

# Molecular mapping and improvement of rust resistance in the Australian wheat germplasm

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## ABSTRACT

Molecular markers make the pyramiding of rust genes possible in adapted elite lines. In this study we report the microsatellite tagging of leaf rust genes *Lr13* and *Lr28* in the Leichardt/WAWHT2071 and Sunland/Arrino populations, respectively. Leichardt and Sunland were the sources of resistance genes for *Lr13* and *Lr28*, respectively. F<sub>2</sub> and F<sub>2,3</sub> populations were used for microsatellite tagging of the genes. Closely linked SSR markers were identified for *Lr13* and *Lr28* on chromosomes 2BS, 4AL, respectively. Molecular markers for a range of other rust resistance genes (*Lr9*, *Lr19/Sr25*, *Lr24/Sr24*, *Lr34/Yr18*, *Lr46/Yr29*, *Lr47*, *Sr26*, *Sr32*, *Sr33* and *Sr36*) are currently being implemented for variety development and germplasm enhancement.

## INTRODUCTION

Pyramiding of rust resistance genes in elite lines using markers that are diagnostic in a range of genetic backgrounds is one of the objectives of Australian breeding programs. Wheat breeders are often challenged with new rust pathotypes and consequently have to deploy additional rust resistance genes in advanced breeding lines. McIntosh *et al.* (1995) has listed and genetically characterized and named rust resistance genes. These genes have been assigned to individual chromosome arms through cytogenetic studies. Linked molecular markers have been identified for some of these genes. The sequence tagged site (STS) and cleaved amplified polymorphic sequence (CAPS) type of markers were developed for some of the resistance genes (*Lr9*, *Lr35*, *Lr37*, *Lr46*, *Lr47*). Validation of markers across different genetic backgrounds is necessary for successful implementation.

The Western Australian Wheat Breeding Program has been deploying the adult plant leaf rust resistance gene *Lr34* and *Sr2* and therefore many advanced breeding lines carry these genes. A molecular marker designed from the chromosome arm 7DS is effectively being used for marker-assisted selection (MAS) of *Lr34* (Lagudah *et al.* 2006). Since additional genes are required to achieve durable rust resistance, this study has been conducted to identify SSR markers linked with *Lr13* and *Lr28*.

Microsatellite markers were shown to be fairly cost effective and amenable to high level of multiplexing with various strategies (Hayden *et al.* 2007). For this reason most recent studies have utilized SSR markers for gene discovery studies.

## MATERIALS AND METHODS

### Plant material

Mapping populations used in this study are listed in Table 1. Phenotypic evaluation was performed according to Shankar *et al.* (this conference).

**Table 1. The list of mapping populations (each included 94 lines), relevant rust genes, donor lines, population analysed.**

Cross	Gene/trait	Donor Line	Pop. type
Leichardt/ WAWHT2071	<i>Lr13</i>	Leichardt	F <sub>2</sub> , F <sub>3</sub>
Sunland/Arrino	<i>Lr28</i>	Sunland	F <sub>2</sub> , F <sub>3</sub>

### Molecular analysis

DNA extraction, from approximately 0.1 g of fresh leaf tissue, obtained from each F<sub>2</sub> plant, was carried out according to Rogowsky *et al.* (1991).

SSR markers were selected from wheat consensus maps (Appels 2003; Somers *et al.* 2004). SSR analysis was performed by PCR amplification of the DNA with primers known to bracket SSR regions (Cakir *et al.* 2003a). Fragments were separated using either polyacrylamide gels with EtBr staining or MRT<sup>TM</sup> (Multiplex-Ready Technology) as described by Hayden *et al.* (2007).

All marker loci were subjected to a Chi-square goodness-of-fit test for segregation analysis to determine whether the alleles distributed in the expected 1:2:1 segregation ratio in the populations. Linkage and quantitative trait loci (QTL) analysis of the markers were conducted according to Cakir *et al.* (2003b) with the software packages Mapmanager (Manly 2001) and Qgene (Nelson 1997). Linkages were established with a minimum LOD score of 3.0. LOD scores lower than 3.0 were used to investigate loose linkages.

## RESULTS AND DISCUSSION

Phenotypic data for these populations reported by Shankar *et al.* (this conference) was used to identify markers closely linked with leaf rust resistance genes *Lr13* and *Lr28* (Table 2). After parental polymorphism study, chromosome specific 14 and 8 polymorphic SSR markers were identified for the mapping of *Lr13* and *Lr28*, respectively. Mapping analysis for each disease data has identified most significant flanking markers (Table 2).

**Table 2. Disease genes, chromosome locations and linked markers.**

Cross	Gene	Chr. Loc.	Linked markers
Leichardt/ WAWHT2071	<i>Lr13</i>	2BS	WMC474, BARC183
Sunland/Arrino	<i>Lr28</i>	4AL	BARC327, BARC343

Marker orders for each chromosome (not shown) were confirmed using two consensus maps of wheat (Appels 2003; Somers *et al.* 2004) and a deletion map of SSR markers (Sourdille *et al.* 2004). The locations of the most significant flanking markers were also in accordance with the previously reported locations of these genes (McIntosh *et al.* 1995).

This study has identified closely linked SSR markers for *Lr13* and *Lr28* leaf rust genes. These markers are currently being implemented in the wheat breeding programs in Western Australia. In addition, markers for a range of other rust resistance genes (*Lr9*, *Lr19/Sr25*, *Lr24/Sr24*, *Lr34/Yr18*, *Lr46/Yr29*, *Lr47*, *Sr26*, *Sr32*, *Sr33* and *Sr36*) are also being implemented for variety development and germplasm enhancement. The impact of these applications to wheat improvement is discussed in detail by Cakir *et al.* (this conference).

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## REFERENCES

- Appels R. 2003. A Consensus map of Wheat-a cooperative international effort. In 'Proceedings of 10<sup>th</sup> International Wheat Genetics Symposium'. Vol.1. Istituto Sperimentale per la Cerealcoltura, Roma, pp. 211-214.
- Cakir M, Appels R, Carter M, Loughman R, Francki M, Li C, Johnson J, Bhawe M, Wilson R, McLean R, Barclay I. 2003a. Accelerated wheat breeding using molecular markers. In 'Proceedings of 10<sup>th</sup> International Wheat Genetics Symposium'. Vol.1. Istituto Sperimentale per la Cerealcoltura, Roma, pp. 117-120.
- Cakir M, Poulsen D, Galwey N, Ablett G, Chalmers KJ, Platz GJ, Park RF, Lance RCM, Panozzo JF, Read BJ, Moody DB, Barr AR, Johnston P, Li CD, Boyd WJR, Grime CR, Appels R, Jones MGK, Langridge P. 2003b. Mapping and QTL analysis in the barley population Tallon X Kaputar. *Australian Journal of Agricultural Research*, 54, 1155-1162.
- Hayden MJ, Nguyen TM, Waterman A, McMichael GL, K. Chalmers KJ. 2007. Application of multiplex-ready PCR for fluorescence-based SSR genotyping in barley and wheat. *Molecular breeding*. DOI;10.1007/s11032-007-9127-5.
- Lagudah ES, McFadden H, Singh RS, Huerta-Espino J, Bariana H, Spielmeier W. 2006. Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistant gene region in wheat. *Theoretical and Applied Genetics*, 114: 21-30.
- Manly KF, Cudmore Jr RH, Meer JM. 2001. Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome*, 12, 930-932.
- McIntosh R.A., Welling CR, Park RF. 1995. Wheat rusts: an atlas of resistance genes. CSIRO, Melbourne, Australia, 200 pp.
- Nelson JC. 1997. QGENE: software for marker-based genomic analysis and breeding. *Molecular Breeding*, 3, 239-245.
- Rogovsky PM, Guidett FLY, Langridge P, Shepherd KW, Koebner RMD. 1991. Isolation and characterization of wheat-rye recombinants involving chromosome arm 1DS of wheat. *Theoretical and Applied Genetics* 82, 537-544.
- Somers DJ, Isaac P, Edwards K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 109, 1105-1114.
- Sourdille P, Singh S, Cadalen T, Gina L. Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M. 2004. Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Functional Integrative Genomics*, 4, 12-25.