# Molecular mapping of durable stripe rust (*Puccinia striiformis* West.) resistance gene(s) in wheat

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## Abstract

The New Zealand spring wheat (*Triticum aestivum* L) cv. Otane confers a quantitative and durable resistance against the stripe rust disease caused by *Puccinia striiformis* West. The objective of this study was to locate and map genes involved in durability of stripe rust resistance. Simple Sequence Repeats (SSRs), also known as microsatellites, were used to cover all three genomes (A, B, D) of a doubled haploid population of 140 lines from a cross between two spring type cultivars, Otane (durably resistant) and Tiritea (susceptible). Glasshouse and field disease reaction assessments of 140 double haploid (DH) lines were carried out using *Puccinia striiformis* pathotype 106E139A$^+$. The transgressive segregation of disease assessment data at the adult plant stage suggested that both cultivars contributed the resistance observed in the DH population. A 215-bp (base pair) allele amplified by microsatellite *gwm332* and a 200-bp allele amplified by microsatellite *gwm282*, both mapped on the short arm of chromosome 7D, were linked to stripe rust susceptibility. Among three different DNA marker systems applied, microsatellites provided more polymorphisms than RFLPs or RAPDs, probably because of the complex nature of the wheat genome.

***Additional key words:*** *microsatellite marker, null alleles, gene mapping.*

## Introduction

Stripe rust, caused by *Puccinia striiformis* West., is one of the most damaging diseases of wheat throughout the world. In New Zealand it was first recorded in 1980 (Beresford, 1982) when up to 60% yield losses in susceptible cultivars, and 20-30% losses in moderately susceptible cultivars, were observed. The disease can be controlled with fungicides, and partly with cultural practices, but the use of resistant cultivars would be an effective, economical, and environmentally sound strategy to protect wheat from stripe rust. However the dearth of information regarding the genetic basis of stripe rust resistance, and the necessity of introgressing resistance genes make this a potentially long process if conventional breeding procedures were used. The recent development of genome maps (Gale *et al*., 1995; Cadalen *et al*., 1997) and quantitative trait loci (QTLs) analyses in wheat (Chantret *et al*., 2000; Messmer *et al*., 2000) offer alternative approaches to locating and subsequently manipulating the resistance genes.

This study was therefore undertaken to identify wheat chromosomal regions carrying resistance genes effective at the adult plant stage by using a newly developed double haploid (DH) population and genomic specific microsatellite markers. The ultimate goal is to map wheat stripe rust resistance genes and to identify molecular markers that can be used as DNA markers for marker assisted selection to introgress resistance genes into New Zealand wheat germplasm.

## Materials and Methods

### Plant materials

An $F_1$ derived double haploid (DH) population of 140 lines was developed for mapping from the cross cv. Tiritrea x Otane using the wheat-maize technique (Laurie and Bennett, 1988). Cv. Otane confers an

known quantitative resistance while cv. Tiritea is susceptible to *Puccinia striiformis* at the adult plant stage (Sparks *et al*., 1987). The DH population was evaluated for its response to wheat stripe rust pathotype 106E139A$^+$ in a glasshouse (15 ± 1 ºC, 16 h light) and in the field (Imtiaz, 2002). Disease was rated by infection type (IT; Line *et al*., 1974).

**DNA isolation**

Leaves of 3-4 week old seedlings of the two parents and all 140 DH lines were cut for DNA extraction according to the protocol of Dellaporta *et al*. (1983). Procedures for the wheat microsatellite (WMS) analysis and the WMS markers employed are described in Röder *et al*. (1998). Based on infection type (IT) recorded at the adult plant stage, DNA bulks of 20 resistant (IT 0-3), 19 moderately resistant (IT 4-6) and 19 susceptible (IT 7-9) DH lines were made by mixing equal amount of extracted DNA (Imtiaz, 2002). The two parents and three bulks were screened with 139 microsatellite markers, 4 to 6 per chromosome, selected from published maps of wheat (Röder *et al*., 1998).

**Linkage and QTL analysis**

Informative markers identified from parental and bulk screening were mapped by representative genotyping using a sub-sample of 50 DH lines (which included DH lines from all phenotypic classes of the whole DH population). For QTL analysis sub population mapping data were extended to the whole DH population of 140 lines through the use of the missing-data method as described by Lander and Botstein (1989). Map manager QTX (Manly *et al*., 2000) and Genstat (Lane *et al*., 1988) were used for genotypic and phenotypic data analysis respectively.

## Results

**Disease reaction**

Cv. Tiritea exhibited susceptibility (IT 8) and cv. Otane showed moderate resistance (IT 6) against pathotype 106E139A$^+$. However the disease reactions of the DH population from the cross cv. Tiritea x Otane against stripe rust pathotype 106E139A$^+$ showed transgressive segregation, as IT ranged from 1 to 9 (data not shown), indicating the presence of resistance factors in both parents.

**Microsatellite markers linked to stripe rust susceptibility**

Out of 139 microsatellite markers tested in this study, *gwm297*, *gwm443*, *gwm282*, *gwm332* and *gwm340* amplified more than one allele from cv. Tiritea that was not present in cv. Otane (null alleles; Fig. 1). Among them only alleles amplified from *gwm282 gwm332* and *gwm340* segregated in the DH sub population when all polymorphic microsatellite markers were tested (Fig. 2A,B). However only *gwm282* and *gwm332* amplified 200 bp (base pairs) and 215 bp specific DNA fragments respectively. These were linked to stripe rust susceptibility, because these alleles were present only in the susceptible DH lines (Fig. 2). Furthermore, when adult plant IT data were subjected to single locus regression (Imtiaz, 2002), both of these markers explained 12% each of the total variation in the stripe rust susceptibility (Table 1).

Linkage analysis showed that the allelic bands amplified by *gwm282* and *gwm332* in both parental cultivars were linked at a LOD (likelihood) score of 12.9 with a 2 cM (centiMorgan) distance between them, and were assigned to chromosome arm 7AL as proposed for these markers (Röder *et al*. 1998; Fig. 3). However, the DNA fragments amplified only from cv.

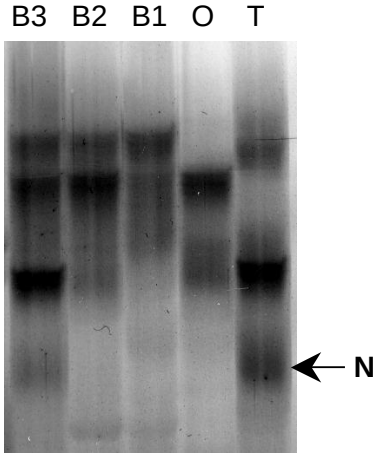
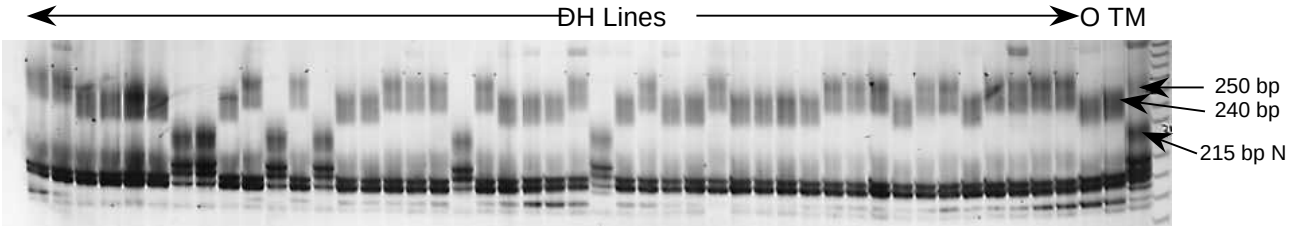
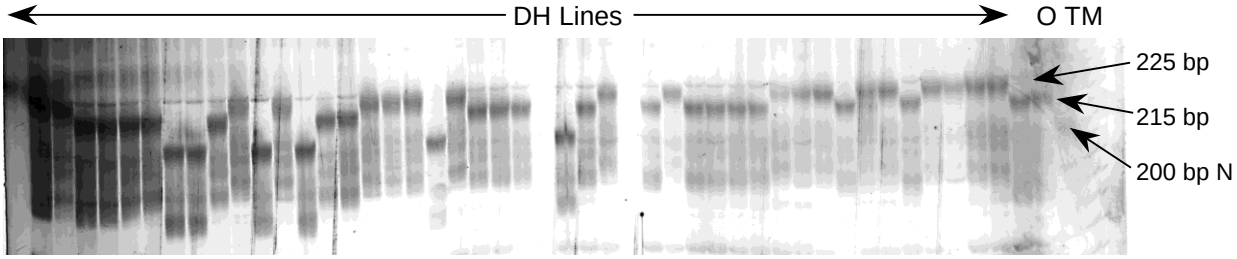



**Figure 1. Segregation patterns of microsattelite markers, *gwm332* and *gwm 282*, in the bulks. B3, B2, B1, O and T represent S, MR, R, cv. Otane and cv. Tiritea, respectively. N represents null alleles.**




A: *Gwm332*




B: *Gwm282*

**Figure 2. Segregation patterns of microsatellite markers in the DH population. O, T, M and N represent cv. Otane, cv. Tiritea, marker and the null alleles respectively. A: *gwm332* segregation and B: *gwm282* segregation in DH lines.**

**Table 1. Chromosomal location, likelihood ratio statistic (LRS), $R^2$, P-value and slopes for markers significantly associated (P<0.05) with stripe rust infection types (IT) in the glasshouse at the wheat adult plant stage in a single locus regression.**

| Marker[+] | Chromosomal location | LRS | $R^2$ | P-value | Slope |
|---|---|---|---|---|---|
| *gwm332* | **7D** | 7.7 | 12 | 0.00552 | -1.50 |
| *gwm282* | **7D** | 7.7 | 12 | 0.00552 | -1.50 |

[+]gwm(Gatersleben wheat microsatellite). Chromosomal locations in bold are putative. Negative and positive slopes indicate that cv. Otane contributed resistant and susceptible QTL alleles respectively.

Tiritea (200 bp and 215 bp), but not from cv. Otane (null alleles) were mapped to chromosome 7D of the cv. Tiritea genome, owing to their linkage with the microsatellite *gwm44* on chromosome 7D (Fig. 3).

## Discussion and Conclusions

This was a preliminary analysis to identify gene(s) linked to the durable stripe rust resistance conferred by cv. Otane. It showed that the DNA fragments amplified by *gwm282* (200bp) and *gwm332* (215bp) could be candidate markers for detecting susceptibility, although they explained only 12% of the total variation. Most of the reported microsatellite markers have been shown to be inherited in a co-dominant fashion (Röder *et al*., 1998; Chantret *et al*., 2000) and the same has been confirmed in this study, as the size of the main locus amplified (Table 2) was in agreement with Röder *et al*. (1998). However these markers, in contrast to Röder *et al*. (1998), but in agreement with

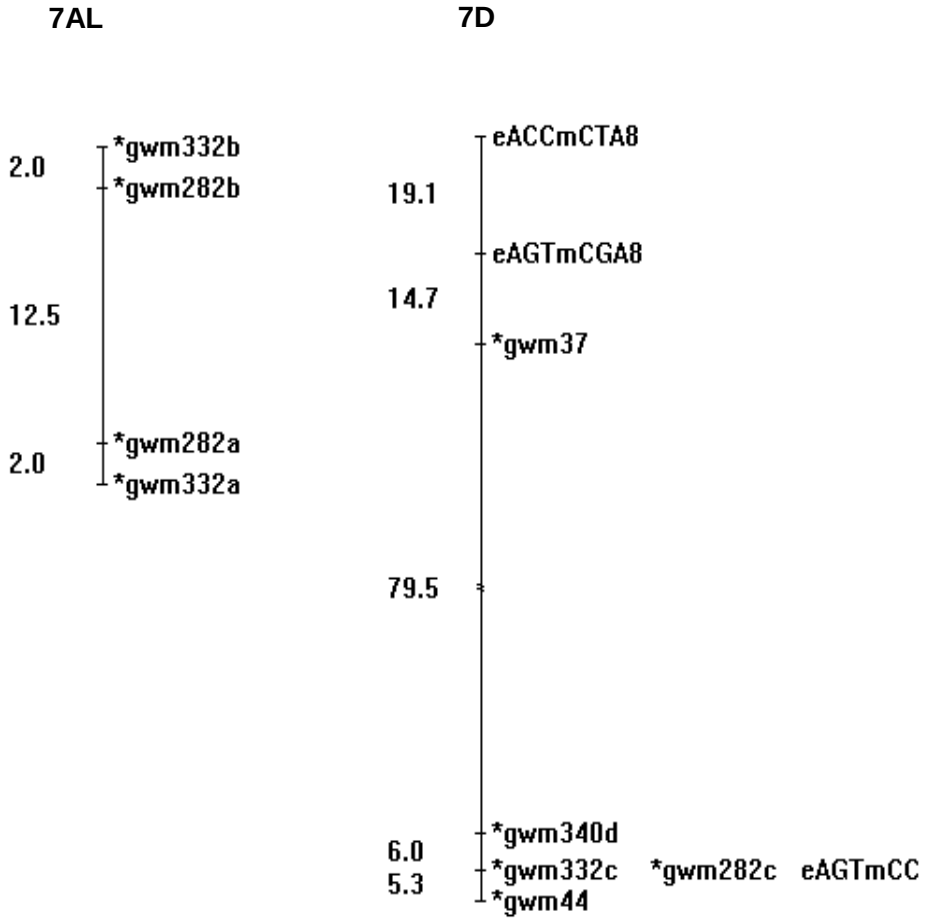



**Figure 3. Linkage groups obtained from the DH population of the cv. Tiritea x Otane cross showing map position of chromosomes 7AL and 7D. Microsatellite markers on the right side of each chromosome were used for linkage map construction. Distances (left side of the maps) are in Kosambi centiMorgans (cM).**

Stephenson *et al*. (1998) and Prasad *et al*. (2000), amplified more than two fragments, where the second fragment (called the null allele; Gill *et al*., 1991) was inherited in a dominant manner because it detected only susceptibility related DNA fragments.

Gill *et al*. (1991) established that the D-genome of wheat possesses a high percentage of null alleles, characterized by the absence of RFLP fragments in one parent that are present in the other, but with no apparent alteration in any other fragments. Recently Liu *et al*. (2001) found the same inheritance pattern with microsatellites where only the resistant band was amplified. In *Arabidopsis*, 12% of the RFLPs were of the null allele type (Chang *et al*., 1988) while in rice two single copy clones detected null alleles in the *japonica* parent (McCouch *et al*., 1988). The possible explanation for the presence of these null alleles could be that insertions-deletions occurred in both parents, which lead to a major sequence rearrangement(s) in the corresponding region of cv. Otane relative to cv.

**Table 2. Chromosomal location of microsatellite markers with expected and observed DNA fragment size (bp) linked with stripe rust susceptibility in two wheat cultivars.**

| Marker | DNA band expected (Röder *et al*., 1998) | Location | DNA band observed in this study Cv. Tiritea | Cv. Otane |
|---|---|---|---|---|
| *gwm282*a,b | 274-193 | 7AL | 225 bp | 215 bp |
| *gwm282*c | ++ | ++ | 200 bp | null |
| *gwm332*a,b | 290-211 | 7AL | 250 bp | 240 bp |
| *gwm332*c | ++ | ++ | 215 bp | null |

++ not reported in Röder *et al*. (1998).

Tiritea, such that the homologous region to these specific microsatellites do not exist in the cv. Otane genome. Although the evidence that the D-genome had a high percentage of null alleles and three of the null alleles were mapped to 7D (Imtiaz, 2002) is convincing, for a more accurate location of these null alleles, the use of nulli-tetra and ditelosomic lines and testing of the whole population with these markers will be required. To answer the question of whether insertion in the cv. Tiritea genome or deletion from the cv. Otane genome has occurred will need phylogenetic studies of the genome of both cultivars. Finally among the different marker systems used (Imtiaz, 2002), microsatellites were found to be more polymorphic and rapid in the generation of results.

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