Genetic and phenotypic mapping for leaf rust resistance, *Lr34* in Indian bread wheat population

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ABSTRACT

The Indian spring bread wheat cultivar 'HD 2189' shows leaf tip necrosis and resistance to leaf rust pathotypes in India since its release in 1980. We studied 128 single seed descent lines of an 'HD2189'×'Agra Local' F_6 population to identify and map quantitative trait loci (QTLs) for leaf rust resistance. Percentage of leaf area infected and the response to infection were evaluated in two field trials in 2006-07 crop season at Directorate of Wheat Research, Karnal (India) and were transformed to the area under the disease progress curve (AUDPC). One–hundred and twenty eight F_6 lines along with both parental lines were analysed with six polymorphic simple sequence repeats (SSRs) markers (GWM1220, XWM1, SWM5, SWM8, SWM10 & WMC463) and one sequence tagged sites (STS) marker, csLV34. A genetic linkage map based on above markers data was established. The region containing *Lr34* was flanked by *XSWM8* (proximal) and *XWMC463* (distal) and spanned 68.7 cM in 'HD 2189'×'Agra Local' population. Marker SWM10 was closely located to *Lr34* in present population. By using composite interval mapping and LOD >4.4, QTL analysis of the linkage group representing chromosome 7DS in the HD 2189×Agra Local population defined a QTL for leaf rust resistance in the interval *XGWM1220* - *XSWM10* which accounted for 14.8 % of observed phenotypic variation for leaf rust resistance (AUDPC).

INTRODUCTION

Bread wheat (*Triticum aestivum* L. em Thell) has an allohexaploid genome (AABBDD, 2n = 6x = 42) with seven group of homeologous chromosomes. It is one of the major food crops in the world. Of the total area (approximately 215 million hectares) sown to hexaploid and tetraploid wheat worldwide, 44 per cent (95 million hectares) is in Asia. Of this, 62 million ha is located in China, India and Pakistan (Singh *et al.,* 2004). The rust diseases of wheat pose a constant threat to sustainable wheat production and thus global food security. Leaf rust caused by *Puccinia triticina* Eriks is a devastating disease of wheat. The leaf rust pathogen is highly variable and has gained additional virulence over a period of time rendering new cultivars grown in India susceptible. The virulence pattern of *P. triticina* in central, peninsular and southern hill zones underwent a shift during 1980 to 2007. New pathotypes virulent on *Lr9*, *Lr19* and *Lr26* have appeared recently (Nayar *et al.,* 2003; Bhardwaj *et al.,* 2005). Although the timely application of fungicides can provide adequate control but their use adds to production costs and they are environment unfriendly. Thus growing resistant varieties is the most effective, environmentally safe and efficient control strategy for wheat rusts.

In contrast to race-specific resistance genes, two adult plant resistance genes (*Lr34* and *Lr46*) have been reported to confer stable resistance to diverse pathotypes of the fungus and are thought to be durable (Singh *et al.,* 1998). Molecular markers are powerful tools for identifying quantitative traits and dissecting these complex traits into Mendelian factors in the form of quantitative trait loci (QTL) as well as for establishing the genomic locations of such genetic loci. Genetic studies using lines from CIMMYT (William *et al.,* 1997), Switzerland (Schnurbusch *et al.,* 2004, Bossolini *et al.,* 2006) or the Indian (Kaur *et al.,* 2000) spring wheat germplasm confirmed the involvement of *Lr34* in the expression of durable leaf rust resistance. The objective of present study was to dissect the quantitative and durable leaf rust resistance of the India cultivar 'HD 2189' in the 'HD 2189' × 'Agra Local' population using quantitative trait locus (QTL) analysis.

MATERIALS AND METHODS

A population of 128 F_6 derived recombinant inbred lines (RILs) of cross between the two Indian spring bread wheat cultivars 'HD 2189' (resistant) and 'Agra Local' (susceptible) was phenotyped for leaf rust resistance at two locations at the Directorate of Wheat Research, Karnal (Haryana) India. Leaf rust epidemic was initiated by inoculating 3 week old plants of spreader rows with urediniospore-water-tween 20 suspension having equal proportions of predominant four leaf rust pathotypes (12-2, 77-2, 77-5 and 104-2). The infection types TR, R, TMS, MS, TS and S) were recorded by following McNeal *et al.,* (1971). Area under disease progress curve (AUDPC) was calculated using a computer package developed at CIMMYT, Mexico (CIMMYT. 1988) based on three leaf rust scorings recorded at 10 days equal interval.

Genomic DNA of 128 RILs and parents, 'HD 2189' and 'Agra Local' was extracted from fresh leaves using cetyltrimethylammonium bromide (CTAB) method. One–hundred and twenty eight F_6 lines alongwith both parental lines were analysed with six polymorphic simple sequence repeats (SSRs) markers (GWM1220, XWM1, SWM5, SWM8, SWM10 & WMC463) and one

sequence tagged sites (STS) marker, csLV34. Microsatellite primers were used for amplification as described in original research papers (Bossolini *et al.,* 2006; Lagudah *et al.,* 2006; Seah *et al*., 1998). PCR products of SSR markers were resolved on polyacrylamide gel (PAGE) separated using LiCor DNA sequencer 4200. Visualization of the amplified PCR products of STS marker, csLV34 was by using a 2.5 per cent high-resolution agarose gel coupled with ethidium bromide staining. The linkage map was constructed using MAPMAKER 3.0b for MS-DOS (Lander *et al.,* 1987). QTL analysis was performed with genotypic data of seven markers and phenotypic data for leaf rust i. e. the Area Under Disease Progress Curve (AUDPC). Interval QTL analysis was carried out with the composite interval mapping (CIM) program, PLABQTL, version 1.1 (Utz and Melchinger, 2000).

RESULTS AND DISCUSSION

One–hundred and twenty eight F_6 lines along with both parental lines were phenotyped for leaf rust under artificially inoculated conditions at two locations in Karnal (India) during 2006-07. AUDPC values ranged from 0 to1082.50 on RILs. AUDPC values of 300 and 820 were calculated on parents 'HD 2189' and 'Agra Local', respectively. One hundred twenty eight lines (F6) from the cross between 'HD 2189' and 'Agra Local' were genotyped with the six SSR markers most closely linked to *Lr34*, alongwith the STS marker csLV34. We found that the region containing *Lr34* and flanked by *XSWM8* (proximal) and *XWMC463* (distal) spanned 68.7 cM in "HD 2189 × 'Agra Local' population. In this population, QTL analysis defined a QTL for leaf rust resistance in the interval *XGWM1220* – *XSWM10*. QTL analysis (composite interval mapping) of the linkage group representing chromosome 7DS in the 'HD 2189' × 'Agra Local' population revealed a QTL at position 20 with *XSWM10*, which accounted for 14.8 % of observed phenotypic variation for leaf rust resistance (AUDPC) with corresponding LOD 4.4. This QTL is considered to be due to the effects of the known slow-rusting gene, *Lr34*. These data indicate the usefulness of SWM10 for marker-assisted breeding programs for *Lr34/Yr18*.

Our finding suggest that leaf resistance locus on 7DS corresponds to the *Lr34* gene. Leaf rust resistance loci were also earlier detected in other wheat populations (Messmer *et al.,* 2000, Suenaga *et al.,* 2003, Schnurbusch *et al,* 2004). *Lr13* had also been postulated in cultivar 'HD 2189' (Nayar *et al.,* 1999). *Lr34* in combination with *Lr13* confers high level of adult plant resistance which is durable in nature in cultivars Frontana and Era (Roelfs, 1988). In an earlier study, *XSWM10* mapped 0.7cM proximal to *Lr34* in 'Arina' × 'Forno' population (Bossolini *et al.,* 2006). A better understanding of the genetic basis of leaf rust resistance in present population would greatly improve the breeding efficiency of this trait for pyramiding of rust resistance genes.

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