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ORIGINAL PAPER

Genetic mapping of adult plant leaf rust resistance genes *Lr48* and *Lr49* in common wheat

U. K. Bansal · M. J. Hayden · B. P. Venkata · R. Khanna · R. G. Saini · H. S. Bariana

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Abstract Hypersensitive adult plant resistance genes *Lr48* and *Lr49* were named based on their genetic independence of the known adult plant resistance genes. This study was planned to determine genomic locations of these genes. Recombinant inbred line populations derived from crosses involving CSP44 and VL404, sources of *Lr48* and *Lr49*, respectively, and the susceptible parent WL711, were used to determine the genomic locations of these genes. Bulked segregant analyses were performed using multiplex-ready PCR technology. *Lr48* in genotype CSP44 was mapped on chromosome arm 2BS flanked by marker loci *Xgwm429b* (6.1 cM) and *Xbarc7* (7.3 cM) distally and proximally, respectively. Leaf rust resistance gene *Lr13*, carried by the alternate parent WL711, was proximal to *Lr48* and was flanked by *Xksm58* (5.1 cM) and *Xstm773-2* (8.7 cM). *Lr49* was flanked by *Xbarc163* (8.1 cM) and *Xwmc349* (10.1 cM) on chromosome arm 4BL. The likely presence of the durable leaf rust resistance gene *Lr34* in both CSP44 and VL404 was confirmed using the tightly linked marker csLV34. Near-isogenic lines for *Lr48* and *Lr49* were developed in cultivar Lal Bahadur. Genotypes combining *Lr13* and/or *Lr34* with *Lr48* or *Lr49* were identified as potential donor sources for cultivar development programs.

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U. K. Bansal · B. P. Venkata · H. S. Bariana (✉)
Faculty of Agriculture, Food and Natural Resources,
University of Sydney PBI-Cobbitty, Private Mail Bag 11,
Camden NSW2570, Australia
e-mail: harbansb@camden.usyd.edu.au

M. J. Hayden
School of Agriculture, Food and Wine, University of Adelaide,
Waite Campus, Urrbrae SA5064, Australia

R. Khanna · R. G. Saini
Department of Plant Breeding, Genetics and Biotechnology,
Punjab Agricultural University, Ludhiana 141004, India

Introduction

Leaf rust caused by *Puccinia triticina* (Pt) is one of the important diseases of wheat globally. More than 50 wheat leaf rust resistance genes have been identified and named (McIntosh et al. 2005). Deployment of hypersensitive resistances individually may lead to increased frequencies of gene-specific virulences, either by selection of pathotypes already present at low frequencies or by selection of new mutational variants. There are numerous examples of breakdown of major genes worldwide. The detection of virulences for *Lr24* after 18 years of use in Australia (Park et al. 2002) and for *Lr26* after 6 years of use in India (Nayar et al. 1991) are important examples. Often, however, the longevity of effectiveness of a single gene is much less.

Over the last decade the emphasis in wheat rust resistance breeding has changed from seedling resistance to adult plant resistance (APR) in many breeding programs throughout the world. APR is controlled by either hypersensitive resistance genes or non-hypersensitive resistance genes. Adult plant hypersensitive resistance genes such as *Lr12*, *Lr22a*, *Lr22b* and *Lr35*, characterised by necrotic flecks or small pustules that are readily detected in monocyclic tests beyond seedling growth stages, behave in a similar way to classical seedling resistance genes. Non-hypersensitive resistance genes *Lr34* and *Lr46* are characterised by susceptible or moderately susceptible responses without necrosis and are detected by lower infection frequencies and by delayed rates of disease development.

Saini et al. (2002) identified hypersensitive adult plant leaf rust resistance genes *Lr48* in CSP44 (a single plant selection from the Australian cultivar Condor) and *Lr49* in Indian wheat cultivar VL404. These genotypes showed resistance against predominating Pt pathotypes in India (Saini et al. 2002) and Australia (Bariana et al. 2007) and can be used as sources of leaf rust resistance in both countries. In the present study, genetic mapping was undertaken to determine the genomic locations of leaf rust resistance genes *Lr48* and *Lr49*.

Materials and methods

Plant material

F_8 recombinant inbred line (RIL) populations were developed from crosses CSP44/WL711 (91 lines) and VL404/WL711 (120 lines) at the Panjab Agricultural University, Ludhiana, India. These RILs and parents were phenotyped for leaf rust responses at the fourth leaf stage in a greenhouse at Cobbitty, Australia and in field at Ludhiana, India. Only 92 lines from the VL404/WL711 RIL populations were used for genotyping.

Pathogen material

Puccinia triticina (Pt) pathotypes 76-1,3,5,10,12 (PBI culture no. 468), virulent for leaf rust resistance gene *Lr13*, carried by WL711, and Pt pathotype 104-1,2,3,(6),(7),11,13 (culture 547) avirulent on plants with *Lr13* in the seedling stage were used. Both Pt pathotypes were avirulent on plants with *Lr48* and *Lr49* at the fourth leaf stage.

Greenhouse studies

Two sets of eight to ten seeds of each RIL from CSP44/WL711 and VL404/WL711 were sown in 9 cm pots, four lines per pot. Pots were kept at 20°C in rust free microclimate rooms after sowing. Parental genotypes CSP44, VL404 and WL711 were included as controls. Ten grams of water soluble fertiliser Aquasol® dissolved in 10 litres of tap water for 100 pots were applied prior to sowing. Two applications of urea at the same rate were applied to seven days and 20 days old seedlings. One set of plants was inoculated at the two leaf stage by spraying a suspension of urediniospores of Pt 104-1,2,3,(6),(7),11,13 suspended in light mineral oil (Shellsol T). The second set was inoculated with Pt 76-1,3,5,10,12 at the fourth leaf growth stage. The inoculated plants were placed in a dew chamber for 24 h prior to moving to greenhouse rooms maintained at 25°C. Infection types were assessed 12–15 days after inoculation using the 0–4 scale described by Stakman et al. (1962). Variations within a given infection type class were indicated by adding '+' (more than average for the class) and '−' (less than average for the class). Heterogeneous uredinial type, including the susceptible types distributed randomly over a leaf were scored as 'X'. The RIL population was classified as homozygous resistant (HR), or homozygous susceptible (HS).

Molecular mapping

DNA was extracted from dried leaves using a Matrix Mill (Retsch MM300) and quantified using a Nanodrop spectrophotometer (Nanodrop Technologies). Bulk segregant analysis (BSA) was used initially to determine the genomic locations of leaf rust resistance genes *Lr48* and *Lr49*. Resistant and susceptible bulks for each RIL population were prepared by mixing equal amounts of DNA from ten homozygous resistant and ten homozygous susceptible lines, respectively. BSA was performed using a multiplex-ready PCR whole genome scan kit consisting of 488 published microsatellite (SSR) markers selected for high polymorphism content in Australian wheat germplasm and genome coverage. To saturate genomic regions associated with the resistance genes, additional SSR markers specifically targeting the regions of interest were screened on the parental lines and contrasting bulks. Markers showing putative linkage to the resistance gene in each cross were mapped on the entire RIL population.

Multiplex-ready PCR assays for BSA and genetic mapping were performed as described by Hayden et al. (2007). Electrophoresis and visualisation of the PCR products was performed on a GelScan2000 (Corbett Research) and ABI3730 DNA fragment analyser (Applied Biosystems). For analysis on the GelScan2000, the PCR products were mixed with an equal volume of gel loading dye (98% formamide, 10 mM EDTA, and 0.5% basic fuchsin as tracking dye), heated for 3 min at 95°C, chilled quickly on ice and separated on a 4% sequencing gel (Sambrook and Russell 2001). For ABI3730 analysis, the procedure described by Hayden et al. (2007) was followed. SSR allele scoring was performed using GeneMapper v4.0 software (Applied Biosystems).

Near-isogenic lines (NILs) for *Lr48* and *Lr49*

CSP44 and VL404 were crossed with the susceptible cultivar Lal Bahadur (LB) and F1 plants were backcrossed with LB. Near-isogenic lines for *Lr48* (CSP44/5*LB) and *Lr49* (VL404/5*LB) were isolated following the fourth backcross. These stocks are submitted to the Australian Winter Cereal Collection, Tamworth, New South Wales, Australia.

Marker csLV34 genotyping

The *Lr34*-linked PCR-based marker csLV34 (Lagudah et al. 2006) was genotyped on the RIL populations and respective parents. This marker was also genotyped on *Lr48* and *Lr49* NILs and Lal Bahadur to test for the possible presence of *Lr34*.

Data analyses and genetic mapping

Goodness of fit of observed segregations to expected genetic ratios was tested using Chi-squared tests. Genetic linkage analysis was performed using MAP MANAGER version QTXb20 (Manly et al. 2001) and the Kosambi (1944) map function. A LOD score of >3.0 was regarded as a reliable statistical indicator of genetic linkage.

Results

Inheritance studies

Genotypes CSP44 and VL404 showed low infection types;1+2 and ;1 respectively, and the susceptible cultivar WL711 showed a high infection type 3+, when tested against Pt pathotype 76-1,3,5,10,12 at the fourth leaf stage. Both RIL populations showed monogenic inheritance of resistance at Ludhiana against Pt pathotype 77-5. Both RIL populations were tested at Cobbitty (Table 1) and the two data sets were in perfect agreement.

Genetic mapping

Lr48

Thirty-one SSR markers showed polymorphism between the parents and bulks and were distributed among 13 chromosomes. All polymorphic markers were tested on the individual lines that constituted the bulks and eight markers from chromosome 2BS were associated with *Lr48*.

Table 1 Frequency distribution of leaf rust response segregation among CSP44/WL711 and VL404/WL711-derived RIL populations

Pathotype/RIL population	Number of lines		$\chi^2_{1:1}$
	Resistant	Susceptible	
76-1,3,5,10,12 (Adult plant)			
CSP44 X WL711	37	49	1.67
VL404 X WL711	61	54	0.43
104-1,2,3(6)(7)11,13 (Seedling)			
CSP44 X WL711	33	42	1.88

Table value of χ^2 at $P = 0.05$ and 1 *d.f.* is 3.84

Additional chromosome 2BS located markers were included in a supplementary screen and 11 markers were polymorphic between the bulks. A total of 19 marker loci (*Xbarc91, Xbarc167, Xbarc349, Xbarc1027, Xbarc55, Xcfd11, Xksm0058, Xbarc318, Xstm773a, Xstm773b, Xbarc7, Xbarc160, Xgwm271a, Xgwm271b, Xbarc95, Xgwm429a, Xgwm429b, Xbarc1064, Xbarc183*) were mapped on the entire RIL population. Two marker loci, *Xgwm429b* and *Xbarc7*, showed linkage with *Lr48* (Fig. 1a, b, respectively), with recombination fractions of 0.061–0.073 and LOD scores of 11.3–11.4, respectively. The locus order and the genetic distances between *Lr48* and SSR markers are shown in Fig. 2a. A third marker locus *Xbarc55* was located 1.9 cM proximal to *Xbarc7*. All three microsatellite markers showed co-dominant inheritance (Table 2) and showed monogenic segregation (Table 3). The $Xgwm429b_{360}$ and $Xbarc7_{309}$ alleles were associated with the parent CSP44 and the parent WL711 amplified $Xgwm429b_{362\ bp}$ and $Xbarc7_{306\ bp}$ alleles. These results conclusively located *Lr48* on chromosome arm 2BS.

Lr49

Of 488 markers 72 were polymorphic between the parents and bulks. Individuals comprising each bulk were tested separately with the polymorphic markers to identify close trait-marker associations. Twelve markers showed close genetic associations between parents and bulks. The entire RIL population was genotyped using the putatively linked markers. Molecular mapping indicated the location of *Lr49* on chromosome 4B. Sixteen additional markers loci (*Xgwm513, Xwmc491, Xbarc301, Xbarc68.1, Xbarc68.2, Xbarc10.1, Xbarc10.2, Xgwm495, Xwmc349, Xwmc397, Xbarc199, Xbarc193, Xksm62, Xbarc163, Xbarc109* and *Xbarc20*) located on the long arm of chromosome 4B were genotyped on the entire population. *Lr49* was mapped to chromosome arm 4BL with *Xbarc163* (8.1 cM) and *Xwmc349* (10.1 cM) being the closest flanking marker loci (Fig. 3). VL404 amplified $Xbarc163_{199}$ and $Xwmc349_{137}$ alleles, whereas the $Xbarc163_{196\ bp}$ and $Xwmc349_{141\ bp}$ alleles were present in WL711. The flanking markers were easily scored, showed codominant inheritance (Table 2) and segregated in a 1:1 ratio without distortion (Table 3). LOD values for flanking markers ranged from 8.2 to 20.0. These results confirmed the location of *Lr49* in chromosome 4BL.

Lr13

The CSP44/WL711 RIL population was tested at the seedling stage with pathotype 104-1,2,3,(6),(7),11,13 and segregated for a single gene (Table 1), presumed to be *Lr13*,

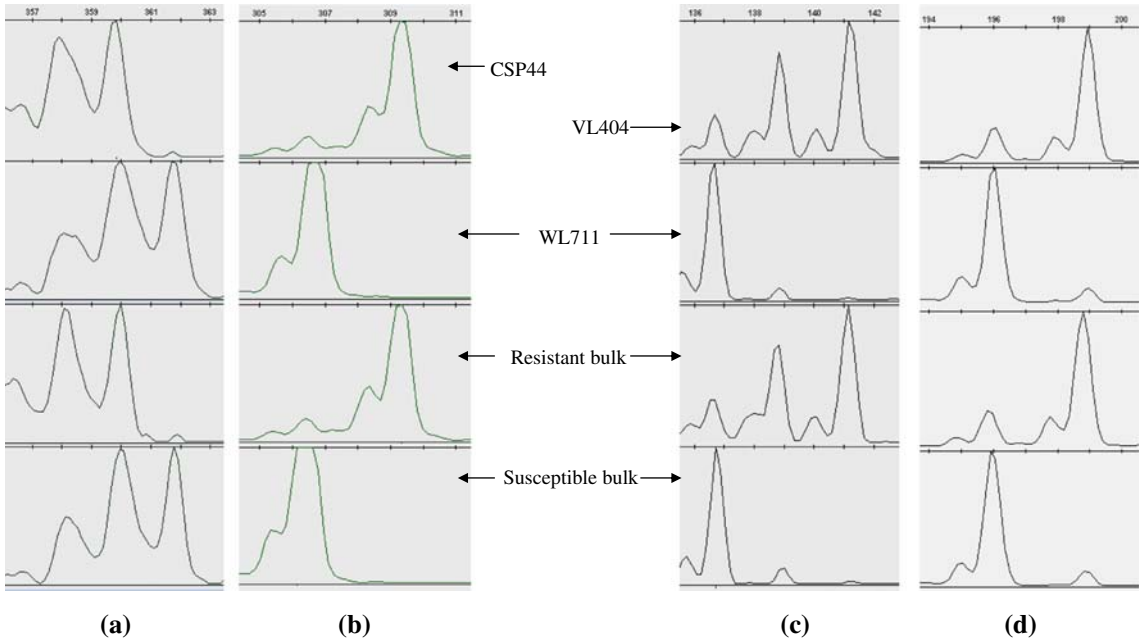

Fig. 1 ABI3730 electrotraces showing multiplex-ready PCR amplification (*from top to bottom*) of resistant parent, susceptible parent, resistant bulk and susceptible bulk for linked SSR loci (**a**) $Xgwm429b_{360}$ (**b**) $Xbarc7_{309}$ linked with *Lr48* and (**c**) $Xwmc349_{141}$ (**d**) $Xbarc163_{199}$ linked with *Lr49*

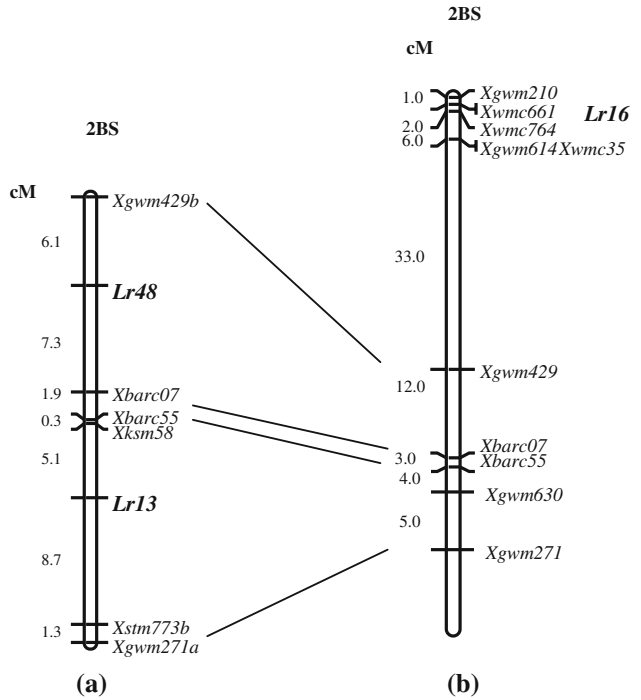

Fig. 2 Partial linkage map showing SSR markers (**a**) linked with *Lr48* and *Lr13* in chromosome arm 2BS in the CSP44/WL711 RIL population, and (**b**) comparative view of *Lr16, Lr48* and *Lr13* on the chromosome arm 2BS consensus map of Somers et al. (2004)

located on chromosome 2BS (McIntosh et al. 1995). *Lr13* mapped 14.6 cM proximal to *Lr48* and was flanked by marker loci *Xksm58* (5.1 cM) and *Xstm773b* (8.7 cM). The relative positions of marker and resistance loci are given in Fig. 2a. A separate estimation of linkage between *Lr48* and *Lr13* using the leaf rust response data was 13.7 cM. This genetic relationship confirmed the location of *Lr48* in chromosome 2BS.

Table 2 Allelic differentiation of parents for linked microsatellite loci

Locus	Allele size (bp)	
chromosome 2BS	CSP44	WL711
Xgwm429b	360	362
Xbarc7	309	306
Xbarc55	162	166
Chromosome 4BL	VL404	WL711
Xbarc193	314	317
Xbarc199	284	288
Xgwm513	178	186
Xgwm495	201	193
Xbarc163	199	196
Xwmc349	141	137

Confirmation of Lr34 in CSP44 and VL404

Saini et al. (2002) reported leaf tip necrosis in genotypes CSP44 and VL404 and suggested the presence of *Lr34* in these genotypes. Closely linked markers for *Lr34*, csLV34 and SWM10 were reported by Lagudah et al. (2006) and

Table 3 Frequency distribution of *Lr48* and *Lr49* linked microsatellite marker alleles among RIL populations

RIL population/Marker	Observed frequency		$\chi^2_{1:1}$
CSP44/WL711	CSP44 allele	WL711 allele	
Xgwm429b	33	44	1.57
Xbarc7	35	50	2.64
Xbarc55	33	49	3.12
VL404/WL711	VL404	WL711	
Xbarc193	42	28	2.80
Xbarc199	49	38	1.39
Xgwm513	56	36	2.27
Xgwm495	50	37	1.94
Xbarc163	48	36	1.71
Xwmc349	48	38	1.16

Table value of χ^2 at $P = 0.05$ and 1 *d.f.* is 3.84

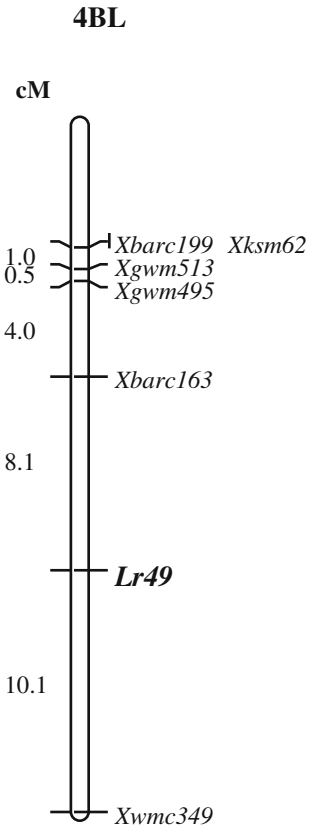

Fig. 3 Partial genetic map showing the location of *Lr49* in chromosome arm 4BL in the VL404/WL711 RIL population

Bossolini et al. (2006), respectively. To confirm the presence of *Lr34*, parents and RILs from both populations were genotyped with the agarose based marker csLV34. Resistant parents CSP44 and VL404 showed the *Lr34*-linked band (150 bp) and the susceptible parent amplified a 229 bp PCR product. RILs segregated in a 1:1 ratio confirming the possible presence of *Lr34* in CSP44 ($\chi^2_{1:1} = 1.49$, non significant at $P = 0.05$ and 1 *d.f.*) and VL404 ($\chi^2_{1:1} = 1.63$, non significant at $P = 0.05$ and 1 *d.f.*). Comparison of *Lr48* and *Lr49* phenotypic segregation data from greenhouse tests and segregation at the *XcsLV34* locus among RILs indicated that *Lr34* did not affect the expression of hypersensitive resistance conditioned by these genes under greenhouse conditions.

Characterisation of NILs with Lr48 and Lr49

NILs, CSP44/5*LB and VL404/5*LB, carrying *Lr48* and *Lr49*, respectively, produced leaf rust responses similar to parental genotypes CSP44 and VL404, when tested against Pt pathotype 76-1,3,5,10,12 under greenhouse conditions at the fourth leaf stage. The *Lr48*-NIL carried the linked $Xgwm429b_{360}$ allele from CSP44 and possessed the Lal Bahadur allele at the *Xbarc7* locus. The *Lr49*-NIL carried linked $Xwmc349_{137}$ allele from VL404 and the Lal Bahadur allele at the *Xbarc163* locus. Tests with the *Lr34*-associated marker csLV34 indicated that VL404/5*LB possibly carried *Lr34*, whereas CSP44/5*LB and Lal Bahadur lacked *Lr34*.

Discussion

The hypersensitive adult plant leaf rust resistance gene *Lr48* was mapped on chromosome arm 2BS. Other leaf rust resistance genes located on this chromosome arm include *Lr13, Lr16, Lr23* and adult plant resistance gene *Lr35* (McIntosh et al. 1995). CSP44 has no seedling resistance to any of the variants of pathotype 77 in India (Saini et al. 2002) or to predominant Pt pathotypes in Australia (Bariana et al. 2007) and hence the presence of these genes can be ruled out. *Lr48* appears to be located at a distinctive locus. The closely linked genes *Lr13* and *Lr23* (McIntosh et al. 1995) are clearly proximal to *Lr48* as indicated in Fig. 2a (13.7 cM apart), whereas *Lr48* was proximal to *Lr16* based on a Canadian report (McCartney et al. 2005). McCartney et al. (2005) mapped *Lr16* distal to the marker wmc764 on chromosome 2BS, a region corresponding to a cluster of closely linked SSRs spanning about a 10 cM region in the microsatellite consensus map of Somers et al. (2004) (Fig. 2b). In the present study, *Lr48* was flanked by the markers gwm429 and barc7. According to the microsatellite consensus map of Somers et al. (2004), the microsatellite locus *Xgwm429* on 2BS is about 37 cM proximal to the *Xwmc764* locus, which mapped closest to *Lr16* (Fig. 2).The order of molecular markers was consistent with the consensus map of Somers et al. (2004). The *Aegilops speltoides*-derived APR gene *Lr35* (Kerber and Dyck 1990) appeared to be present on a distal portion of chromosome 2BS (Dundas et al. 2007) and is linked with

stem rust resistance gene *Sr39*. In wheat genetic nomenclature the naming of alien-sourced genes located in non-recombining chromosome segments is not based on recombination data. *Lr48* was not associated with stem rust resistance and therefore cannot be *Lr35*. The widely distributed gene from bread wheat, *Lr13*, was flanked by the markers ksm58 and stm773b, and mapped proximal to *Lr48* in the present study (Fig. 2a). Therefore, the most likely gene order of the leaf resistance genes on the chromosome arm 2BS is *Lr16-Lr48-Lr13* with *Lr16* being distal and *Lr13* proximal loci. The proposed gene order is supported by the concordance of the map order obtained in the present study with published genetic maps (Fig. 2).

Molecular mapping using the VL404/WL711 RIL population located the adult plant resistance gene *Lr49* on chromosome 4BL, flanked by markers barc163 and wmc349 (Fig. 3). Chromosome 4B carries *Lr12* and *Lr31* on the short arm and *Lr30* on the long arm (McIntosh et al. 1995). *Lr31* interacts with *Lr27* in a complementary manner to produce a seedling resistance phenotype when challenged by pathotypes avirulent for *Lr12* (Singh et al. 1999). *Lr12* is an independent hypersensitive APR gene that confers resistance to avirulent pathotypes but the Indian Pt 77-5 was virulent on plants with *Lr12* (Saini et al. 2002). A Thatcher derivative with *Lr30* (Tc + *Lr30*) expressed seedling resistance to Indian Pt pathotype 77-5, whereas VL404 was susceptible, thereby indicating that VL404 does not carry *Lr30* (Kaur 2004; Saini et al. 2002). No other named gene for leaf rust resistance has been reported on chromosome 4BL.

In conclusion, this study successfully mapped the adult plant leaf rust resistance genes *Lr48* in chromosome 2BS and *Lr49* in chromosome 4BL. *Lr13* mapped 14.6 cM proximal to *Lr48*. Genotypes carrying *Lr13* and possibly *Lr34* together-with *Lr48* or *Lr49* were identified as potential donor sources. The future aim of our group is to develop markers linked closely with these resistance genes to facilitate their pyramiding with other resistance genes in cultivar development programs.

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