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Marker Assisted Gene Pyramiding of Leaf Rust Resistance Genes *Lr24* and *Lr28* in the Background of Wheat Variety DWR 162 (*Triticum aestivum* L.)

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ABSTRACT

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Two highly effective genes for leaf rust resistance viz., *Lr24*, *Lr28* were selected for pyramiding in the background of a susceptible but high yielding bread wheat variety DWR 162. The screening against most virulent pathotype of leaf rust 77-5 (121R63-1) indicated that both the genes confer a high degree of seedling resistance. The use of molecular markers, namely, SCS719, SCS1302607, SCS421570 and Xwmc313 validated the presence of resistance genes, *Lr24* and *Lr28* in DWR 162. The application of molecular markers facilitated identification of individual plants in BC2 -F1 and BC2 -F2 generations possessing the targeted genes. Finally 60 plants were selected in BC2 -F2 generation carrying the desired resistance genes, *Lr24* and *Lr28* in the background of DWR 162. The availability of combination of major rust resistance genes in desirable background would facilitate the strategic deployment of wheat varieties to achieve durable resistance.

Introduction

Rusts are the most devastating fungal diseases posing a threat towards realizing the potential yield in wheat, worldwide. Wheat is attacked by three rust species: leaf rust (*Puccinia triticina* Eriks.), stem rust (*P. graminis* f. sp. *graminis* Eriks. & E. Henn) and stripe (yellow) rust (*P. striiformis* Westend.). Leaf rust caused by *Puccinia triticina* is the most common and widely distributed of the three wheat rusts.

Chemical control of rust pathogens is inefficient, expensive and cannot be adopted

by small and marginal farmers. Therefore, an effective, economical, and ecologically safe method to control leaf rust epidemics is the breeding and cultivation of resistant wheat varieties.

Improving resistance to rust fungi is one of the major tasks facing wheat breeders all over the world. Although conventional gene transfer offers useful means of introgressing or pyramiding more than one well characterized resistance gene into susceptible genetic background, however, when no

distinguishing virulence is available for pyramiding two effective major genes, conventional technique is not useful for precisely pyramiding these genes in single genetic background. The use of molecular markers facilitates the incorporation of the major leaf rust resistance genes (*Lr* genes) responsible for resistance into new varieties and the pyramiding of these genes.

Breeding programs have successfully implemented molecular markers to assist in the development of cultivars with stem, leaf and stripe rust resistance genes. Rust pathotypes however overcome resistance genes through mutation and genetic recombination (Dadkhodaie *et al.*, 2011). It is thus necessary to constantly improve the genetic resistance of commercially grown wheat cultivars and to explore novel sources of genetic resistance.

Several rust resistance genes are available in the common wheat background originating from *Triticum* and its wild relatives like, *Agropyron*, *Aegilops* and *Secale*. Wild relatives of wheat serve as an abundant genetic resource for improving the genetic variation in cultivated wheat (Dadkhodaie *et al.*, 2011). Plant breeders have access to the primary (*Triticum* spp.), secondary (*Aegilops* spp.) and tertiary (*Tritiaceae* spp.) gene pools of wheat, primarily constituted of wild wheat species and related grasses. A greatest hope for improving the rust resistance in wheat lies in exploiting some of these valuable species possessing useful genes. A large number of *Lr* genes providing resistance to leaf rust and stripe rust pathogens world over have been documented (McIntosh *et al.*, 2007). Among these, the alien leaf rust resistance genes, *Lr24* derived from *Agropyron elongatum* and *Lr28* originating from *Aegilops speltoides* provide effective resistance against all the Indian leaf rust pathotypes (Tomar and Menon, 2001). The alien segment carrying *Lr24/ Sr24* does not impose any deleterious

effect on yield as several cultivars carrying *Lr24* have been released for cultivation in India (Singh *et al.*, 2007). Although, *Lr28* has not yet been commercially deployed on large scale, but a cultivar MACS6145 (=HW2034) carrying this gene has been released for North Eastern zone in India (Rao *et al.*, 2007).

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. The duration and efficiency of the rust resistance genes utilized within a wheat cultivar can be improved by pyramiding multiple resistance genes in the same cultivar (Simons *et al.*, 2011).

Materials and Methods

The bread wheat genotype DWR 162, which is susceptible to leaf rust, was selected as recipient parent for pyramiding the leaf rust resistance genes, *Lr24*, *Lr28*. 'PBW 343' near isogenic lines with *Lr24* and *Lr28* developed through marker assisted backcross breeding were used as donor parent for introgression of leaf rust resistant genes. The parents DWR 162 and NIL PBW 343, BC2F1 (generated by Arati *et al.*, 2013), BC2F2, were sown and desired selections were made at All India Coordinated Wheat Improvement Project (AICWIP), Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad. Normal agronomical practices were followed for raising the crop.

Seedling test and field inoculations

Three weeks old seedlings of parental lines and segregating generations were inoculated in the field with selected pathotypes of leaf rust 77-5 (121R63-1). The pure inoculum of

rust pathotypes was obtained from the Directorate of Wheat Research, Regional Station, Flowerdale, Shimla Rust severity was recorded according to the modified Cobb's scale described and was estimated on the basis of percentage area covered with pustules (Peterson *et al.*, 1948).

PCR analysis

DNA was isolated from young leaves by a modified CTAB method (Dellaporta *et al.*, 1983). PCR reactions were performed in a total volume of 25 μ l, containing 1 \times PCR buffer, 200 μ M of each dNTP, 20 ng of each primer, 1 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) and 100 ng of genomic DNA in a PTC-200 thermal cycler (MJ Research).

Primers were synthesized from Sigma Aldrich Pvt. Ltd., Bangalore, India. The sequence of primers and the PCR conditions used for amplification presented in (Table 1). Amplified PCR products were resolved in 2% agarose gel, stained with ethidium bromide.

Results and Discussion

The recipient parent DWR 162, donor parent NIL PBW 343, were validated for the presence/absence of *Lr24* and *Lr28* genes with the help of SCAR markers linked to leaf rust resistance genes, *Lr24* and *Lr28*. These markers showed amplification of specific marker fragments of 719bp size with the SCAR marker SCS73719 and 607bp fragment with the SCAR marker SCS1302, both linked to *Lr24*, in the donor parent NIL PBW 343 and 570bp fragment with SCAR marker SCS421 and 320bp fragment with SSR marker Xwmc313, both linked to *Lr28*, in the donor parent NIL PBW 343 (Plate 1 to 3). There was absence of amplification of specific marker fragments of sizes 719bp with the SCAR marker S73719 and 607bp with the

SCAR marker SCS1302 linked to *Lr24* and 570 bp fragment with the SCAR marker S421 and 320bp fragment with SSR marker Xwmc313 linked to *Lr28*, in the recipient parent DWR 162. This confirms the presence of resistance genes in the donor parent NIL PBW 343 and their absence in recipient parent DWR 162. Thus these markers can be effectively utilized marker assisted selection of both the leaf rust resistance genes *Lr24* and *Lr28*.

Field screening of the donor parent, NIL PBW 343, and recipient parent, DWR 162 for seedling and Adult Plant Resistance (APR) with most virulent leaf rust pathotype 77-5, showed that DWR 162 is highly susceptible with a very high seedling infection score of '3+' and adult plant reaction indicating that this variety is highly susceptible (Table 2).

BC₂F₁ progeny generated by Arati *et al.* (2013) was subjected to marker assisted foreground selection using SCAR and SSR markers. Genotypes with the leaf rust resistance genes *Lr24* and *Lr28* using linked SCAR markers SCAR: SCS719 and SCS1302 for *Lr24* and SCAR: SCS421 for *Lr28* respectively were advanced to BC₂F₂ generation.

One hundred and twenty BC₂F₂ plants were subjected to foreground selection with markers linked to leaf rust resistance genes *Lr24* and *Lr28*. Foreground selection of plants with SCAR markers SCS1302 linked to *Lr24* and SCAR marker SCS421 linked to *Lr28* showed 60 plants carrying both *Lr24* by amplifying 607 bp size band (Plate 1) and *Lr28* by amplifying 570 bp size band (Plate 2), leaf rust resistance genes, 20 plants with only *Lr24* gene, 23 plants with only *Lr28* and 11 plants showed absence of both the genes. Foreground selection with another SCAR marker SCS73719 linked to *Lr24* showed the presence of the gene by amplifying 719bp

size band in all the plants carrying *Lr24* confirmed by SCS1302. The expected segregation of the SCAR markers SCS1302 and SCS421 in the ratio of 9:3:3:1 in BC₂F₂ generation (Table 3) was recorded indicating the goodness of fit with a calculated χ^2 value of 2.76 (table χ^2 value 7.82) as revealed from the results of χ^2 test.

Since Xwmc313 is a co-dominant marker it facilitated the identification of plants as homozygous or heterozygous for leaf rust resistance gene *Lr28*. A perusal of table 4 shows that molecular marker Xwmc313 identified 23 plants as homozygous resistant and 48 plants as heterozygous resistant carrying *Lr28*. SCAR marker SCS1302 identified 80 plants positive for leaf rust resistance gene *Lr24* out of 120 plants studied. Since, SCS1302 is a dominant marker, these 80 identified individuals included both homozygous and heterozygous resistant plants for *Lr24*. Joint segregation analysis of two markers *i.e.*, one co-dominant marker Xwmc313 for *Lr28* and a dominant marker SCS1302 for *Lr24* was undertaken to identify plants with different genotypic constitution with respect to the two rust resistance genes. The expected and observed frequencies of different genotypic classes are shown in table 4. Out of 23 plants identified as homozygous resistant for *Lr28*, 16 were observed to carry leaf rust resistance gene *Lr24* as well, either in homozygous or heterozygous condition. Remaining 7 plants, homozygous for *Lr28* did not carry *Lr24* gene. Out of 48 plants identified as carrying *Lr28* in heterozygous state, 36 also carried leaf rust resistance gene *Lr24* either in homozygous or heterozygous condition as against an expected frequency of 45. 12 plants were observed to carry only *Lr28* in heterozygous condition as against an expectation of 15. Twenty three plants were observed to carry only *Lr24* either in homozygous or heterozygous state. Out of 120 plants 11 did not carry either *Lr24* or

Lr28 as against expected value of 7.5. The six categories of genotypic classes expected from segregation of a dominant and a co-dominant marker are shown in the table 4. A perusal of the table shows that the observed values of the six genotypic classes fits well with the expected ratio of 3:1:6:2:3:1 with a non-significant χ^2 value of 5.96.

Individual plants of BC₂F₂ generation were studied for their performance in the presence of individual leaf rust resistant genes *Lr24* or *Lr28* and in the presence of both *Lr24* and *Lr28* together (Table 5). Individual plants were compared with each other for agronomic characters such as days to 50 percent flowering, number of tillers per plant, spike length, spikelets per spike, grain yield per plant, thousand grain weight and leaf rust resistance to know the effect of these genes in the background of DWR 162.

Perusal of results indicated that the plants carrying *Lr24* gene were on par with plants which carry *Lr28* for characters such as days to 50 percent flowering and plant height. For leaf rust resistance, plants with *Lr24* recorded very low coefficient of infection as compared to plants with *Lr28* (Table 5). The alien segment carrying *Lr24* does not impose any deleterious effect on yield as several cultivars carrying *Lr24* have been released for cultivation in India (Singh *et al.*, 2007).

Plants carrying *Lr28* gene were superior for all agronomic characters such as number of tillers per plant, spike length, spikelets per spike, grain yield per plant and thousand grain weight. This could be attributed to the presence of *Lr28* gene which contributes for superior agronomic traits along with rust resistance. The similar reports of increased grain yield, 1000-grain weight and number of effective tillers per plant due to the presence of *Lr28* gene has been reported by Kumar and Raghavaiah (2004). For leaf rust, high resistance has been recorded in plants with

Lr28, which controls one of the important resistances in the Indian subcontinent against the most prevalent *Puccinia triticina* pathotype 77-5. *Lr28* gene has been reported

to contribute for superior agronomic traits along with rust resistance (Kumar and Raghavaiah, 2004) without any deleterious effects.

Table.1 Molecular markers for foreground selection of rust resistance genes used in the study

Genes tagged	Molecular markers	Primer sequence 5'---3'	Amplification product size(bp)	References
<i>Lr24</i>	SCAR:SCS719	F: TCG TCC AGA TCA GAA TGT G R: CTC GTC GAT TAG CAG TGA G	719	Prabhu <i>et al.</i> , (2004).
<i>Lr24</i>	SCAR:SCS1302	F: CGC AGG TTC CAA TAC TTT TC R: CGC AGG TTC TAC CTA ATG CAA	607	Gupta <i>et al.</i> , (2006).
<i>Lr28</i>	SCAR:SCS421	F: ACA AGG TAA GTC TCC AAC CA R: AGT CGA CCG AGA TTT TAA CC	570	Cherukuri <i>et al.</i> , (2005).
<i>Lr28</i>	SSR:Xwmc313	F: GCAGTCTAATTATCTGCTGGCG R: GGGTCCTTGTCTACTATGTCT	320	Annapurnalily <i>et al.</i> , (2011)

Table.2 Screening of parental lines under artificial inoculated conditions at Seedling and adult stage

Parental lines	Reaction to leaf rust	
	Seedling score	APR
DWR 162	3+	80S
PBW 343	;0	;0

APR= Adult plant response; S=Susceptible (Large uredia with or without necrosis or chlorosis); 0= No infection

Table.3 Observed and expected number of plants with *Lr24*, *Lr28* and *Lr24* and *Lr28* in BC₂F₂ generation of DWR 162 X NIL PBW 343 based on joint segregation of linked SCAR markers SCS1302 (*Lr24*) and SCS421(*Lr28*)

Sl. No.	Genotypic class	Number of plants		Cal χ^2	Tab χ^2
		Observed	Expected		
1	A_ B_(9)	60	67.5	0.84	
2	A_ bb (3)	20	22.5	0.28	
3	aaB_(3)	23	22.5	0.01	
4	aabb (1)	11	7.5	1.64	
	Total	120	114	2.76	7.82

A= *Lr24*, B= *Lr28*

Table.4 Joint segregation of linked SSR marker Xwmc313 (*Lr28*) and SCAR marker SCS1302 (*Lr24*) in BC₂F₂ generation of DWR 162 X NIL PBW 343

Genotypic Frequency	Expected	No of Plants		Cal χ^2	Tab χ^2
		E	O		
AAB-	3/16	22.5	16	1.88	
AAbb	1/16	7.5	7	0.03	
AaB-	6/16	45	36	1.80	
Aabb	2/16	15	12	0.60	
aaB-	3/16	22.5	23	0.01	
aabb	1/16	7.5	11	1.63	
Genotypic ratio:3:1:6:2:3:1 A= <i>Lr28</i> , B= <i>Lr24</i>		120	105	5.96	

Table.5 Performance of individual plants of BC₂F₂ in different gene combinations

Character	<i>Lr24</i>	<i>Lr28</i>	<i>Lr24 and Lr28</i>	Without <i>Lr24</i> or <i>Lr28</i>	DWR 162	NIL PBW 343
	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Days to 50 per cent flowering	72.68 \pm 4.46	71.67 \pm 2.40	70.70 \pm 2.89	71.05 \pm 0.55	64.50 \pm 2.12	83.8 \pm 1.6
Plant height (cm)	76.67 \pm 3.67	74.86 \pm 4.58	88.25 \pm 8.56	69.90 \pm 0.60	94.5 \pm 2.29	86.4 \pm 3.37
Tillers per plant	18.67 \pm 2.68	20.43 \pm 4.28	20.00 \pm 5.14	11.93 \pm 2.67	19.50 \pm 0.96	11.7 \pm 0.83
Spike length (cm)	9.03 \pm 0.44	11.04 \pm 0.81	11.75 \pm 0.23	9.11 \pm 0.07	12.60 \pm 0.50	10.4 \pm 1.02
Number of spikelets per spike	17.53 \pm 0.58	19.23 \pm 0.88	22.40 \pm 0.60	16.44 \pm 0.43	21.90 \pm 0.36	17.9 \pm 0.22
Grain yield per plant (g)	16.90 \pm 2.67	21.32 \pm 2.15	23.55 \pm 1.67	12.26 \pm 0.72	20.21 \pm 2.06	16.3 \pm 1.23
Thousand grain weight (g)	36.55 \pm 2.04	39.87 \pm 2.66	42.17 \pm 1.96	34.04 \pm 2.78	38.02 \pm 1.62	41.2 \pm 1.62
Average coefficient of infection of leaf rust	0.12 \pm 0.10	4.55 \pm 3.73	0.07 \pm 0.23	65.00 \pm 1.52	31.5 \pm 1.58	5.33 \pm 1.32

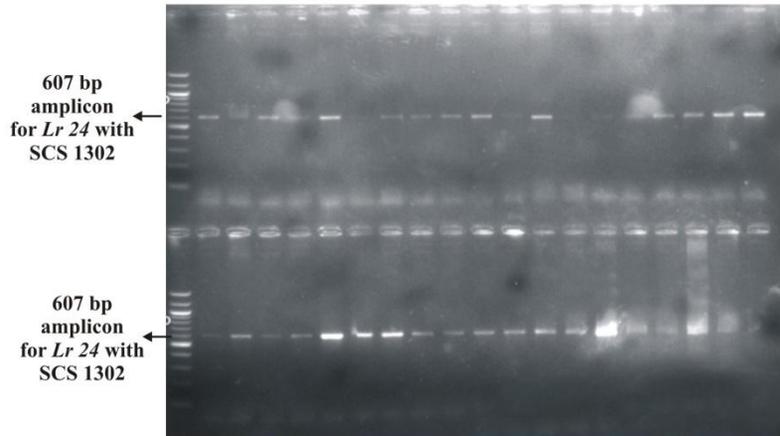


Plate 1: Molecular confirmation for the presence of *Lr24* in BC_2F_2 generations using SCAR marker SCS 1302

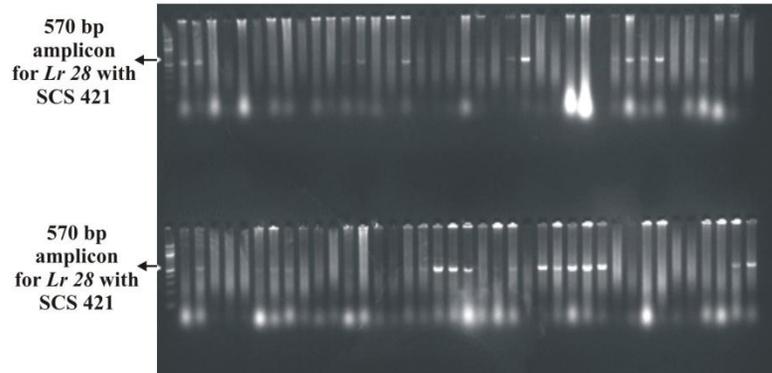
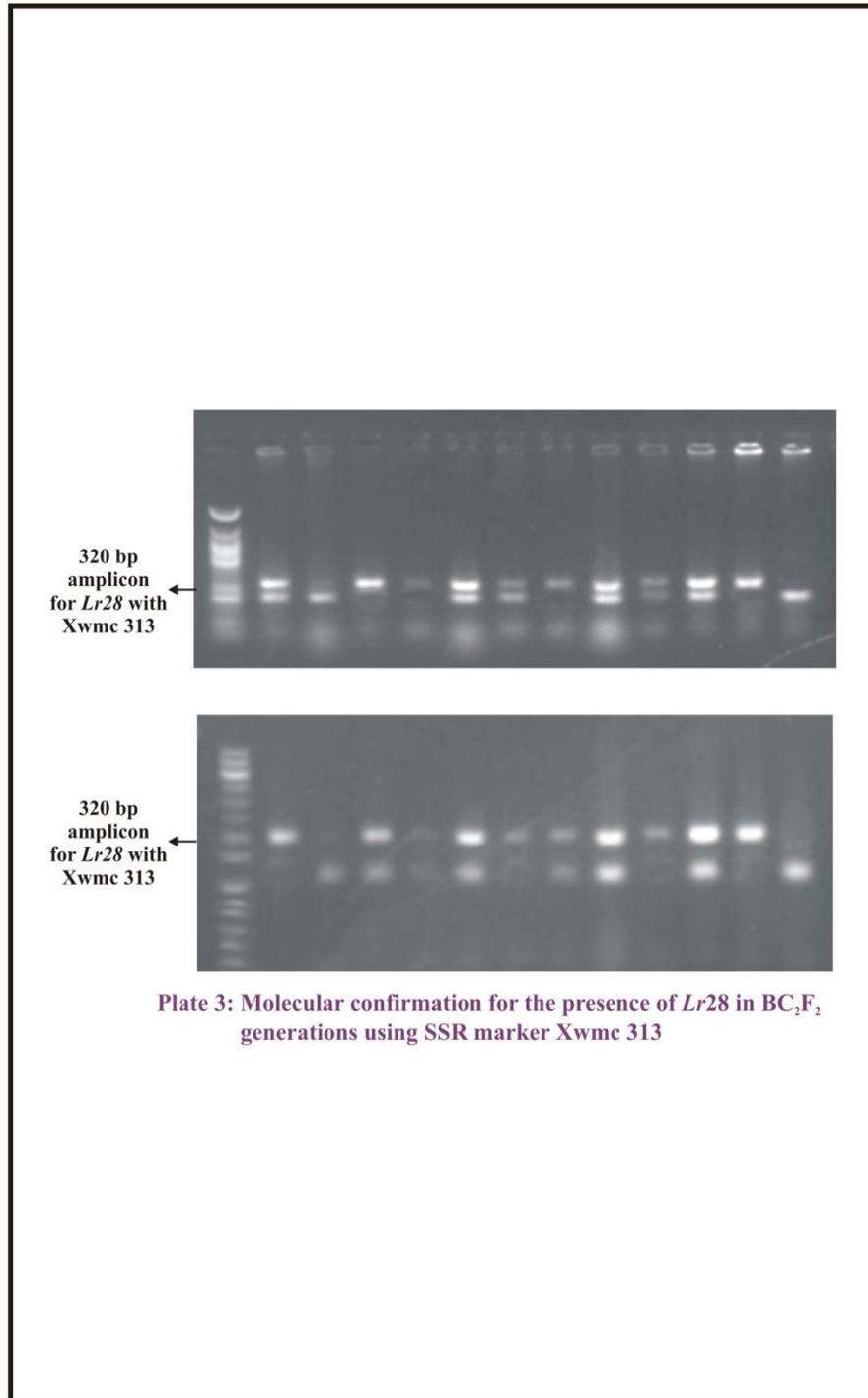


Plate 2: Molecular confirmation for the presence of *Lr28* in BC_2F_2 generations using SCAR marker SCS 421



Plants with both leaf rust resistance genes *Lr24* and *Lr28* were superior in performance for all the characters along with rust resistance (with zero coefficient of infection for leaf rust) as compared to plants with individual leaf rust resistance genes, *Lr24* or *Lr28*. Similar results of 'PBW 343' lines pyramided with *Lr24* and *Lr28* showing broad spectrum rust resistance with newly introduced leaf rust resistance genes has been reported by Chhuneja *et al.*, (2011). This kind of interaction where the combination of genes give higher level of resistance than when present separately have been reported earlier by several authors (Kolmer *et al.*, 1991; Kloppers and Pretorius 1997; Dyck and Samborski 1982) and often forms the basis of durable resistance.

On the contrary, plants which do not carry both *Lr24* and *Lr28* genes yielded less grain yield due to high susceptibility to leaf rust (Table 5).

Observations recorded on individual plants of BC₂F₂ for morphological characters to study the phenotypic similarity of BC₂F₂ with DWR 162 suggested good resemblance of BC₂F₂ population with DWR 162 (Table 5).

High phenotypic association of BC₂F₂ with DWR 162 for most of the agronomic characters like days to 50 percent flowering, number of tillers per plant, spike length, spikelets per spike and grain yield per plant suggested that, more number of backcrosses is a worthwhile exercise for higher recovery of all the desirable traits of recurrent parent(s). High phenotypic association of BC₂F₂ plants with the recurrent parent HD 2877, indicating good recovery of all the desirable traits has been reported by Revathi *et al.*, (2010).

For number of tillers per plant, number of spikelets per spike, thousand grain weight and grain yield per plant, BC₂F₂ population was

superior to DWR 162. This could be attributed to the desirable gene combination of both DWR 162 and NIL PBW 343 and the leaf rust resistance genes *Lr24* and *Lr28*. Superior performance of backcross lines of 'PBW 343' pyramided with leaf rust resistance genes *Lr24* and *Lr28* was reported by Chhuneja *et al.*, (2011).

For leaf rust infection, the coefficient of infection in BC₂F₂ generation which was on par with resistant parent NIL PBW 343. This indicated the introgression of leaf rust resistance genes *Lr24* and *Lr28*. Similar reports of improved leaf rust resistance through marker assisted introgression of *Lr24* and *Lr28* genes was reported by Revathi *et al.*, (2010) and Chhuneja *et al.*, (2005).

The present study highlighted the usefulness of DNA markers linked to resistance genes and revealed the importance of MAS in pyramiding the gene combinations of *Lr24*, *Lr28* for leaf rust resistance in wheat. Since *Lr24* is linked with stem rust resistance gene *Sr24*, the newly pyramided lines are expected to show additional resistance. The *Lr28* gene remained effective for many decades in India and elsewhere, however, Bhardwaj *et al.*, 2010 recently reported a new virulent pathotype 121 R60-1 from India, which appears closely related to the most prevalent pathotype 121R 63-1 (77-5).

Nevertheless, the lines obtained with different combination of rust resistance genes in DWR162 background are likely to provide enhanced durable resistance. These lines can be used for gene deployment after the testing of their yield potential at multi-locations.

The introgressed backcross segregants with high recovery of recurrent parent genome can be used in further studies to develop near isogenic lines of DWR162 with *Lr24* and *Lr28*.

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