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Alternate schemes for combining leaf rust resistance genes through molecular marker

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ABSTRACT

The molecular marker for *Lr9*, *Lr19* and *Lr24* were validated in the parents and segregating F_2 populations derived from the cross between PBW343 and the donors of leaf rust resistance genes *Lr9* (HP1633), *Lr19* (Tc*Lr19*) and *Lr24* (HP1776). The markers for *Lr9*, *Lr19* and *Lr24* were able to discriminate the lines with specific genes from the lines that did not carry these genes. Further, molecular markers and the resistance genes co-segregated. Three different breeding schemes were followed to develop lines containing combination of *Lr9+Lr19+Lr24*. Frequency of high yielding lines in the three selection schemes ranged from 10 to 20%. Genetic stocks with combined resistance genes *Lr9+Lr19+Lr24+Lr26+Sr24+Sr25+Sr31+Yr9+Yr27* were developed in the background of PBW343.

Key words: Gene pyramiding, *Lr19* gene, *Lr24* gene, Marker validation, Molecular markers,*Triticum aestivum*

Leaf rust *(Puccinia triticina* Eriks.) occurs throughout wheat growing regions on the globe and it is one of the most important foliar diseases of wheat in India. Rusts can be managed most effectively and economically through cultivation of resistant varieties. Cultivars with diversified sources of resistance genes offer better shield against notorious races of the pathogen. Monogenetically inherited *Lr* resistance genes have usually been rapidly overcome by rust pathotypes. One of the effective remedies for increasing the longevity of resistance genes is to develop varieties with more than one effective resistance gene. Lack of differential host-pathogen interaction puts a limitation on conventional techniques to stack two or more effective rust resistance genes because seedling resistance genes normally act in an epistatic manner. Several PCR-based STS or SCAR markers have been developed for *Lr* genes namely, *Lr9* (Schachermayr *et al*. 1994), *Lr19* (Prins *et al*. 2001), *Lr24* (Mago *et al*. 2005). Some of these markers have been validated in parental lines and utilized for either authenticating incorporation of resistance genes or pyramiding of resistance genes (Slikova *et al*. 2004; Datta *et al*. 2006). For the present study a popular bread wheat cultivar, PBW343 (ND/VG9144//KAL/ BB/3/ YACO/4/VEE#5) was used as the recipient parent for incorporating three leaf rust resistance genes namely, *Lr9*, *Lr19* and *Lr24*. PBW343 has several resistance genes, viz *Lr26*, *Sr2*, *Sr31*, *Yr9*, *Yr27* (Nayar *et al*. 2001; Mc Donald *et al*. 2004). Apart from these postulated resistance genes, it carries additional temperatures sensitive factors against leaf rust (Datta *et al*. 2009). In the present report, molecular markers reported for *Lr9, Lr19* and *Lr24* were validated in the segregating population involving PBW343 and thereafter genetic stocks with combined resistance for *Lr9+Lr19+Lr24* were developed through marker-assisted selection.

MATERIALS AND METHODS

Plant materials for molecular marker validation

PBW343/HP1633 *(Lr9*), PBW343/HP1776 *(Lr24*) and PBW343/Tc*Lr19* F_2 populations were used for validation of *Lr9*, *Lr24* and *Lr19* markers, respectively. Lines carrying *Lr9*, viz. CS*Lr9* and HP1633 *(Lr9*) ; lines carrying *Lr19* viz Tc+*Lr19*, and FLW8; lines carrying *Lr24*, viz Agent, Arkan and HP1776 were kept as control for each marker.

Plant materials for applying marker-assisted selection

A high-yielding popular common wheat cultivar 'PBW343' (carrying *Lr26+LrPB1+LrPB2+Sr31+Yr9+Yr27*) was used as the recipient parent for the incorporation of leaf rust resistance genes *Lr9*, *Lr19* and *Lr24*. Homozygous lines of *Lr9*, *Lr19* and *Lr24* were derived from PBW343*4/ HP1633 *(Lr9*), PBW343*4/HP1776 *(Lr24*/*Sr24*) and PBW343*4/Tc*Lr19* crosses. Among these lines, further selections were made for presence of PBW343 genes, viz

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Lr26+LrPB1+LrPB2+Sr31+Yr9+Yr27. In the next step, line containing *Lr9* and *Lr19* were intercrossed to generate homozygous lines for *Lr9+Lr19*. Finally, a fixed line of 'PBW343'*4/HP1776 *(Lr24*) was crossed with a fixed line of *Lr9+Lr19* to generate population segregating for leaf rust resistance genes *Lr9*, *Lr19*, *Lr24* and their combination thereof. Three different breeding schemes were followed to develop lines containing combination of *Lr9+19+24*. In each of the schemes the initial F_2 population size was 300.

In the first scheme, three hundred F_2 seedlings were tested with a mixture of *Puccinia triticina* Eriks. pathotypes 121R127 and 77–8. Resistant seedlings were transplanted in the field and subjected to molecular marker analysis for the detection of *Lr9*. The seedlings positive for *Lr9* were tested for the presence of *Lr19* gene. The *Lr19* positive seedlings were tested for *Lr24.* The plants positive for all the markers were selected and advanced to next generation. The F_3 progenies were tested at the seedling stage with leaf rust pathotypes 121R127+77–8 and the non-segregating families were transplanted in the field. Ten plants from each of the non-segregating F_3 progenies were tagged for agronomic superiority and DNA was isolated from the tagged plants. The F_3 families that did not segregate for *Lr9* marker were selected. Among each of the selected families, individuals were identified that were positive for *Lr19* and *Lr24* markers and advanced to the next generation. In the F_4, DNA was isolated from 10 agronomically desirable plants from each of the F_4 progenies. The families that did not segregate for *Lr19* marker were identified. Among the non-segregating *Lr19* families, the plants that were also positive for *Lr24* marker were selected and advanced to the next generation. In the F_5, DNA was isolated from 10 agronomically desirable plants from each of the F_5 progenies. The families that were non-segregating for *Lr24* marker were selected for generation advancement. Incorporation of specific resistance genes were confirmed in the F_6 generation through progeny testing of 20 individuals/family.

In the second scheme F_2 seedlings were tested for the presence of *Lr9*, *Lr19* and *Lr24* markers sequentially. The seedlings positive for all three markers were transplanted in the field. The F_3 families were tested for the presence of markers and families homozygous for all three markers were selected and transplanted in the field. Visually superior plants were selected from F_3 progenies. Single plant selection was followed up to F_6 generation wherein best plants were selected from the superior progenies. In the F_7, superior progenies were identified and harvested in bulk.

In the third scheme F_2 seedlings were tested with a mixture of *Puccinia triticina* pathotypes 121R127 and 77–8 and resistant seedlings were transplanted in the field. F_3 families were tested in the seedling stage and all resistant seedlings were transplanted in the progeny rows. One agronomically superior plant was selected at maturity from each of the transplanted F_3 families. The procedure was repeated up to F_7 generation. Each of the F_7 lines were harvested as single plant and five seeds from each plant was pooled to form F_8 bulk. The F_8 lines were tested for the presence of *Lr9*, *Lr19* and *Lr24* markers.

Agronomic traits were evaluated at Regional Station, Directorate of Wheat Research, Flowerdale in augmented design. A two-row plot of 2 m length represented each entry. Yield/m row was recorded on whole plot basis. Lines were categorized into four arbitrary groups of very low (less than 110 g/m row, low (111–125 g/m row), medium (125–140 g/ m row) and high (greater than140 g/m row) yield.

Pathological testing conditions

The F_2 and F_3 seedlings were raised in the aluminum bread pan trays comprising 10 rows with the 7th row of each tray as a susceptible check, Agra Local. Fully expanded primary leaves were inoculated with uredospores suspended in light weight, non-phytotoxic isoparaffinic oil (soltrol). The inoculated seedlings were kept in a saturated humid glass chamber for 48 hr. The seedlings were then transferred to the glass house benches at 20–22°C. Infection types (IT) were recorded 14 days after inoculation and classified according to the standard method. The IT's 0; (naught fleck),; (fleck),;- (fleck minus),;1 (fleck one), 1 (one), 2 (two) and x (mesothetic) were classified as the resistant reactions whereas IT 3 (three) and 3+ (three plus) were classified as susceptible reactions.

Molecular markers

Molecular markers of *Lr9* (Schachermayr *et al*. 1994), *Lr19* (Prins *et al*. 2001) and *Lr24* (Mago *et al*. 2005), were validated in the segregating populations of PBW343/HP1633, PBW343/Tc*Lr19* and PBW343/HP1776. After validation these markers were utilized for marker-assisted selection of *Lr9*, *Lr19* and *Lr24* genes.

DNA isolation and PCR amplification

DNA was extracted by CTAB method. Amplifications were performed in PTC-200 Thermal cycler (MJ Research, Waltham, MA). PCR products of *Lr9* and *Lr24* were analyzed in 1.5% agarose and *Lr19* in 3% agarose gel in 0.5X TAE buffer. The PCR conditions for different molecular markers are presented in Table 1.

RESULTS AND DISCUSSION

The 1.1 kb band was specifically amplified in the lines carrying *Lr9* and absent in the negative control (Fig1). In the segregating population (F_2) of the cross PBW343/HP1633 *(Lr9*), 83 seedlings were resistant and 35 were susceptible. The resistant seedlings were positive for the *Lr9* marker. A 132bp fragment was amplified in the lines with *Lr19* (Fig 2). In the F_2 of the cross PBW343/Tc*Lr19*, 75 seedlings were resistant and 23 were susceptible. The *Lr19* specific fragment was present in all resistant seedlings, whereas the marker

Table 1 PCR profile of molecular markers for genes *Lr9*, *Lr19* and *Lr24*

Gene	Component		Cycle
Lr9 (SCAR)	F: 5'CCA CAC TAC CCC AAA GAG ACG 3'R: 5'TCC TTT TAT TCC GCA CGC CGG 3'	2mM $MgCl_2$, 100uM dNTP, 40Nm primer, 0.5U Taq Polymerase, 50ng DNA	1× 94°C 6m'40 × 94°C 1m'; 62°C 1m';72°C 2m'1× 72°C 4m'
Lr19 (SCAR)	F: 5' CAT CCT TGG GGA CCT C 3'R: 5' CCA GCT CGC ATA CAT CCA 3'	2mM $MgCl_2$, 0.2mM dNTP, 12.5p moles primer, 0.6U Taq Polymerase, 50ng DNA	1× 94°C 4m'30 × 94°C 1m'; 60°C 0.5m'; 72°C 0.5m'1× 72°C 5m'
Lr24/Sr24 (SCAR)	F: 5' CAC CCG TGA CAT GCT CGT A 3' R: 5' AAC AGG AAA TGA GCA ACG ATG T 3'	0.25mM of each dNTP, 2mM $MgCl_2$, 1U *Taq* polymerase, and 50 ηg of template DNA	94°C for 3 min. followed by seven cycles of 30 s at 94°C, 30s at 65°C, 40s at 72°C, primer extension temperature was reduced by 1°C/cycle for next six cycles; next 30 cycles of 30 s at 94°C, 30s at 58°C, 40s at 72°C, followed by a final extension step at 72°C for 5 min.

was absent in the susceptible seedlings. The *Lr24* marker (Dedryver *et al*. 1996) was validated in different wheat lines (Datta *et al*. 2007). A marker (Mago *et al*. 2005) for stem rust resistance gene *Sr24* was also validated as *Sr24* and *Lr24* are tightly linked and co-segregates. The 500bp marker was specifically amplified in the cultivars known to posses *Lr24* and absent in the lines devoid of *Lr24 viz. Lr9* and *L19* (Fig 3). In the F_2 of the cross PBW343/HP1776 *(Lr24)*, 91 seedlings were resistant as well as positive for the specific fragment, whereas 29 seedlings were susceptible and negative for the marker. Results of our experiments show the usefulness of the STS markers for identification of leaf rust resistance genes *Lr9*, *Lr19* and *Lr24* in different wheat genetic backgrounds.

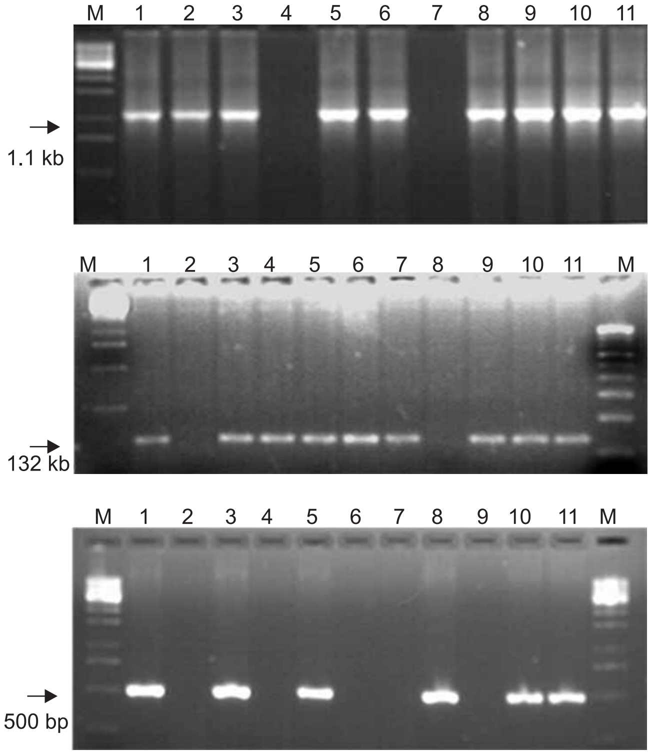

Fig 1–3. **1.** 1-Cs*Lr9*, 2-HP1633 *(Lr9)*, 3-FLW6 *(Lr9+24)*, 4-Chinese Spring, 5-FLW23 (PBW343/HP1633), 6–11: segregating population of PBW343/HP1633; **2.** 1- Tc*Lr19*, 2- Thatcher, 3-FLW8 (HI1077/Tc*Lr19*), 4- FLW24 (PBW343/Tc*Lr19*), 5–10: segregating population of Tc/Tc*Lr19*; **3.** 1-Agent *(Lr24)*, 2- Thatcher, 3- HP1776 *(Lr24)*, 4–12: segregating population of Tc/HP1776

In the first scheme three hundred seedlings out of the 258 F_2 were resistant to pathotypes 77–7 (virulent on *Lr9*) and 77–8 (virulent on *Lr19*). Among the resistant seedlings 185 were positive for *Lr9* marker. One hundred fiftysix of the *Lr9* carrying seedlings were positive for *Lr19* marker as well. Among the *Lr9+Lr19* carrying plants, 112 were also positive for *Lr24* marker. In the F_3, 41 families were homozygous resistant (HR) to mixture of pts. 77–7+77–8. Among the HR families, 19 were homozygous for *Lr9* marker. The plants from homozygous *Lr9* were also positive for *Lr19* and *Lr24* markers. Ten selected plants from each of the 19 F_3 families were advanced to F_4. Among the 190 F_4 progenies 103 were HR for *Lr19* marker. Eightyone *Lr19* HR plants were also positive for *Lr24* marker. Among the 81 F_5 progenies 62 were homozygous for *Lr24* marker. Two agronomically superior plants from each of the 62 families were advanced to F_6 as progeny rows. Ten superior progenies were identified on the basis of progeny yield in the F_6. Five high-yielding plants were selected from each of the 10 progenies and advanced to F_7. Ten superior progenies were identified (on the basis of progeny yield) and harvested in bulk. In the second scheme 133 seedlings were positive for *Lr9*, *Lr19* and *Lr24* markers in the F_2 and advanced to the next generation. In the F_3, 25–30 seedlings were transplanted and 10 random plants were tested for the presence of *Lr9* marker. Fortyseven F_3 families were homozygous for *Lr9*. These were tested for the presence of *Lr19* marker. Among the *Lr9* homozygous families, 13 were also homozygous for *Lr19*. The *Lr9+Lr19* homozygous families were tested for the presence of *Lr24* marker. Five F_3 families were homozygous for *Lr24* as well. Ten visually superior plants from each of the homozygous *Lr9+Lr19+Lr24* F_3 families were advanced to F_4. Fifty visually superior plants were selected from the F_4 generation and advanced to F_5. Fifty visually superior plants were selected from the F_5 generation and advanced to F_6. Ten superior progenies were identified on the basis of progeny yield in the F_6. Five superior plants were selected from each of the 10 progenies and advanced to F_7. Ten superior progenies were identified (on the basis of progeny yield) and harvested in bulk.

In the third scheme, 264 out of 300 F_2 seedlings were resistant to pts. 77–7 (virulent on *Lr9*) and 77–8 (virulent on *Lr19*). The resistant seedlings were transplanted in the field. Selection procedure as described in the materials and methods was followed to derive F_6 progenies. Among the 264 progenies 188 were positive for *Lr9* marker. These were tested for the presence of *Lr19* marker. One hundred twentyeight progenies were positive for *Lr19* as well. Among 128 progenies, 54 were also positive for *Lr24* marker. Ten progenies of each of the 44 selected lines were tested for homozygosity at *Lr9*, *Lr19* and *Lr24* loci. Thirtyeight homozygous lines were selected for further analysis.

In the selection schemes 1 and 2, ten lines were generated that possessed combination of *Lr9+Lr19+Lr24*, whereas in the scheme 3, thirtyeight lines carried three *Lr* gene combination. In the scheme 1, two lines were grouped in medium yield category and one line gave high yield (Table 2). In the selection scheme 2, four lines gave medium yield and two lines were grouped in high yield category. In the third scheme 10 lines were grouped in medium yield category and seven lines gave high yield. Though more number of high-yielding lines were generated in scheme 3 but their frequency was similar in all the schemes (10–20%) in all three schemes. Though some of the lines were high yielding but none of them were superior than PBW343, the high-yielding parental check. Such results were expected because the initial population size was low. The results further indicated that if the objective is to generate pre-breeding lines the best option is third scheme which generated reasonable number of high-yielding lines carrying three leaf rust gene combinations (*Lr9+Lr19+Lr24*) with minimum efforts.

Table 2 Yield potential of lines generated from different selection schemes

Group	No. of lines generated from different selection schemes	Y/MR Range (g)
I (very low)	S1-2 lines S2-3 linesS 3-8 lines	<110
II (low)	S1-5 lines S2-1 lineS 3-13 lines	111-125
III (medium)	S1-2 lines S2-4 linesS 3-10 lines	126-140
IV (high)	S1-1 line S2-2 linesS 3-7 lines	>140
	Parents	
	Tc*Lr19*	69.5
	PBW343	165.4
	HP1633	132.2
	HP1776	133.3
	PBW343*4/HP1776 (*Lr24*)	154.6
	PBW343*4/HP1633// PBW343*4/Tc*Lr19*	150.5

Y/MR= Yield/m row

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