Inheritance and genetic mapping of leaf rust resistance genes in the wheat cultivar Buck Manantial

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ABSTRACT

Leaf rust, caused by the biotrophic fungus *Puccini triticina*, is one of the most important diseases of wheat worlwide. The use of resistance genes is of great interest in numerous breeding programs. Our objectives were to determine the number and characterization of resistance genes to wheat leaf rust present in Buck Manantial, an Argentinian cultivar that shows durable resistance. Also, we sought to identify molecular markers that can be used in marker assisted selection for these resistance genes. A genetic linkage map of 533 Amplified Fragment Length Polymorphism (AFLP) and microsatellite markers was developed. We identified and mapped the genes *Lr*3, *Lr*16, *Lr*17 and an adult plant resistance gene (APR) named BMP1. The BMP1 showed a high effective resistance to natural infection of wheat leaf rust in field test in three different locations during 3 years. The study of traditional wheat varieties of South America origin showing durable resistance is of great value, as they can provide new sources of resistance to this disease.

INTRODUCTION

Leaf rust incited by *Puccinia triticina* Eriks., is a worldwide disease of wheat and causes important yield looses in temperate regions where wheat is grown. Resistance to leaf rust in wheat cultivars had always been one of the main objectives in breeding programs. The use of resistance genes represents a cost effective and environmental-friendly way to control this disease in wheat. However, this approach demands a constant effort to identify, characterize and incorporate resistance genes, mainly due to the great capability of of rust populations to change (Ingala et al, 2003, McCallum et al, 2005). Durable resistance, based on the action of minor genes, and composed of seedling and APR genes, is a desirable trait in current breeding progams compared with resistance given by simple race-specific genes. In Argentina, several old cultivars that show durable resistance as Sinvalocho MA, Pergamino Gaboto, El Gaucho and Buck Manantial have been identified (Favret et al 1983). Buck Manantial (released in 1964) has been used as a source of resistance to wheat leaf rust not only in Argentina, but also in North America and Eastern Europe. The complex genotype of this cultivar, composed of several resistance genes, makes difficult the study and characterization of APR genes, probably, the main component of durable resistance. Dyck (1989) determined by genetic analysis the presence of the seedling resistance genes *Lr3, Lr16* and *Lr17*, the adult resistance gene *Lr13* and one unidentified adult plant gene, suggesting the presence of *Lr34* based on infection type and leaf rust reaction under natural infection. Saione et al (1993) concluded that 4 to 7 genes must be defeated to overcome resistance in Buck Manantial, at seedling stage.

The use of molecular markers and mapping softwares can help to develop saturated linkage maps and determine the genetic position of resistance genes and study for the presence of putative QTLs. Linked molecular markers can be used in the introduction and selection of genes in breeding programs.

The objectives of the present work were to determine the number and chacterization of resistance genes to wheat leaf rust present in Buck Manantial and also identify molecular markers that can be used in marker assisted selection.

MATERIALS AND METHODS

An F8 population of 118 recombinant inbred lines (RILs), coming from a cross between Buck Manantial and the susceptible genotype Purplestraw was phenotyped at seedling and flag leaf stage with different races of *P.triticina* to characterize and map the resistance genes. Procedures for infections and scorings were made following procedures described by Dieguez et al, (2006), both for seedling and adult resistance genes.

A genetic linkage map of 533 AFLP and SSR (Single Sequence Repeat) molecular markers was developed with JoinMap v3.0. The AFLP and SSR analysis was carried out according to Vos et al.(1995), and Roder et al.(1998) and Somers at al. (2004), respectively.

RESULTS

Three races, coming from the Instituto de Genética "E:A.Favret" (INTA) *P.triticina* collection, were used to infect the RILs population and the near-isogenic Thacther lines. These races allowed the identification of seedling resistance genes present in Buck Manantial. The race Ma-05 Onix detected the *Lr*16 ($P\chi^2$1:1= 0.92), the race Rq-05 Cronox detected *Lr16* + *Lr*17 ($P\chi^2$3:1= 1) and the race 66 that detected *Lr*3, *Lr*16 and *Lr*17 ($P\chi^2$7:1= 0.66). Another race, Ca2Lr17, was used to detect an adult plant resistance gene, named BMP1.($P\chi^2$7:1= 0.47). For genetic mapping of *Lr*17 and *Lr*3 genes, the suscetible RILs to Ma-05 Onix and Rq-05 Cronox were used, respectively, to obtain the phenotypic data.

The *Lr*3 gene mapped on the distal end of chromosome 6BL (Fig. 1a), as previously reported Dieguez et al, (2006), the *Lr*16 gene on the distal end of 2BS (Fig. 2) and the *Lr*17 on the distal end of the 2AS (fig. 1b) also as previously reported McIntosh et al, (1995). The BMP1 gene mapped on chromosome 2B and for this reason this chromosome was saturated with molecular markers (31 AFLPs and 19 SSRs ,Fig. 2). The BMP1 mapped at 1.3 cM from *Lr*16, suggesting a closely linked gene to *Lr*16.

The BMP1 gene showed a high correlation for resistance under natural infection conditions of wheat leaf rust, as demonstrated in field tests in three different locations during 3 years (Test F for pustules/cm^2= 0.001).

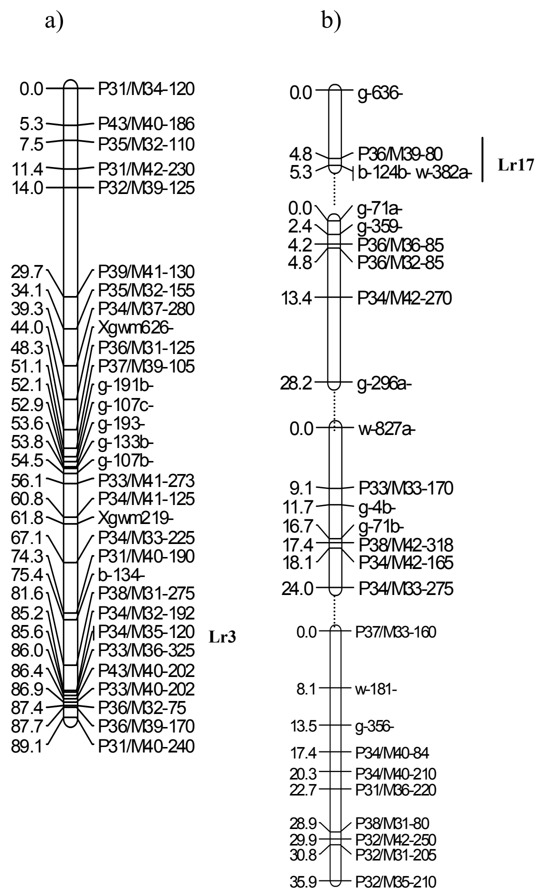

Fig. 1 **a** Linkage group of chromosome 6B and **b** linkage groups of chromosome 2A. Dashed lines represents gaps between linkage groups. Genetic distances in centimorgans (cM) by Kosambi function. In bold, leaf rust resistance genes.

Another race was used that identified the *Lr13* gene for adult plant resistance, but failed to show the presence of this gene in the Buck Manantial in the present study. Further analysis is being carried out to confirm the presence of *Lr13*. The closely linked marker csLV34 (codominant sequence tagged site, Lagudah et al, 2006) to *Lr*34 (located on chromosome 7D) was used to detect the presence of this resistance gene in Buck Manantial as hypothesized by Dyck (1989). The presence of *Lr*34 could not be confirmed in Buck Manantial based on the allele detected by this molecular marker (Fig. 3).

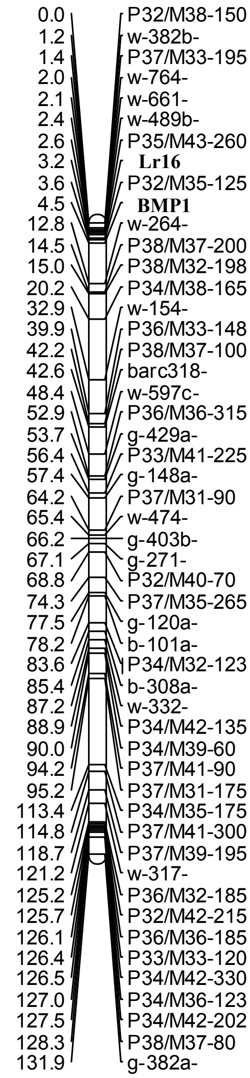

Fig. 2 Linkage group of chromosome 2B. In bold, leaf rust resistance genes.

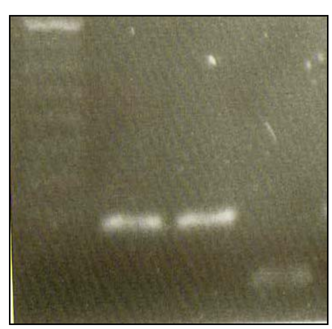

Fig. 3 PCR amplification of Buck Manantial (BM), Purlpestraw (P) and the near isogenic Thatcher line + *Lr*34, using the csLV34 primers. M-100 bp ladder molecular size markers. Amplification product of 230 bp is associated with lines that lack *Lr*34 and the product of 150 bp is associated with wheat lines that carry *Lr*34.

DISCUSSION

The study of traditional wheat varieties from South America showing durable resistance to leaf rust is of great value, as they can provide new sources of resistance to this disease. It is probable that in the development of Buck Manantial seedling and adult plant resistance genes of major effects have been accumulated, so the pathogen population could not overcome resistance in this wheat cultivar. It is of interest to characterize resistance genes in Buck Manantial and to have molecular markers linked to these genes. In the present work three of the known leaf rust resistance genes were mapped to their respective chromosome arms and linked molecular markers were found. In addition we could saturate chromosome 2B (Fig. 2) with molecular markers that should be valuable for linkage with *Lr*16, *Lr13* (although not confirmed in this study) and the BMP1. The order of SSRs and the length of the 2B was quite similar to that determined by Somers et al, (2004) in a consensus map of wheat.

It is striking to note that the highly effective resistance to natural leaf rust infection conferred by *Lr*16, or the complex of *Lr16* together with BMP1 (if both genes are alleles or a closely linked cluster needs a more detailed study) was also shown by the cultivar Americano 25e (Kolmer et al, 2007). In addition Americano 25e carries *Lr*3 gene, one unidentified seedling resistance gene and at least one APR gene. Americano 25e is the ancestor of many early South American wheats, including Buck Manantial.

There are still APR genes to be identified in Buck Manantial, as suggested by the segregation for race 77, in the RILs population. This race apparently identifies 2 epistatic genes (duplicate genes), approaching a 3:1 segregation. However, races that can separate both genes are not available in our Institute. A future experiment, using only susceptible families, which lack seedling resistance genes, is underway to elucidate both the presence of *Lr13* gene as well as other unidentified genes in Buck Manantial.

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