Genetics of rust resistance in the Australian wheat germplasm

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ABSTRACT

F_2 and $F_{2:3}$ populations targeting leaf rust resistance genes (*Lr13*, *Lr21 Lr28*) and stem rust resistance genes (*Sr32* and *Sr33*) were phenotyped for seedling resistance. In populations targeting *Lr13* (Leichardt/WAWHT2071), *Lr21* (Tincurrin+Lr21/EGA2248) and *Lr28* (Sunland/Arrino), parents Leichardt, Tincurrin+Lr21 and Sunland were resistant (R) while parents WAWHT2071, EGA 2248 and Arrino were susceptible (S) to leaf rust. F_2 progeny in crosses Leichardt/WAWHT2071 and Sunland/Arrino showed a 3R:1S segregation ratio (χ^2 = 0.3 and 1.3; *P* = 0.6 and 0.3) while F_3 families segregated as 1:2:1 (true breeding R (TR):segregating (seg):true breeding S (TS)) (χ^2 = 1.8 and 1.0; *P* = 0.4 and 0.6) indicating the single dominant nature of *Lr13* and *Lr28*. Population Tincurrin+Lr21/EGA 2248 targeting *Lr21* showed a 13R:3S F_2 segregation (χ^2 = 0.4; *P* = 0.5) indicating the presence of one dominant and one recessive independent genes. The hypothesis was confirmed in F_3 where families arising from resistant F_2 plants segregated in a ratio of 7:6 (TR:seg) while families from susceptible F_2 plants were all true breeding susceptible (χ^2 = 1.4; *P* = 0.5). In populations targeting *Sr32* and *Sr33* parents C77.19/3*77W:549-163658 and *Sr33*/2*Shortim//4*Jacup were used as the sources of resistance, respectively, while parents WAWHT2046 and Calingiri were susceptible to stem rust. The F_2 progeny in both crosses segregated into a 3R:1S ratio (χ^2 = 0.1 and 3.3; *P* = 0.8 and 0.1) and the F_3 families showed a segregation of 1:2:1 (TR:seg:TS) (χ^2 = 5.5 and 1.2; *P* = 0.1 and 0.6) indicating the single dominant nature of *Sr32* and *Sr33*.

INTRODUCTION

Resistance to stem rust (*Puccinia graminis* Pers. f. sp. *Tritici* Eriks. & E. Henn.) and leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. & Henn.; Prt) is of high priority in the InterGrain wheat (*Triticum aestivum* L.) breeding program. Breeding for durable resistance against these diseases is based on the combination of different resistance genes in one cultivar (Van Ginkel and Rajaram, 1993). The selection of genotypes containing several rust resistance genes using infection with rust isolates with defined avirulence genes is very time-consuming. The development of molecular markers for specific genes allows the detection of these genes independently of the phenotype. Detailed genetic knowledge increases the efficiency of development of molecular markers which can be used in marker-assisted selection for an efficient combination of genes in the pyramiding strategy to create a more durable resistance (Roelfs et al., 1992).

The objectives of this study were genetic analysis of F_2 and $F_{2:3}$ breeding populations for leaf rust and stem rust resistance and provision of phenotypic data for the development and validation of molecular markers linked to known rust resistance genes in the Australian germplasm.

MATERIALS AND METHODS

F_2 and $F_{2:3}$ populations were developed from the following crosses:

(a) Leichard/WAWHT2071 for targeting leaf rust resistance gene *Lr13*

(b) Tincurrin+Lr21/EGA2248 for targeting leaf rust resistance gene *Lr21*

(c) Sunland/Arrino for targeting leaf rust resistance gene *Lr28*

(d) C77.19/3*77W:549-163658//WAWHT2046 for targeting stem rust resistance gene *Sr32*

(e) Sr33(R.L.5405)/2*Shortim//4*Jacup/3/Calingiri for targeting stem rust resistance gene *Sr33*

Generation and management of plant material. Ninety four lines from each F_2 population and parental lines were grown in a glasshouse with 22/18°C day/night temperatures and natural lighting in 96-cell trays containing a sand-loam mix with 1 g of Osmocote (slow release fertiliser). A single seed was planted per cell. A set of susceptible and resistant lines were included with each experimental set as controls.

Inoculation and scoring. Plants were inoculated at the two-and-a-half-leaf stage with a spore suspension of urediniospores in paraffin oil using an air brush. For crosses targeting leaf rust resistance genes *Lr13* and *Lr21 and Lr28* urediniospore suspension of *P. recondita* f.sp. *tritici* pathotype 104-1,2,3,(6),(7),11 +Lr37 was used while for crosses target ting stem rust resistance genes *Sr32* and *Sr33* urediniospore suspension of *P. graminis* f.sp. *tritici* pathotype 98-1,2,3,5,6,7 was used. Inoculated plants were placed in a humid chamber at 22°C for 48 hours for establishment of infection. Disease was assessed 12 to 14 days after inoculation using a 0 to 4 scale (McIntosh et al. 1995), where a scores of 0, 1 and 2 was classified as resistant (R) and 3 and 4 as susceptible (S). Infection type 3n (pustule accompaned by necrosis) was also classified as R.

Plants were grown to maturity and single heads harvested from each F_2 plant. Twelve $F_{2:3}$ seed per family were sown in 10-cm pots, inoculated and assessed as described above. Segregation of resistance alleles in the F_2 and $F_{2:3}$ was analysed by comparing the observed ratio of resistant:susceptible with the expected ratio by the chi-square method (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

In populations targeting *Lr13* (Leichardt/WAWHT2071), *Lr21* (Tincurrin+Lr21/EGA2248) and *Lr28* (Sunland/Arrino), parents Leichardt, Tincurrin+Lr21 and Sunland were resistant (R) while parents WAWHT2071, EGA2248 and Arrino were susceptible (S) to leaf rust. F_2 progeny in crosses Leichardt/WAWHT2071 and Sunland/Arrino showed a 3R:1S segregation ratio (χ^2 = 0.3 and 1.3; *P* = 0.6 and 0.3) (Table 1) while F_3 families segregated as 1:2:1 (true breeding R (TR):segregating (seg):true breeding S (TS)) (χ^2 = 1.8 and 1.0; *P* = 0.4 and 0.6) indicating the single dominant nature of *Lr13* and *Lr28*. However, in concurrent studies conducted on F_2 populations Strzelecki/WAWHT2454 and EGA Gregory/Ajana where the resistant parents Strzelecki and EGA Gregory are known to carry *Lr13* in combination with *Lr23* (not effective against 104-1,2,3,(6),(7),11 +Lr37) it appears recessive with a 1R:3S reaction observed in both populations. For 196 F_2 individuals of Strzelecki/WAWHT2454 chisquare was 1.9 and p value 0.17 while for 196 F_2 individuals of EGA Gregory/Ajana chisquare was 0.5 and p value 0.5. Although *Lr23* is not effective against leaf rust pathotype 104-1,2,3,(6),(7),11 +Lr37 it appears that it has some sort of an epistatic effect on *Lr13* or perhaps the two genes are present in repulsion.

Population Tincurrin+Lr21/EGA 2248 targeting Lr21 showed a 13R:3S F_2 segregation (χ^2 = 0.4; P = 0.5) (Table 1) indicating the presence of one dominant and one recessive independent genes [13(A_B_+A_bb+aabb):3(aaB_)]. The hypothesis was confirmed in F_3 where families arising from resistant F_2 plants segregated in a ratio of 7:6 (TR:seg) while families from susceptible F_2 plants were all true breeding susceptible (χ^2 = 1.4; *P* = 0.5). Although, a fraction (1/12) of F_3 families arising from suceptible F_2 plants (those of genotype aaBb) were expected to be resistant, we did not come across these due to the limited numbers tested.

In populations targeting *Sr32* and *Sr33* C77.19/3*77W:549-163658 and *Sr33*(R.L.5405)/2*Shortim were the resistance parents while parents WAWHT2046 and Calingiri were susceptible to stem rust. C77.19 is a cleaner threshing *Sr32* carrying line derived from Chinese Spring/*Triticum speltoides* cross. R.L.5405 is a Tetra Canthatch/*Triticum tuschii* derivative with *Sr33* (Kerber and Dyck 1979). The F_2 progeny in both crosses segregated into a 3R:1S ratio (χ^2 = 0.1 and 3.3; *P* = 0.8 and 0.1) (Table 1) and the F_3 families showed a segregation of 1:2:1 (TR:seg:TS) (χ^2 = 5.5 and 1.2; *P* = 0.1 and 0.6) indicating the single dominant nature of *Sr32* and *Sr33*.

Phenotypic leaf rust and stem rust data of the above populations was used to develop closely linked markers to genes *Lr13*, *Lr21*, *Lr28*, *Sr32* and *Sr33* (Cakir et al. this conference). These markers are currently being implemented in the InterGrain wheat breeding program.

Table 1. Frequency distribution of F_2 and F_3 generations of of various crosses targetting leaf rust and stem rust resistance genes.

Cross	Gene	Gener-ation	Segregation Ratio	χ^2	*P* value
Leichardt/ WAWHT2071	*Lr13*	F_2	3R[1]:1S[2]	0.3	0.6
		F_3	1TR[3]:2seg[4]:1TS[5]	1.8	0.4
Tincurrin+Lr21/ EGA2248	*Lr21*	F_2	13R:3S	0.3	0.6
		F_3	7TR:6Seg:3TS	1.4	0.5
Sunland/Arrino	*Lr28*	F_2	3R:1S	1.3	0.3
		F_3	1TR:2seg:1TS	1.0	0.6
C77.19/3*77W:549-163658	*Sr32*	F_2	3R:1S	0.1	0.8
		F_3	1TR:2seg:1TS	5.5	0.1
Sr33(R.L.5405)/ 2*Shortim	*Sr33*	F_2	3R:1S	3.3	0.1
		F_3	1TR:2seg:1TS	1.2	0.6

[1]R = resistant; [2]S = susceptible; [3]TR = true breeding resistant; [4]seg = segregating; [5]TS = true breeding susceptible

ACKNOWLEDGEMENTS

Funding for this research was provided by GRDC (Grains Research and Development Corporation) through Australian Winter Cereal Molecular Marker Program, and Molecular Plant Breeding CRC.

REFERENCES

Kerber ER and Dyck PL. 1979. Resistance to stem rust and leaf rust of wheat in Aegilops squarossa and transfer of a gene for stem rust resistance to hexaploid wheat. In 'Proceedings of the Fifth International Wheat Genetics Symposium'. (Ed. S. Ramanujam). Pp358-364. (Indian Society of Genetics and Plant Breeding, New Delhi, India).

Roelfs AP, Sing RP, Saari EE. 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico DF.

Snedecor, WG and Cochran, G. 1967. Statistical Methods. pp. 228--253. Oxford and IBH Publishing Co., New Delhi, India.

Van Ginkel M, Rajaram S. 1993. Breeding for durable resistance to diseases in wheat: An international perspective. In: Jacobs T, Parlevliet JE (eds) Durability of Disease Resistance, pp. 259– 72. Kluwer Academic Publishers, Dordrecht.