Molecular mapping of leaf rust resistance gene *Lr15* in bread wheat

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INTRODUCTION

Wheat leaf rust, also known as brown rust, is a serious fungal disease affecting wheat and rye caused by *Puccinia triticina*. Leaf rust has potential to cause losses up to 40% whereas up to 100% losses due to stem rust have been reported[1]. The wheat leaf rust fungus is adapted to a range of different climates and the disease can be found in diverse wheat growing areas throughout the world thereby affecting the productivity and quality of wheat worldwide. Wheat cultivars that are susceptible to leaf rust regularly suffer yield reductions depending on the stage of the crop development when infection occurs. Depending on the severity and duration of infection, losses can vary up to 50% depending on the leaf rust resistance genes deployed and the composition of virulence in the region[2, 3].

Growing resistance cultivars is an effective and economical method of reducing losses to leaf rust. To date, 50 leaf rust resistance genes have been identified in wheat and related species[3]. However, the constant search for the novel resistance genes is essential in order to cope with the dynamic and rapidly evolving pathogen population. The demands for the increasing global crop production have promoted the development of new approaches relying on molecular markers technologies to investigate and improve plant genome organization. Development of genetic resistance in new cultivars contributes to the reduction in economic losses, production costs and risk of environmental pollution due to fungicide usage. As the cultivars with single resistance genes have been successfully attacked by emergence of virulent pathotypes, pyramiding of rust resistance has been the better approach[4]. However, pyramiding of rust resistance genes through traditional phenotypic methods is difficult when different resistance genes produce similar types of infection. Identification of molecular markers linked to rust resistance genes facilitates marker assisted selection (MAS) and gene pyramiding in breeding programs[5, 6]. Additionally, the efficiency of introgression of alien genes to hexaploid wheat can be improved by use of molecular markers[7]. Various molecular markers have been widely used to tag and map resistance genes in wheat, however, simple sequence repeat (SSR) have emerged as the choice of markers in gene mapping studies. In recent efforts, molecular markers linked to rust resistance genes such as *Lr3a,*[8] *Lr19,*[9, 10] *Lr34,*[11] *Lr39,*[12] *Sr2,*[13] *Sr39,*[14] have been reported. In our earlier efforts, we have also reported tagging of leaf rust and stem rust resistance genes[8]. Here we have attempted to identify tightly linked flanking markers for *Lr15* and their utility in marker assisted selection (MAS) with gene pyramiding efforts is discussed.

MATERIALS AND METHODS

Plant material and phenotypic evaluations:

The segregating population for *Lr15* was developed from a cross between susceptible variety Thatcher (Tc) and resistant NIL Tc-*Lr15* which consisted of 221, F_2 plants developed at Punjab Agricultural University, Ludhiana. Plants were grown in pots under controlled environmental conditions. The parents and populations were evaluated for seedling resistance gene *Lr15* against the leaf rust, *Pucciniaa triticiana* pathotype 162(A).

DNA extraction:

Genomic DNA was extracted from young leaf tissue of parents and segregating progeny according to the protocol[15]. DNAs from other wheat genotypes and double crossed lines were also extracted.

SSR analysis:

Sixty-five SSR primers from the 2DS chromosome were synthesized (Sigma Inc. USA) and utilized in this study. The polymerase chain reaction with 15ul total reaction volume was performed using SSR primers in MJ Thermocycler[16] (MJ Research, USA). The PCR products were separated on 6% PAGE gel in 0.5X TBE (Tris borate, EDTA pH 8.0) at 1600V for 3h and visualized by silver staining[17].

Data analysis:

Linkage analysis was carried out using the MAPMAKER v. 3.0[18]. The marker order was established using multipoint analysis at LOD 3.0 or above. Kosambi mapping function was used to determine the distances in centimorgans (cM) between the two markers[19]. Linkage groups constructed at LOD 3 and flanking markers were tested for higher LOD (> 3).

RESULTS AND DISCUSSION

Phenotypic evaluation:

Disease scoring revealed monogenic nature of *Lr15* forming two distinct phenotypic groups viz resistant as Tc*Lr*15 and susceptible as Thatcher. Out of 221 F_2 plants, 148 plants showed resistance and remaining 73 plants were susceptible ($\chi^2_{3:1}$= 6.91, non significant at 1 df and $P = 0.05$) [10].

Microsatellite analysis:

In the present study, we employed 64 2DS specific microsatellite primers [20, 21, 22] of which, 24 markers (37% polymorphism) showed polymorphism in Thatcher and its NIL having resistance gene *Lr15*. Later, these polymorphic markers were used on F_2 population of 221 plants. The high polymorphism (37%) in present mapping population in spite of being NILs can be attributed to the increased recombination events in the gene rich region.

Mapping of *Lr15*:

For all mapping analysis, phenotype of F_2-derived F_3 families was used. All the polymorphic SSRs were used to analyze the population segregating for *Lr15*. Based on marker segregation, linkage group was constructed with 19 SSRs at LOD 4.0, representing 2D chromosome (Fig. 1). The closest markers flanking *Lr15* locus, were Xgwm4562 and Xgwm102 at a distance of 3.1 cM and 9.3 cM, respectively till LOD 9.0. Further, to confirm linkage of marker for *Lr15*, data was analyzed considering only homozygous resistant: homozygous susceptible (A:B) lines. This analysis revealed presence of same flanking markers, Xgwm4562 and Xgwm102, to *Lr15* at a distance of 1.9 cM and 8.1 cM, respectively, till LOD 24.0. This is the first report of tagging seedling resistance gene *Lr15* in wheat.

Pyramiding of *Lr15* and *Lr34:*

Genotype NI5439, which is adapted to rain-fed and low irrigation conditions, is recommended for central and peninsular zones in India. Although its yield potential is high with one or two irrigations, it is completely susceptible to prevalent races in this region. Incorporation of *Lr34* in NI5439 along with the seedling resistance gene *Lr15* will not only enhance the resistance of this variety significantly but this will ensure sustained high productivity also. To pyramid the leaf rust resistance genes *Lr15* and *Lr34*, doubled crosses were made using NI5439, TcLr15 and TcLr34; and seeds from these crosses were tested for presence of *Lr15* and *Lr34*. The genomic DNA was extracted from 97 doubled cross lines with *Lr15* and *Lr34* genes, generated at ARI, Pune along with the parental lines i.e. NI5439, TcLr15, TcLr34 and Tc. These lines were screened with flanking markers for *Lr15* (Xgwm4562 and Xgwm102) and *Lr34* (csLV34[11] and SWM10[23]) to identify the lines carrying these genes. Marker segregation data of the doubled cross lines revealed total 40 lines having both the R alleles with the markers. Further, these 40 lines will be analysed for csLV34 and SWM10 for presence of *Lr*34. The genetic background of those lines will also be analysed using markers all over the genome. *Lr*15 gene shows spectrum of resistance in presence of APR gene such as *Lr34* and is linked to stem rust resistance gene *Sr*6 which is effective in India. Further, gene pyramiding of seedling resistance gene and adult plant resistance gene in drought tolerant cultivar NI5439 will provide long term resistance, thereby overcoming the ever increasing deficit of wheat crop enabling to bridge the gap between the demand and supply of wheat.

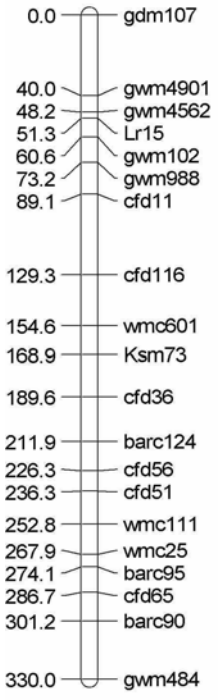

Fig. 1: Genetic linkage map of markers linked with *Lr15* on chromosome 2DS in the cross Tc x Tc-*Lr*15. Marker loci are indicated on right side and the genetic distances (cM) on the left side of the map.

REFERENCES

1. Singh RP, Huerta-Espino J, Roelfs AP (2002) The wheat rusts. FAO Corporate Document Repository. http://www.fao.org
2. Nagarjan S, Joshi LM (1975) A historical account of wheat rust epidemics in India and their significance. Cereal Rusts Bull 2:29-33
3. McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO Australia/ Kluwer Academic Publishers, Dordrecht, The Netherlands.
4. Messmer M, Seyfarth R, Keller M, Schachermayr GM, Winzeler M, Zanetti S, Feuillet C, Keller B (2000) Genetic analysis of durable leaf rust

resistance in winter wheat. Theor Appl Genet 100:419–431

5. Sharp PJ, Johnston S, Brown G, McIntosh RA, Pallota M, Carter M, Bariana HS, Khatkar S, Lagudah ES, Singh RP, Khairallah M, Potter R, Jones GK (2001) Validation of molecular markers for wheat breeding. Aust J Agric Res 52(12):1357-1366
6. Babu R, Nair SK, Prasanna BM, Gupta HS (2004) Integrating marker-assisted selection in crop breeding-prospects and challenges. Curr Sci 87(5):607-619
7. Asiedu R, Mujeeb-Kazi A, ter Kuile N (1989) Marker-assisted introgession of alien chromatin into wheat. In: Mujeeb-Kazi A, Sitch LA (eds) Review of advances in plant biotechnology, 1985-1988. 2nd Int Symp Genetic Manipulation of Crops. CIMMYT, Mexico City, and IRRI, Manila, pp 133-144
8. Khan RR, Bariana HS, Dholakia BB, Naik SV, Lagu MD, Rathjen AJ, Bhavani S and Gupta VS. (2005) Molecular mapping of stem and leaf rust resistance in wheat. Theor Appl Genet 111:846-850
9. Prins R, Groenwald JZ, Marias GF, Snape JW, Koebner RMD (2001) AFLP and STS tagging of *Lr*19, a gene conferring resistance to leaf rust in wheat. Theor Appl Genet 103:618-624
10. Gupta SK, Charpe A, Prabhu KV, Haque QMR (2006) Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat Theor Appl Genet 113:1027-1036
11. Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theor Appl Genet 114:21-30
12. Raupp WJ, Singh S, Brown-Guedira GL, Gill BS (2001) Cytogenetic and molecular mapping of the leaf rust resistance gene *Lr*39 in wheat. Theor Appl Genet 102:347-352
13. Hayden MJ, Kuchel H, Chalmers J (2004) Sequence tagged microsatellites for the Xgwm 533 locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr*2 in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1641-1647
14. Gold J, Harder D, Townley-Smith F, Aung T, Procunier J (1999) Development of a molecular marker for rust resistance genes *Sr*39 and *Lr*35 in wheat breeding lines. EJB Elect. J Biotech, ISSN: 0717-3458, 2, April 15, pp 1-6. http://www.ejb.org/content/vol2/issue1/full/1
15. Stein N, Herren G, Keller B (2001) A new DNA extraction method for high-throughput marker analysis in a large-genome species such as *Triticum aestivum*. Plant Breeding 120, 354-356
16. Ammiraju, JSS, Dholakia BB, Jawedkar G, Santra DK, Singh H, Lagu MD, Tamhankar SA, Dhaliwal HS, Rao VS, Gupta VS, Ranjekar PK (2002) Inheritance and identification of DNA markers associated with yellow berry tolerance in wheat (*T. aestivum* L.). Euphytica 123, 229-233.
17. Sanguinetti CJ, Dias Neto E, Simpson AJ (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17, 914-921
18. Lander ES, Green P, Abrahamson J, Barlow A, Day MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1, 174-181.
19. Kosambi DD (1994) The estimation of map distances from recombination values. Ann Eugen 12:172-175
20. Röder MS, Korzun V, Wendehale K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007-2023
21. Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697
22. Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105-1114
23. Bossolini E, Krattinger SG, Keller B (2006) Development of simple sequence repeat markers specific for the *Lr34* resistance region of wheat using sequence information from rice and *Aegilops tauschii* Theor Appl Genet 113:1049-1062