Genetic analysis of wheat rust resistance genes segregating in a Kariega x Avocet S population

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ABSTRACT

Complete adult plant resistance (APR) to stripe rust of the wheat cultivar Kariega was previously ascribed to two major quantitative trait loci (QTL) on chromosomes 2B and 7D and three minor QTL. In the present study the Kariega x Avocet S doubled haploid population was increased from 150 to 254 individuals and the map improved by adding Diversity Array Technology (DArT) markers. Additional field and greenhouse phenotypic data for stripe rust were collected. The major QTL regions detected previously were validated, but different minor QTL compared to the previous study were identified. In the field, the chromosome 2B QTL region explained more of the phenotypic variance for host reaction type scores (RT), compared to the 7D QTL region. For the field leaf area infected score (LAI) both the major QTL regions explained more variance over time. A minor QTL on chromosome 4A of Kariega was consistently detected for LAI (up to 25.9%) and the two early RT (up to 12.2%) scores. In addition we used an accelerated greenhouse scoring method for APR to stripe rust, which detected both major QTL, the 4A QTL and another minor QTL. Using an adult plant screening method and different pathotypes of *Puccinia triticina*, several leaf rust resistance genes have been detected in the mapping population. This study has been valuable in confirming and expanding information on the leaf rust resistance genes and QTL for adult plant resistance to stripe rust in wheat.

INTRODUCTION

The Kariega x Avocet S partial linkage map contributed significantly towards understanding the adult plant resistance (APR) of Kariega to stripe rust (Ramburan et al., 2004). This analysis also identified doubled haploid (DH) lines for studying mechanisms of stripe rust resistance controlled by some of the major QTL derived from Kariega (Moldenhauer et al., 2008). The aims of this study were to: i) extend the partial linkage to improve genome coverage, ii) test the efficiency of the new accelerated greenhouse method to detect stripe rust APR, iii) validate the existing stripe rust QTL field data, and iv) determine the leaf rust resistance genes segregating in the Kariega x Avocet S population.

MATERIALS AND METHODS

Mapping population

The mapping population described by Ramburan et al. (2004) and Prins et al. (2005) was extended from 150 to 254 Kariega x Avocet S DH lines.

Phenotyping

Four sets, each consisting of 254 mapping population entries, Kariega and Avocet S, were grown to the adult plant stage as described by Pretorius et al. (2007). Sets were inoculated with *Puccinia striiformis* f. sp. *tritici* pathotype 6E22A+ and pathotypes UVPrt2, 9 and 13 of *P. triticina*, respectively. Plants were kept under appropriate greenhouse conditions and flag leaf infection types (IT, 1 to 9 scale, McIntosh et al., 1995) were recorded 16 days after inoculation for stripe rust and 11 days after inoculation for leaf rust. Whole plant reaction type (RT) to stripe rust, viz. resistant (R), moderately resistant (MR), moderately susceptible (MS) or susceptible (S) (McIntosh et al., 1995), was also recorded.

The same population was phenotyped to pathotype 6E22A+ in a replicated field trial at Greytown, South Africa in 2006. Stripe rust responses were recorded on three occasions. A mean severity rating (leaf area infected=LAI) and associated response class (R, MR, MS and S) were allocated for each entry. The different stripe rust disease scores were computed as in Ramburan et al. (2004).

DNA marker analysis

The existing partial linkage map consisting of 203 markers (SSR, AFLP) and genes was improved by adding DArT markers (Triticarte P/L and Diversity Arrays Technology P/L, Canberra, Australia).

Linkage map construction

MapManager QTXb20 (Manly et al., 2001) was used to curate the partial linkage map (Prins et al., 2005; Ramburan et al., 2004) and to add the DArT markers. Ordering of the markers within linkage groups was performed with RECORD (Van Os et al., 2005). The

QTL maps were prepared with MapChart V2.1 (Voorrips, 2002).

QTL analysis

MapManager QTXb20 and Windows QTL Cartographer V2.5 (Wang et al., 2007) were used for single marker regression. Composite interval mapping (CIM) was performed with Windows QTL Cartographer V2.5. To determined the significance of the detected QTL, 1000 permutations were run (P=0.05) for all traits.

RESULTS AND DISCUSSION

Following map curation and the addition of the DArT markers, 466 markers were placed in 31 chromosome anchored groups covering ~1365 cM. Although the D genome remains sparsely covered by markers, the DArTs contributed towards covering telomeric regions of some chromosomes.

Stripe rust

The major QTL for LAI and RT on chromosome 2B and 7D were validated in this study. The 2B QTL interval (*gwm682-gwm148*) is only detected for the stripe rust IT score in the greenhouse, whilst a larger interval (*gwm682-psp3030*) is detected for all other stripe rust traits. This suggests an investigation into the presence of more than one QTL as this is a fairly large region (~23 cM).

A previously described minor QTL on chromosome 4A of Kariega was consistently detected for LAI (explained up to 25.9% of the phenotypic variance) and the two early RT (12.2%) scores across the three different scoring dates. Although this QTL was also detected in the greenhouse test, little phenotypic variance for IT (3.9%) and RT (3.8%) scores was explained. This QTL may be indicative of a slow-rusting response that retards disease progress during the early stages of infection in the field.

The QTL on 2B and 4A are currently further investigated using various finer mapping and host-pathogen interaction approaches. The detection of a combination of QTL in Kariega, with seemingly different resistance mechanisms, emphasizes the value of unravelling these complex resistances as it provides a foundation for long-term efficiency in marker-assisted resistance breeding.

Leaf rust

Assessment of leaf rust responses suggested the segregation of three resistance genes each for the pathotypes UVPrt2 and UVPrt9, whereas two genes were detected by UVPrt13. Based on genes known to occur in the parents, the most likely postulation is that *Lr13* from Avocet S was detected by UVPrt2 and UVPrt9 and that *Lr34* from Kariega was effective against all three pathotypes. Marker analysis confirmed resistance to these pathotypes on chromosome 7D (*Lr34*) in Kariega and to UVPrt2 and UVPrt9 on chromosome 2B (*Lr13*) in Avocet S. In addition, significant variation for resistance to UVPrt9 and UVPrt13 was contributed by a linkage group temporarily assigned '6B', based on the presence of several previously mapped 6B DArT markers (Akbari et al., 2006). Markers did not detect the third gene revealed by UVPrt2, which is probably a result of insufficient marker coverage in the region of interest.

CONCLUSION

An increase in the size of the mapping population from 150 to 254 individuals and the addition of DArT markers have improved the genetic map. Similarly, the addition of an early scoring date for stripe rust has improved the QTL analysis considerably. The greenhouse method also proved to be valuable as both the major QTL were clearly detected.

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