Genetic map of wheat chromosome 3BS including SV2, an adult plant leaf rust resistance gene

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ABSTRACT

Durable leaf rust resistance in Sinvalocho MA could be explained by the combination of adult plant resistance genes and genes expressed at seedling stage. Two genes, SV1 and SV2, fully expressed at flag leaf stage, were identified previously and SV2 was mapped on chromosome 3BS where no other adult plant leaf rust resistance gene was previously reported (Ingala *et al.*, 2005).
Using an F9 population of 91 recombinant inbred lines (RILs), a linkage map of chromosome 3B from the cross Sinvalocho MA and Gamma 6 was constructed. Thirty AFLP and ten SSR markers were allocated in three intervals of 104, 10.7 and 58.96 cM. The more distal interval of 104 cM includes the SV2 gene.
In order to develop a fine map of this region, an F2 population of 1108 individuals was used. Three microsatellites, gwm533.2, cft3417 and cft5010 and the SV2 gene were mapped in an interval of 6.24 cM in distal 3BS.

INTRODUCTION

Leaf rust, incited by the biotrophic fungus *Puccinia triticina*, is one of the most important diseases of wheat worldwide, causing in Argentina annual yield losses of about 5-10%. The large amount of genetic variation for pathogenicity commonly observed in rust populations, as well as the speed at which these populations adapt to resistance genes in wheat cultivars, makes the genetic control of the disease difficult to achieve. Most of the new commercial varieties are resistant to rust populations because they carry different resistant genes combinations. However, these varieties frequently become susceptible when widely grown over the years because of the occurrence and selection of new virulent strains. In spite of this situation some wheat varieties remained resistant for a long time. This kind of resistance was operationally defined as "durable" by Johnson (1981). Some old South American varieties as Sinvalocho MA, Buck Manantial, La Prevision 13 and Pergamino Gaboto showed durable resistance and were used as sources of resistance in breeding programs locally and worldwide. This type of resistance could be explained by the combination of adult plant resistance genes and genes expressed at seedling stage (Favret *et al*., 1983; Sawhney *et al*., 1989). In Sinvalocho MA, two dominant genes expressed at flag leaf stage, SV1 and SV2, were identified and SV2 was mapped to chromosome 3BS where no other adult plant resistance gene was previously reported (Ingala *et al*., 2005). A linkage map of 3BS using an F9 population of 91 recombinant inbred lines (RILs) from the cross Sinvalocho MA and Gamma 6 was constructed with thirty AFLP and ten SSR markers.
The objective of the present work was to develop a fine map of the region where SV2 was previously mapped. Fine mapping is a prerequisite for positional cloning, provided that the genetic map is representative of physical distances.
It should be noted that SV2 maps to a region where other pathogen resistance genes were detected: the *Sr*2 gene of durable adult plant stem rust resistance caused by *Puccinia graminis f. sp. tritici* (Kota *et al*., 2006) and *Qfhs.ndsu-3BS*, a major QTL for *Fusarium gramineum* resistance (Liu *et al*., 2006). In addition, a BAC library of chromosome 3B was constructed (Safar *et al.*, 2004) and approximately 19 400 BAC-end sequences were generated, representing a cumulative length of nearly 11 Mb, 1.1% of the chromosome length, which allowed the development of chromosome-specific markers (Paux *et al*., 2006).
The use of resistance genes, particularly from varieties that show durable resistance, may be an outstanding contribution for controlling this disease.

MATERIALS AND METHODS

Two mapping populations were used: an F9 population of 91 recombinant inbred lines (RILs) from the cross Sinvalocho MA and Gamma 6 and, for fine mapping, an F2 subpopulation of 1108 susceptible plants selected among 5861 individuals from the same cross.
Both populations were grown in the greenhouse and artificially inoculated at flag leaf stage with *Puccinia triticina* strain F05, which gives an incompatible reaction in the presence of SV2 gene only at this late developmental stage. Three weeks later, disease symptoms were observed and plants were scored as resistant or susceptible.
AFLP reactions were carried out using *Pst*I and *Mse*I, following the procedures described by Vos *et al* (1995).
Microsatellites gwm and cft were amplified as described by Röder *et al*., 1998 and Paux *et al*., 2006, respectively**.**
Amplification products were visualized on silver stained denaturing 5%-polyacrylamide gels.
Linkage analysis and distances were estimated using Gqmol program, Version 2006 9.1 (Genética Quantitativa e Molecular. Lab. de Bioinformática. Universidade Federal de Viçosa, Brasil).

RESULTS AND DISCUSSION

A genetic map of 352 molecular markers (AFLPs and SSRs) with almost full genome coverage was devised

using a 91 RIL population from the cross of Sinvalocho MA and Gamma 6. Particularly in chromosome 3B, 10 SSRs and 30 AFLPs were allocated using the GQMol program (figure 1, left). In this chromosome map, the four SSR markers proximal to SV2 gene showed an inverted order with respect to previous reports (Röder *et al*., 1998 and Feuillet *et al*., unpublished results).

In order to develop a fine map of the region surrounding SV2, an F2 population of 5861 individuals from the cross Sinvalocho MA and Gamma 6 was grown in the greenhouse and inoculated at flag leaf stage with *Puccinia triticina* strain F05 to identify SV2 gene. The scoring gave 4753 resistant and 1108 susceptible plants. This segregation did not fit an expected 3:1 segregation for a single dominant gene ($p< 0.01$) but it should be noted that adult plant inoculations are operationally more difficult and several plants may have escaped the infection and were therefore misclassified as resistant. However, this does not affect the results since only the 1108 susceptible individuals were used as mapping population because of the certainty about the homozygous recessive state of SV2 gene.

Microsatellites gwm533.2 (Röder *et al*., 1998) and cft3417 and cft5010 (Paux *et al*., personal communication) were genotyped in this subpopulation. The resulting map was constructed using the GQMol program (figure 1, right).

Both maps showed the same marker order. The distance between SV2 gene and gwm533.2 was quite similar, but higher in the fine map, probably due to an increased probability of recombination. However, due to the lack of additional markers in-between, the distance SV2-cft markers might have been underestimated in the fine map. In addition, this map allowed the resolution of the two cft markers.

At present, the mapping of additional markers is under way to allow the saturation of this interval and therefore a more accurate estimation of the distances. It should be noted the importance of the identification of close flanking markers encompassing an interval suitable for future positional cloning.

An important feature of wheat genome organization is that gene density and recombination rates are not uniform along the chromosomes and increase with the distance to the centromere (Gill *et al*. 1993; Lukaszewski and Curtis 1993; Akhunov *et al*. 2003). Mean recombination rates range between 0.06 and 0.87 cM/Mb for the proximal and distal intervals, respectively (Akhunov *et al*. 2003). Taking into account that SV2 is distally located and assuming no variation of the recombination rate at this region of 3BS, we can estimate a length of approximately 1.3 Mb for the distance between SV2 and the nearest marker gwm533.2.

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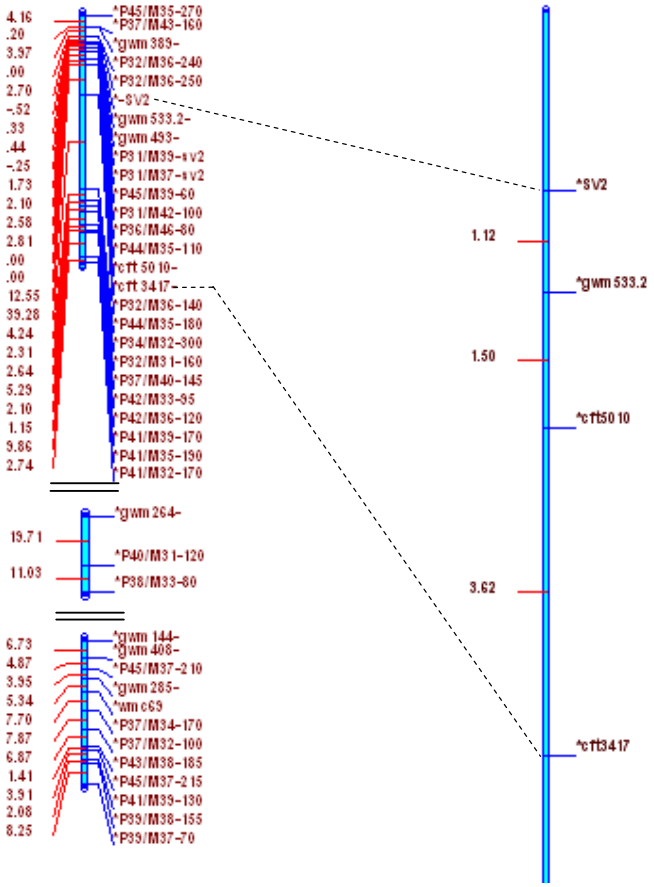

Figure 1: Genetic maps of chromosome 3B. The map on the left is a low resolution map done with 91 RILs and the one on the right is a fine map based on an F2 population of 1108 individuals. Genetic distances (in cM) are shown on the left of each map. AFLP markers are codified by the primers used for its amplification (Pst/Mse) followed by the size of the polymorphic band (in bp)