µE/m^2/s. Plants were fertilized with soluble all-purpose Thrive (Yates) at a concentration of 0.8 g/L and a rate of 60 mL/pot on a weekly basis and with a trace element solution of Liberal BMX (BASF) at a concentration of 0.5 g/L and a rate of 30 mL/pot on a fortnightly basis. Flag leaves of individual pots were inoculated at half spike emergence (Feekes stage 10.3) as above and rated on a percentage scale 14 days after inoculation.

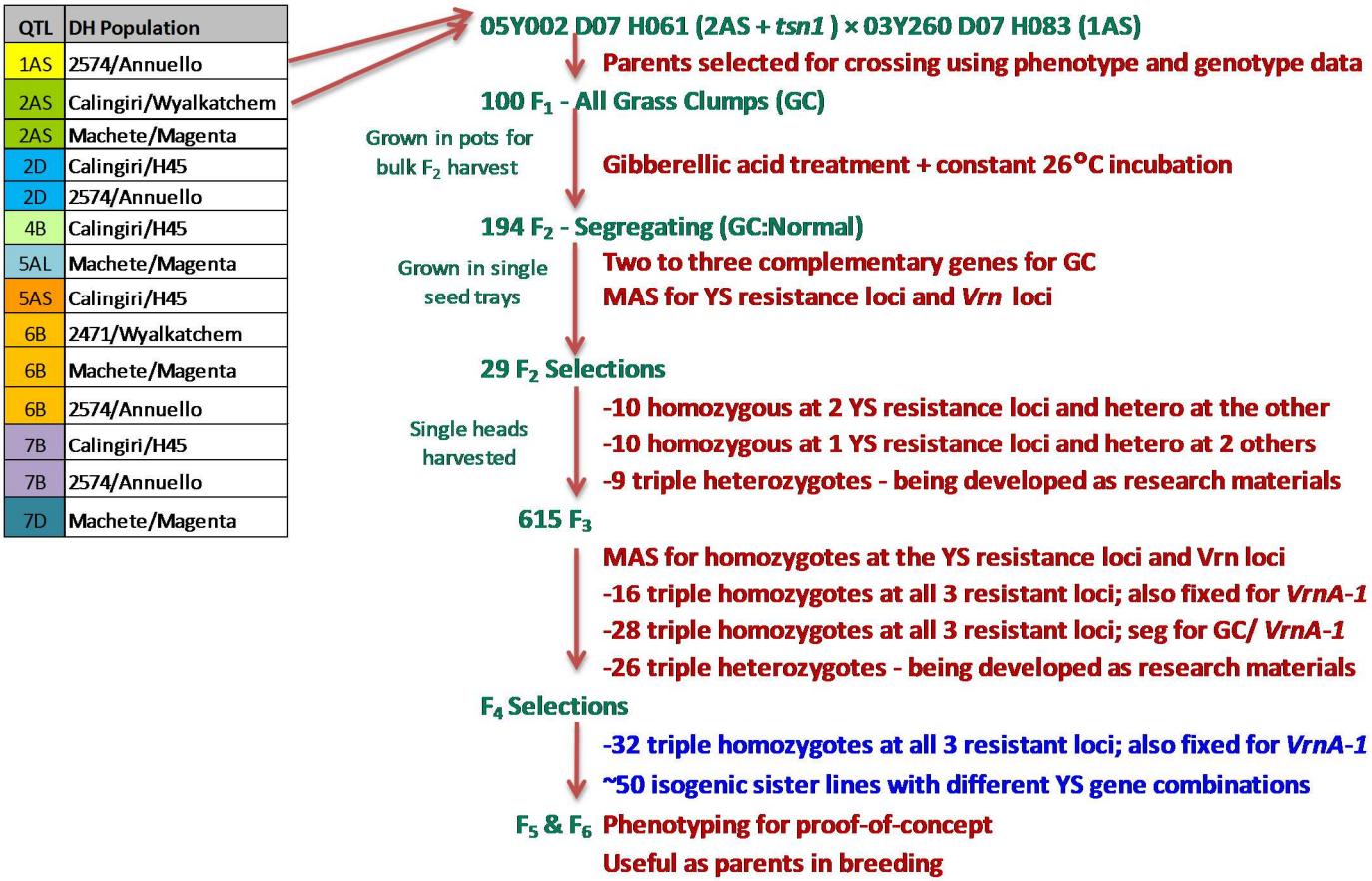

Fig. 1. Stacking of yellow spot resistance genes on chromosomes 1AS and 2AS with known resistance gene *tsn1* on chromosome 5BL using single seed descent

Results

Resistance at various growth stages and environments in 16 F_5 lines with stacked resistance loci are presented as blue bars in Fig. 3. Orange bars are individual selections from the two populations which were used in crossing for gene stacking while the red and green bars are the parents of the two DH populations. Some very resistant individuals amongst the F_5 lines indicate significant genetic gain as compared to the parents and grandparents. These lines will also be assessed under a ring test at various growth stages, environments and national sites during 2015 and are potential parental stocks enriched for resistance.

Conclusion

Genes for yellow spot resistance can be successfully combined into fixed lines using single seed descent and marker assisted selection. Fixed lines with stacked resistance genes express significantly higher resistance at both seedling and adult plant stages that is effective in various environments. These lines are important resources that can be used by breeders for rapid development of varieties with high levels of resistance. Newly developed resistant varieties will greatly reduce the estimated annual losses of $212M per annum caused by this disease in Australia (Murray and Brennan 2009).

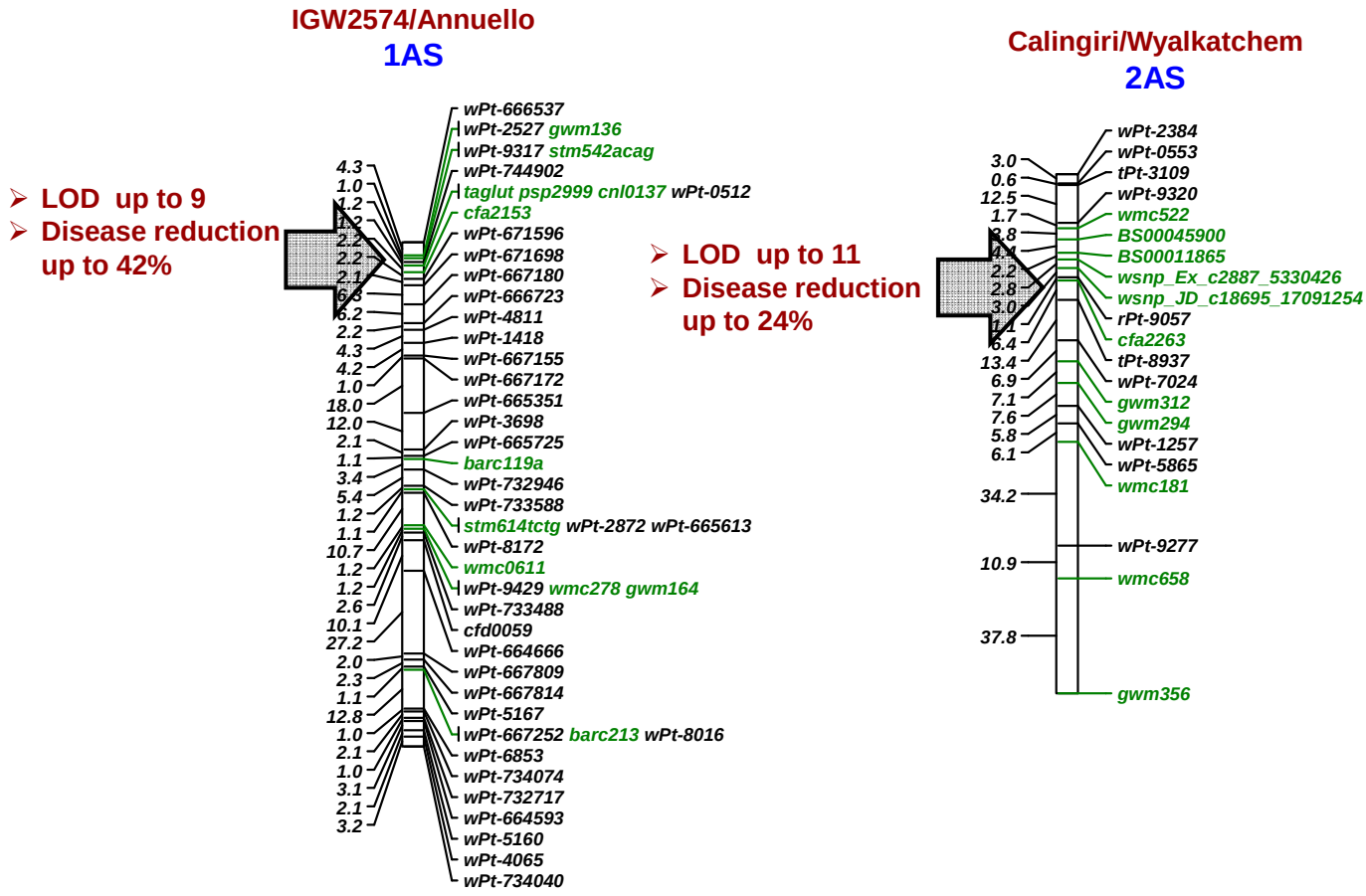

Fig. 2. QTL on 1AS in IGW2574/Annuello and 2AS in Calingiri/Wyalkatchem. Markers in green are SSRs, STSs or SNPs while those in black are DArT markers.

References

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Key words

Phenotyping, quantitative trait loci, resistance, tan spot, yellow leaf spot

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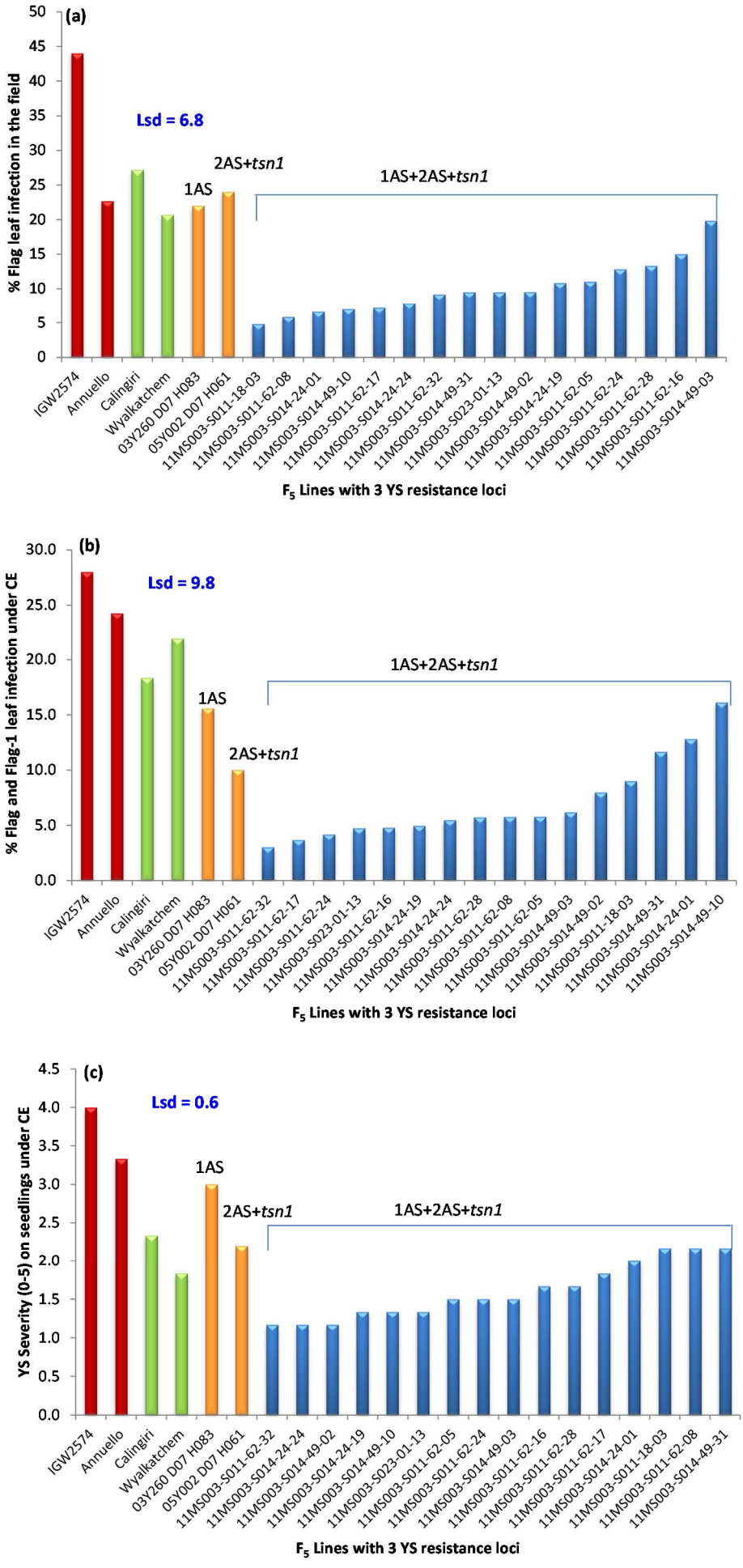

Fig. 3. Assessment of yellow spot resistance in 16 F_5 lines with stacked resistance loci at (a) adult stage in the field, (b) adult stage under controlled environment (CE) and (c) seedling stage under CE.