Mapping resistance gene to leaf rust in wheat line KS91WGRC11 using quantitative bulked segregant analysis and DArT platform

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ABSTRACT

Leaf rust caused by *Puccinia triticinia* is one of the most devastating foliar diseases of wheat. Major leaf rust resistance gene *Lr42* identified on chromosome 1D of *T. tauschii* was transferred to common wheat line KS91WGRC11 (= Century*3/*T. tauschii* TA2450, *T. tauschii* TA2450) (Kansas State University, Manhattan, KS, USA). Recently, using molecular markers resistance gene *Lr42* was located on chromosome 2D. According to our preliminary mapping neither 1D nor 2D harbor resistance gene to leaf rust in line KSWGRC11. Based upon novel marker technology DArT (Diversity Arrays Technology) and quantitative bulked segregant analysis we can conclude that resistance is conditioned by a gene located on chromosome 3D. However, this notion has to be confirmed in further mapping experiments.

Keywords: DNA markers, *Puccinia triticina*, resistance locus, *Triticum aestivum*

INTRODUCTION

Leaf rust (*Puccinia triticina* Erks.) is one of the most important foliar diseases of wheat (*Triticum aestivum* L.) worldwide. High genetic variability of the pathogen prompts breeders to stack few major resistance genes into a single cultivar. To effectively manipulate resistance genes, tightly linked molecular markers to resistance loci are required. Recently, a novel marker system called Diversity Arrays Technology (DArT) has been devised. Such system provides a practical and cost-effective whole-genome fingerprinting tool without the requirement of DNA sequence information comparing to other DNA markers (Jaccoud et al. 2001).

Wild relative of common wheat *T. tauschii* (genomes DD, 2n = 14) is a valuable source of resistance genes. Major leaf rust resistance gene *Lr42* identified in *T. tauschii* was transferred to common wheat line KS91WGRC11 (= Century*3/*T. tauschii* TA2450, *T. tauschii* TA2450) (Cox et al. 1994). According to monosomic analysis resistance in line KSWGRC11 was conditioned by single partially dominant gene located on chromosome 1D (Cox et al. 1994). Recently, this line was used as source of resistance to leaf rust in mapping experiments and a resistance locus (putative gene *Lr42*) was located on chromosome 1D (Sun and Bai 2007), but also on 2D (personal communication, Robert Bowden and Sukhwinder Singh, Department of Plant Pathology, Kansas State University, Manhattan KS 66506, USA).

The objective of our study was to clarify the location of *Lr42* gene in wheat genome and mapping closely linked molecular markers suitable for marker assisted selection.

MATERIALS AND METHODS

Wheat line KS91WGRC11 carrying the putative *Lr42* gene was kindly provided by Wheat Genetics Resource Center (Kansas State University, Manhattan, KS66506, USA). From a cross between the German winter wheat cultivar Aristos (susceptible parent) and KSWGRC11, we produced a mapping population comprising 154 $F_{2:3}$ families. Seedlings of the F_2 population and F_3 derived families were evaluated for reaction to leaf rust using one isolate of *P. triticinia*. DNA was extracted from all F_2 plants using Plant DNeasy Mini Kit (Qiagen Inc., 27220 Turnberry Lane, Valencia, CA 91355, USA).

Initially for mapping, we used 65 microsatellites specific for wheat chromosomes 1D and 2D (Röder et al. 1998; Somers et al. 2004; Song et al 2005). For technical reasons, the maping population was reduced to 94 F_2 plants. Amplification and detection of PCR products were performed according to procedures described earlier (Czembor et al. 2003). Polymorphic markers were used to build up maps using JoinMap 4 software (Van Ooijen 2006). Next, in mapping resistance locus *de novo*, we decided to apply quantitative bulked segregant analysis based (BSA) on DArT platform (Wenzl et al. 2007). Six DNA samples were prepared: susceptible and resistant parents, two random bulks (mix of susceptible and resistant F_2 plants), resistant bulk (29 homozygous resistant F_2 plants) and susceptible bulk (29 homozygous susceptible F_2 plants). DArT-BSA assay was performed by Triticarte Pty Ltd (Diversity Arrays Technology P/L - Triticarte P/L, 1 Wilf Crane Crescent, Yarralumla ACT 2600, Australia).

RESULTS AND DISCUSSION

Observed segregation ratio among F_3 families (37 susceptible, 67 heterozygous and 30 resistant) indicated that resistance was controlled by a single dominant gene. We could establish genetic maps for chromosomes 1D and 2D, comprising 13 and 9 microsatellite markers, respectively. The order of markers on each chromosome

was consistent with the consensus genetic map of wheat published by Somers et al. (2004). The resistance locus (putative *Lr42*) could not be placed reliably on neither of the two chromosomes. We report a successful application of BSA strategy coupled with scanning of wheat DNA samples with thousands of polymorphism-enriched clones. In our experiment, we identified 30 clones that differ significantly (over 50%) in allele frequency between bulks and parents (figure 1). Only 13 of these 30 clones have been mapped and 11 of them are located on chromosome 3D. The other two are mapped on chromosomes 1B and 7D.

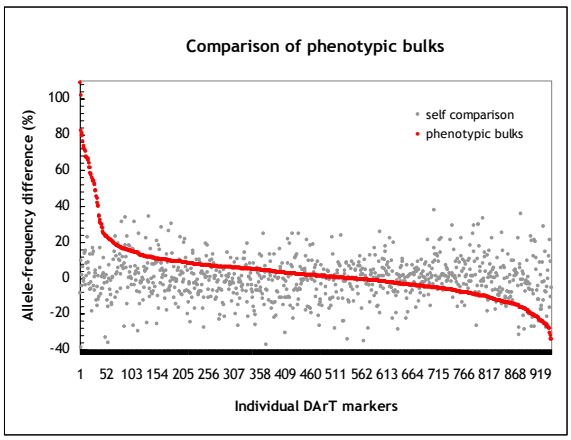

Figure 1. DArT-BSA genome scan for leaf rust resistance gene in the Aristos/KSWGRC11 population.

DArT-BSA assay confirmed our previous finding that resistance to leaf rust in line KS91WGRC11 is probably not located on chromosome 1D or 2D, but on chromosome 3D. The same source of resistance was used in mapping experiments and tight linkage with DNA markers was identified on chromosomes 1D (Sun and Bai, 2007) and 2D (personal communication, Robert Bowden and Sukhwinder Singh). However, in the later case it is likely that the resistance gene mapped on 2DS is different from gene *Lr42*. We can only speculate on the reasons for discrepancy on the location of the resistance to leaf rust in line KS91WGRC11. It is likely that different genetic materials were evaluated and described in the above mapping experiments or different resistance genes were transferred to line KS91WGRC11 form *T. tauschii*. Interestingly, resistance gene to leaf rust *Lr32* derived from *T. tauschii* is also located on chromosome 3D (Kerber 1987, 1987). Further genetic and molecular analyses are required to discover possible gene(s) contributing to leaf rust resistance in line KS91WGRC11. In the next step, we will try to map this resistance gene on chromosome 3D using microsatellite markers.

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