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# Marker-assisted pyramiding of leaf rust resistance genes *Lr*24 and *Lr*28 in wheat (*Triticum aestivum)*

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ABSTRACT

A study was undertaken to pyramid two effective leaf rust resistance genes *Lr24* and *Lr28* in 'PBW 343' wheat (*Triticum aestivum* L. emend. Fiori & Paol.) background, a ruling variety of North Western Plains Zone using marker assisted selection. 'PBW343' near isogenic lines with *Lr24* and *Lr28* developed through backcross breeding, were intercrossed and the marker assisted selection was conducted in the $F_1$ to $F_4$ segregating generations using SCAR markers $S73_{719}$ and $S421_{570}$ linked to *Lr24* and *Lr28* respectively. Homozygous pyramid lines were developed and evaluated in yield trials to assess the effect of these genes on yield parameters. Some of the pyramid lines were observed to have better yield potential than the recipient parent 'PBW 343' and no yield penalty was apparent in these lines. Selected lines are being used for combining stripe rust and stem rust resistance genes with leaf rust resistance through marker assisted selection.

**Key words**: Leaf rust resistance, *Lr24, Lr28*, Marker-assisted selection, Pyramiding, *Triticum aestivum*

Leaf rust or brown rust caused by *Puccinia triticina* is one of the most common diseases affecting wheat production worldwide. The impact of leaf rust on yield reduction in wheat ranges from 10% under moderate conditions to 65% under intense epidemics. An effective, economical and ecologically safe method to control leaf rust epidemics is the cultivation of resistant cultivars. Sixty seven leaf rust resistance genes have been designated so far (McIntosh *et al*. 2008, 2010) and most of the genes condition hypersensitive reaction and interact with the pathogene in gene-for-gene fashion. Deployment of single resistance gene will not be effective because large-scale and long-term cultivation of such resistant varieties may result in significant shifts in the virulence pattern of the pathogen population leading to breakdown of resistance. Pyramiding multiple resistance genes in a single variety is an attractive strategy to prevent or delay the breakdown of resistance. Gene pyramiding is difficult using conventional breeding methods, however, the availability of molecular markers closely linked with the target genes makes the identification of plants with two and three genes possible (Gupta *et al*. 2009). A set of alien leaf rust resistance genes effective in India (*Lr19*, *Lr24* and *Lr28*) were initially mobilized from the winter wheat stocks to improved genetic backgrounds (Tomar and Menon 1998). These near isogenic lines (NILs) constitute the basic parental material for the transfer and pyramiding of these genes into other cultivated backgrounds. *Lr24* transferred from *Agropyron elongatum* to wheat chromosome 3DL and *Lr28* transferred from *Aegilops speltoides* to 4AL are highly effective against the prevalent pathotypes of leaf rust in Indian subcontinent. The present marker-assisted selection programme was undertaken to pyramid these genes into *T. aestivum* cultivar 'PBW343' to have more durable resistance for leaf rust.

The landmark variety 'PBW343' released for cultivation in north-western plains zone (NWPZ) in 1995 has 1BL.1RS translocation carrying a group of disease resistance genes, viz *Lr26*/*Sr31*/*Pm8*/*Yr9*. The leaf rust resistance of 'PBW 343' has shown signs of breakdown in the field. So with an aim to restore the leaf rust resistance of 'PBW 343', leaf rust resistance genes *Lr24* and *Lr28* were transferred to PBW 343 in two separate backcross programmes (Chhuneja *et al*. 2005) and the advance backcross progenies formed the basic material for pyramiding these two alien leaf rust resistance genes in 'PBW343' background.

In the present study the pyramiding of leaf rust resistance

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genes *Lr24* and *Lr28* in PBW343 background using SCAR markers linked to the target genes and their agronomic evaluation is being reported.

## MATERIALS AND METHODS

'PBW343' NILs in $BC_5F_1$ for *Lr24* and $BC_3F_1$ for *Lr28* developed in two separate backcross breeding programmes (Chhuneja *et al*. 2005) were crossed to pyramid these two leaf rust resistance genes in 'PBW343' (ND/VG1944//KAL/BB/3/YACO'S'/4/Veery#5'S') background. $F_1$ was selfed to generate $F_2$. The selectd $F_2$ plants were carried forward to $F_3$ and then to $F_4$ to develop homozygous pyramid lines with *Lr24* and *Lr28*. Leaf rust screening along with molecular marker analysis was used to follow the genes during these selfing generations.

The segregating progenies were screened at the seedling stage for leaf rust resistance with leaf rust pathotype 77-5, virulent on recurrent parent 'PBW343'. Screening at the seedling stage was done following the procedure of Nayar *et al*. (1997) in controlled conditions. Fourteen days after inoculations the infection types were recorded using 0–4 scale proposed by Stakman *et al*. (1962). The seedling-screened plants were transplanted in the field and evaluated for terminal disease severity at the adult plant stage. In the field screening, data were recorded as percentage of leaf area covered with leaf rust according to a modification of the Cobb scale as described by Peterson *et al.* (1948).

A simplified procedure was followed to obtain crude DNA suitable for PCR analysis. A single leaf of wheat seedling was harvested and placed in a 1.5 ml centrifuge tube on ice. The sample was ground in a spot test plate with a polished glass rod after the addition of 400µl of extraction buffer (50 mM Tris-HCl, pH 8.0, 25 mM EDTA, 300 mM NaCl and 1% SDS). After grinding, another 400µl of DNA extraction buffer was added to the well and mixed. From the well, 400µl of lysate was transferred to the original tube. The lysate was extracted with 400µl of chloroform. The aqueous supernatant was transferred to another 1.5 ml tube, and DNA was precipitated with ethanol and finally washed with 70% ethanol. The DNA pellet was air-dried and was suspended in 50µl of Tris EDTA (TE). Aliquots of 1µl was taken for PCR analysis.

SCAR markers, $S73_{719}$ and $S421_{570}$ were employed to follow leaf rust resistance genes *Lr*24 and *Lr*28, respectively during marker-assisted pyramiding of these genes (Prabhu *et al*. 2003, 2004). PCR amplification was carried out in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 µM of each primer (forward and reverse), 50 ng of genomic DNA and one unit of Taq polymerase/25 reaction. PCR was performed in thermocycler (Eppendorff) with initial denaturation for 2 min. at 94°C, followed by 35 cycles of 1 min at 94°C, annealing for 1 min. at 58°C, 1 min. at 72°C and a final 7 min. extension at 72°C. Amplification products were separated on 1.5% agarose gel for 2-3 hr at 5V/cm constant voltage and viewed under UVP gel-documentation system.

Twenty four 'PBW343' pyramid lines along with near isogenic lines (NILs) with *Lr28* singly were evaluated for various agronomic parameters to assess the yield potential of these lines and to determine the extent of linkage drag, if any.

### *Results and Discussion*

The present work was to pyramid two leaf rust resistance genes in a popular wheat cultivar of NWPZ, 'PBW343' which has been in cultivation for last 15 years on major wheat growing area of this region. Monoculture of this cultivar has resulted in breakdown of resistance for leaf rust as well as stripe rust. Marker-assisted pyramiding of multiple effective disease resistance genes is one of the best strategies to achieve durable rust resistance which can provide resistance to fast evolving pathogens.

$BC_5F_1$ plants of 'PBW343' with leaf rust resistance gene *Lr24* and $BC_3F_1$ plants of 'PBW343' with *Lr28* were crossed and self progenies analyzed for the presence of leaf rust resistance genes *Lr24* and *Lr28* using a combination of rust screening and marker analysis. In $F_1$ to $F_4$ self generations, selection for the leaf rust resistance genes was performed with SCAR markers for identifying plants carrying both *Lr24* and *Lr28*, followed by visual selection for the better phenotype among the plants carrying both the genes. The homozygous pyramid lines were developed and finally evaluated under yield trials.

### *Selections in $F_1$ to $F_4$ from pyramid crosses*

Since the parental plants used for crossing were heterozygous for leaf rust resistance genes *Lr24* and *Lr28*, the $F_1$ from pyramid crosses, segregated for leaf rust resistance. A total of 94 plants were analyzed at the seedling stage against leaf rust pathotype 77-5 in the crop season 2002–03. All the screened plants were transplanted in the field and out of these 74 leaf rust resistant plants were analyzed for the presence of gene-specific SCAR markers; $S73_{719}$, linked to *Lr24* and $S421_{570}$ linked to *Lr28*. Twentyfive plants were observed to be positive for *Lr24*, 16 were positive for *Lr28* and 17 plants were positive for both *Lr24* and *Lr28* (Table 1). Being the dominant markers both of the markers did not show any amplification in the leaf rust susceptible plants. Plants having both the leaf rust resistance genes (*Lr24lr24Lr28lr28*) were carried forward. $F_2$ progeny of 14 plants were evaluated for leaf rust resistance and for the presence of *Lr24* and *Lr28* in 2003–04. Molecular marker analysis was performed on selected 321 resistant plants to identify the plants carrying both the leaf rust resistant genes 85 plants were observed to carry *Lr24*, amplifying 719bp fragment with SCAR marker $S73_{719}$, 123 plants carried *Lr28*, amplifying 570bp fragment with SCAR marker $S421_{570}$. Out of 321 plants analyzed, 113 plants showed positive

amplification for both the genes (Table 1). It was not possible to distinguish heterozygous and homozygous plants based on these SCAR markers. Then the selection was made for the plant type to identify the plants having maximum recipient parent genotype recovery. Twentyone best $F_2$ plants were carried forward to $F_3$ progenies.

A total of 456 plants from 21 $F_3$ families were screened for leaf rust resistance. Eighteen families found to be homozygous resistant for leaf rust were further characterized with SCAR markers. The 17 $F_3$ progenies showed amplification with both the SCAR markers. Out of these, 15 progenies showed segregation for one or the other SCAR marker indicating that the $F_2$ plants were heterozygous for *Lr24* and/or *Lr28* (*Lr24Lr24/Lr28lr28*; *Lr24lr24/Lr28Lr28* or *Lr24lr24/Lr28lr28)*. Only two of the progenies were found to give uniform amplification with both the markers indicating that the parental $F_2$ plant was homozygous for both the genes (Table 1). $F_4$ seed was harvested from the selected plants positive for both the genes, from segregating progenies as well as from homozygous progenies. The presence of *Lr24* was also confirmed through GISH using *Agropyron intermedium* DNA as probe (data not given).

In $F_4$, a total of 42 progenies were selected for marker analysis, out of which 12 progenies segregated for *Lr24* only and showed uniform amplification with *Lr28* SCAR indicating that these have been derived from *Lr24lr24/Lr28Lr28* $F_3$ plant. Three progenies segregated for *Lr28* and were homogenous for *Lr24* and hence are the derivatives of the $F_3$ plant with genomic constitution of *Lr24Lr24/Lr28lr28* at the target loci. Ten progenies showed segregation both for *Lr24* as well as *Lr28* confirming the genotype, *Lr*24*lr*24/*Lr*28*lr*28, of the parental plant. Seventeen progenies were found to be homozygous for both the target loci (Table 1).

*Yield trial of pyramid lines*

Single plant progenies after seed multiplication at offseason nursery were evaluated under replicated yield trials at main campus at PAU, Ludhiana. Near isogenic lines with single leaf rust resistance genes *Lr28* were also included in the trials during 2005–06 and 2006–07 to estimate the effect of these alien leaf rust resistance genes singly and in combination on grain yield and other yield component traits. The yield evaluation of the NILs for *Lr24* has been reported in Chhuneja *et al*. (2005). The prevalent apprehension was that the genes when pyramided in a single cultivar, may prove taxing to yield or any of the yield components leading to the yield penalty, especially in the absence of rust but at the same time it could be useful to sustain yield levels in case of rust epidemic. In 2005–06, the lines 'K40' and 'K76' had significantly high yield (Table 2, 3.752kg/plot and 3.101 kg/plot, respectively) than the recipient parent 'PBW343' (2.101kg/plot). Line K112 (*Lr28* only, 3.101 kg/plot) also had significantly high yield than the best check. Other lines had yield at par with 'PBW343', clearly indicating no penalty of the rust resistance genes (whether present in combination or alone) on yield or any of the yield components. In 2006–07, the lines 'K22', 'K40' and 'K87' had yield (3.852kg/plot, 3.865 kg/plot and 3.852kg/plot, respectively) were at par with check 'PBW343' (3.842kg/plot). Lines 'K 22' and 'K 40' had both *Lr24* and *Lr28* pyramided in 'PBW343' and line 'K87' had only *Lr28*. Same kind of inference indicating no penalty of these rust resistance genes (present in combination or alone) on yield as well as any of the yield components was observed in the second year also. The positive effect of *Lr28* in NILs in increasing yield through yield components has been reported by Kumar *et al*. (2004). High-yielding lines along with 'K75' and 'K76' have been selected for further improvement.

Table 1 Marker analysis in $F_1$ to $F_4$ populations derived from pyramid crosses of 'PBW343'+*Lr24*/'PBW343'+*Lr28*

| Generation | Total leaf rust resistant plants analyzed[$] | Marker-assisted selection in $F_1$–$F_2$ generations: Resistant plants with+ive amplification for | | |
|---|---|---|---|---|
| | | $S73_{719}$ only[#] | $S421_{570}$ only | $S73_{719}$ and $S421_{570}$ |
| $F_1$ | 58 | 25 | 16 | 17 |
| $F_2$ | 321 | 85 | 123 | 113 |
| | *Total progenies analyzed[$]* | *Progenies segregating for* | | *Progenies homozygous for* |
| Generation | | $S73_{719}$ (homozygous for $S421_{570}$) | $S421_{570}$ (homozygous for $S73_{719}$) | $S73_{719}$ and $S421_{570}$ | $S73_{719}$ and $S421_{570}$ |
| $F_3$ | 18 | 0 | 1 | 15 | 2 |
| $F_4$ | 42 | 12 | 3 | 10 | 17 |

[$] In $F_1$ and $F_2$ data for individiual plants and in $F_3$ and $F_4$ data for progenies is presented
[#]$S73_{719}$ and $S421_{570}$ are the SCAR markers linked to *Lr24* and *Lr28*, respectively

Table 2 Agronomic evaluation of the pyramid lines with *Lr24* and *Lr28* and NILs with *Lr28* in PBW343 background

| ID | Days to flowering | | Plant height (cm) | | Tillers/m | | Spikelets/ spike | | Days to maturity | | Yield (kg/ plot#) | | Leaf rust | | Stripe rust | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2005-06 | 2006-07 | 2005-06 | 2006-07 | 2005-06 | 2006-07 | 2005-06 | 2006-07 | 2005-06 | 2006-07 | 2005-06 | 2006-07 | 2005-06 | 2006-07 | 2005-06 | 2006-07 |
| 'K22' | 97 | 102 | 75 | 90.1 | 101 | 118.0 | 20 | 18.2 | 148 | 145 | 1.685 | 3.852 | 0 | 0 | 0 | 10S |
| 'K23' | 100 | 110 | 70 | 86.0 | 106 | 124.3 | 18 | 17.8 | 150 | 148 | 2.682 | 3.612 | 0 | 0 | 0 | 10S |
| 'K28' | 97 | 106 | 79 | 86.0 | 118 | 125.5 | 16 | 17.0 | 148 | 148 | 1.869 | 3.511 | 0 | 0 | 0 | 10S |
| 'K29' | 99 | 104 | 78 | 83.5 | 90 | 115.0 | 15 | 16.8 | 150 | 145 | 2.111 | 3.671 | 0 | 0 | 0 | 5S |
| 'K30' | 98 | 105 | 79 | 83.5 | 105 | 118.8 | 20 | 18.8 | 145 | 145 | 1.875 | 3.825 | 0 | 0 | 0 | 10S |
| 'K32' | 98 | 105 | 79 | 79.5 | 115 | 124.0 | 19 | 15.4 | 148 | 142 | 2.289 | 3.125 | 0 | 0 | 0 | 20S |
| 'K35' | 98 | 105 | 79 | 86.0 | 80 | 114.0 | 20 | 16.4 | 148 | 140 | 2.252 | 3.425 | 0 | 0 | 0 | 5s |
| 'K38' | 92 | 95 | 84 | 94.0 | 85 | 90.25 | 19 | 17.4 | 145 | 140 | 1.451 | 3.475 | 0 | 0 | 0 | 10S |
| 'K40' | 94 | 97 | 86 | 97.0 | 95 | 104.3 | 20 | 16.6 | 145 | 145 | 3.752 | 3.865 | 0 | 0 | 0 | 5S |
| 'K48' | 96 | 106 | 78 | 94.0 | 99 | 131.5 | 19 | 17.2 | 142 | 142 | 1.785 | 3.522 | 0 | 0 | 0 | 10s |
| 'K53' | 93 | 90 | 71 | 95.0 | 88 | 109.5 | 22 | 18.0 | 140 | 145 | 2.125 | 3.512 | 0 | 0 | 0 | TS |
| 'K55' | 98 | 94 | 72 | 94.0 | 90 | 108.3 | 21 | 15.4 | 140 | 148 | 2.296 | 3.351 | 0 | 0 | 0 | TS |
| 'K56' | 99 | 93 | 69 | 96.0 | 83 | 116.0 | 20 | 16.4 | 145 | 148 | 1.175 | 3.562 | 0 | 0 | 0 | TS |
| 'K61' | 93 | 93 | 80 | 94.0 | 88 | 102.3 | 22 | 15.6 | 142 | 145 | 2.111 | 3.265 | 0 | 0 | 0 | 5S |
| 'K67' | 93 | 93 | 84 | 95.0 | 80 | 125.3 | 19 | 16.0 | 145 | 145 | 2.425 | 3.462 | 0 | 0 | 0 | 5S |
| 'K68' | 93 | 105 | 76 | 89.0 | 78 | 113.0 | 16 | 15.8 | 142 | 142 | 2.225 | 3.725 | 0 | 0 | 0 | 10S |
| 'K70' | 94 | 106 | 82 | 97.5 | 78 | 121.0 | 16 | 14.0 | 142 | 140 | 2.268 | 3.165 | 0 | 0 | 0 | 5S |
| 'K71' | 95 | 107 | 77 | 99.0 | 99 | 125.0 | 15 | 15.6 | 142 | 140 | 2.225 | 3.225 | 0 | 0 | 0 | 10S |
| 'K72' | 96 | 107 | 71 | 99.5 | 91 | 112.8 | 16 | 14.8 | 145 | 145 | 2.213 | 3.162 | 0 | 0 | 0 | 10S |
| 'K73' | 93 | 105 | 76 | 99.5 | 85 | 123.0 | 17 | 14.6 | 142 | 142 | 2.011 | 2.237 | 0 | 0 | 0 | 10S |
| 'K74' | 93 | 105 | 65 | 91.1 | 80 | 121.8 | 16 | 17.8 | 142 | 148 | 2.225 | 3.651 | 0 | 0 | 0 | 10S |
| 'K75' | 93 | 105 | 70 | 90.5 | 115 | 104.8 | 17 | 16.4 | 145 | 150 | 2.635 | 3.612 | 0 | 0 | 0 | 10S |
| 'K76' | 98 | 105 | 80 | 90.0 | 99 | 113.5 | 17 | 17.8 | 145 | 148 | 3.101 | 3.737 | 0 | 0 | 0 | 40S |
| 'K77' | 99 | 110 | 74 | 88.0 | 85 | 122.5 | 17 | 17.0 | 145 | 150 | 2.115 | 2.912 | 0 | 0 | 0 | 10S |
| 'K84' | 98 | 110 | 77 | 92.5 | 85 | 127.8 | 20 | 18.8 | 145 | 145 | 2.011 | 3.712 | 0 | 0 | 0 | 10S |
| 'K85' | 98 | 113 | 78 | 98.5 | 100 | 129.8 | 18 | 19.8 | 145 | 148 | 1.885 | 3.411 | 0 | 0 | 0 | 5S |
| 'K86' | 96 | 106 | 78 | 94.0 | 74 | 120.6 | 18 | 19.0 | 148 | 148 | 2.320 | 3.752 | 0 | 0 | 0 | 5S |
| 'K87' | 99 | 115 | 77 | 95.0 | 85 | 116.5 | 18 | 18.6 | 150 | 145 | 2.451 | 3.852 | 0 | 0 | 0 | 0/5S |
| 'K93' | 96 | 106 | 81 | 89.8 | 102 | 104.6 | 20 | 18.2 | 145 | 145 | 2.125 | 3.775 | 0 | 0 | 0 | 5S |
| 'K100' | 96 | 110 | 93 | 90.0 | 105 | 110.9 | 19 | 17.4 | 148 | 142 | 2.715 | 3.512 | 0 | 0 | 0 | 5S |
| 'K106' | 98 | 108 | 74 | 89.0 | 80 | 110.5 | 17 | 16.6 | 145 | 140 | 2.111 | 3.475 | 0 | 0 | 0 | TS |
| 'K111' | 98 | 108 | 85 | 97.0 | 96 | 126.0 | 20 | 15.4 | 148 | 140 | 2.961 | 3.725 | 0 | 0 | 0 | 5S |
| 'K112' | 98 | 108 | 86 | 96.5 | 100 | 103.3 | 22 | 14.6 | 148 | 145 | 3.101 | 3.101 | 0 | 0 | 0 | 0 |
| 'PBW343' | 102 | 108 | 79 | 83.0 | 108 | 115.0 | 19 | 20.0 | 150 | 148 | 2.101 | 3.842 | 10S | 30S | 0 | 40S |
| CD (*P*=0.05) | 2.83 | 1.03 | 9.15 | 10.11 | 17.51 | 15.21 | 1.88 | 1.52 | 2.83 | 1.12 | 2.23 | 1.06 | | | | |

$ Lines K22 to K77 are pyramid lines with *Lr24* and *Lr28* and lines from K84 to K112 are NILs with *Lr28* only.
# Plot Size=1.38×4.5 meters

Closely linked DNA markers are available for several rust resistance genes. In several countries including Australia, USA, Canada, Mexico (CIMMYT), Argentina, UK, France, Turkey, China and India, marker-assisted breeding in the public and private sectors has resulted in the development of improved varieties (Gupta *et al*. 2009). In India also, two varieties have been released after pyramiding 2–3 bacterial blight resistance genes in 'Pusa Basmati 1' (Gopalakrishnan *et al*. 2008) and in Sambha Mahsuri (Sundaram *et al*. 2008) at IARI, New Delhi and Directorate of Rice Research, Hyderabad respectively. In wheat multiple traits including disease resistance, resistance against abiotic stresses and quality traits are being targeted for MAS. A number of rust resistance genes such as *Lr9, Lr19/Sr25, Lr24/Sr24, Lr34/Yr18, Lr37/Yr17/Sr38, Lr46/Yr29, Lr47, Sr26, Sr32, Sr33 and Sr36* have been selected for variety development and germplasm enhancement through MAS (Gupta *et al*. 2009).

In the present study we have successfully pyramided two leaf rust resistance genes in wheat cultivar 'PBW343' using SCAR markers. Although codominant DNA markers are preferred but our studies showed that dominant markers can also be employed successfully for marker-assisted selection following simultaneously screening for disease reaction and genotyping. Pyramided 'PBW343' lines showed broad

spectrum of rust resistance and newly introduced leaf rust resistance genes do not impart any linkage drag on the recipient parent indicating that these improved lines can be used as base genotype for incorporating other desirable traits such as stripe rust resistance, quality traits etc. These lines are already being used by wheat breeders for pyramiding stripe rust resistance genes *Yr10* and *Yr15* (data not given) along with leaf rust resistance which will be reported somewhere else.

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