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Pyramiding of leaf rust resistance genes in bread wheat variety DWR 162 through marker assisted backcrossing

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Abstract

A popular widely grown but leaf rust susceptible Indian bread wheat variety was pyramided with two leaf rust resistance genes, *Lr24* and *Lr28* by Marker Assisted Backcrossing (MABC). The near isogenic line of PBW 343 introgressed with *Lr24* and *Lr28* was used as a donor. The MABC approach employed one SSR marker, Xwmc313 and one SCAR marker SCS421 with its locus linked to *Lr28* and two SCAR markers, SCS719 and SCS 1302 linked to *Lr24* for foreground selection to select plants carrying *Lr24* and *Lr28* genes. Marker assisted background selection in BC_2F_2 to recover the genome of recipient parent involving 42 polymorphic SSR markers dispersed throughout the genome facilitated mean recurrent parent genome (RPG) recovery of 80.3% with a range from 67.5-94.7%. BC_2F_2 plants were characterized for DUS (Distinctiveness Uniformity Stability) for phenotypic identification of plants that mostly resemble DWR 162 variety. Field evaluation of BC_2F_2 plants for yield and its component traits to assess the effect of these two genes in the background of DWR 162 revealed high yield potential of selected plants along with leaf rust resistance and no yield penalty was apparent. This study successfully demonstrate the practical utility of MABC in wheat rust resistance breeding for developing resistant lines in the background of any elite and popular wheat cultivar with relatively higher speed and precision.

Key words: Leaf rust, MANC, foreground selection, background selection, genome recovery

Introduction

In India wheat is grown over an area of 31.5 million hectare, with production of about 93 million tonnes with an average productivity of 3140 kg per hectare (Anonymous 2015). Rust diseases caused by *Puccinia* spp. inflict significant yield losses to wheat crop throughout the world (Kolmer 2005; Tomar et al. 2014). Wheat leaf rust (brown rust) caused by *P. triticina* Eriks is a serious fungal disease of wheat. Judicious deployment of rust resistance genes through resistance breeding is the most effective and economical strategy to protect wheat varieties against rust. More than 76 leaf rust resistance genes have been catalogued (McIntosh et al. 2016) and most of them have been identified in a hexaploid background. Dynamic change in the virulence characteristics of rust pathogen poses a continuous threat to the effectiveness of rust resistance genes. However, many of the seedling resistance genes when incorporated singly tend to become ineffective due the constantly evolving physiological races of the pathogen. To suppress such reviving pathogenesis, an approach to pyramid more than one gene into the same background for deployment of combinations of effective resistance genes has been suggested (Sohail et al. 2014). *Lr24* is an effective resistance gene transferred into bread wheat from *Agropyron elongatum* which confers seedling resistance. *Lr24* is being used in major wheat breeding and pyramiding programmes as a means to provide resistance to susceptible cultivars (Tomar and Menon 1998; Tomar et al. 2014). Another gene *Lr28* is also reported as an effective resistance gene against prevailing leaf rust pathotypes and, therefore, represents a good candidate for gene pyramiding (Tomar and Menon 2001; Sohail et al. 2014).

Pyramiding major genes for resistance in single genotype by conventional breeding may be time consuming and laborious when one or more of the genes are effective against all known isolates of the pathogen (Kurupathy et al. 2011). In such cases, the

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presence of exclusive DNA based markers acts as indices for each *Lr* gene. Marker-assisted selection (MAS) is a very useful technique to pyramid two or more genes in elite crop varieties (Tyagi et al. 2014). Marker assisted selection (MAS) for pyramiding desired genes along with background selection can result in rapid recovery of the recurrent parent genome in a short span of 2-3 generations (Ribaut et al. 2002). In wheat, MAS has been utilised successfully for pyramiding leaf rust resistance genes (Gupta et al. 2005; Singh et al. 2004; Nocente et al. 2007; Revathi et al. 2010; Chhuneja et al. 2011). DWR 162 (Vinayak) variety developed at UAS, Dharwad (Hanchinal et al. 2005) was a popular bread wheat cultivar of peninsular zone. Evolution of new virulent leaf rust pathotype, 77-5 (121R-63-1) with matching virulence knocked down the combined resistance of *Lr*23 and *Lr*26 genes present in DWR 162 (Nayar et al. 1993). Hence, the present investigation was aimed to introgress effective leaf rust resistance genes *Lr24* and *Lr28* in an elite, but susceptible bread wheat cultivar, DWR 162 employing Marker Assisted Backcross Breeding (MABB).

Materials and methods

Plant materials and scheme

NIL PBW 343 (Near Isogenic Line PBW 343) introgressed with *Lr24* (from *Agropyron elongatum*) and *Lr28* (from *Aegilops speltoides*) was developed through MABB (Chhuneja et al. 2011). In the recent past, by using NIL PBW 343 as donor parent**,** BC_1F_1 and BC_1F_2 plants introgressed with *Lr24* and *Lr28* leaf rust resistance genes were developed in the background of an elite but susceptible bread wheat variety DWR 162 at University of Agricultural Sciences, Dharwad (Yadawad et al. 2015). In continuation of this work, second backcrossing followed by selfing to identify homozygous plants with *Lr24* and *Lr28* genes was attempted in this experiment to generate BC_2F_1 and BC_2F_2 generation during 2012 and 2013 at UAS, Dharwad. The entire crossing programme followed for pyramiding *Lr24* and *Lr28* genes into DWR 162 is presented in Fig. 1.

Leaf rust inoculation

Screening for leaf rust resistance was done at both Dharwad and IARI, RS, Wellington, The Nilgiris, Tamil Nadu. Disease severity was assessed at the adult plant stage in field condition by spray-inoculating with mixture of urediniospores of virulent leaf rust pathotypes, suspended in water (1 g inoculum per 10 L of water, using one drop of Tween-20 as dispersant). On the appearance of symptoms, rust severity (percentage) and response of the plants to disease were assessed using a modified Cobb's scale (Peterson et al. 1948).

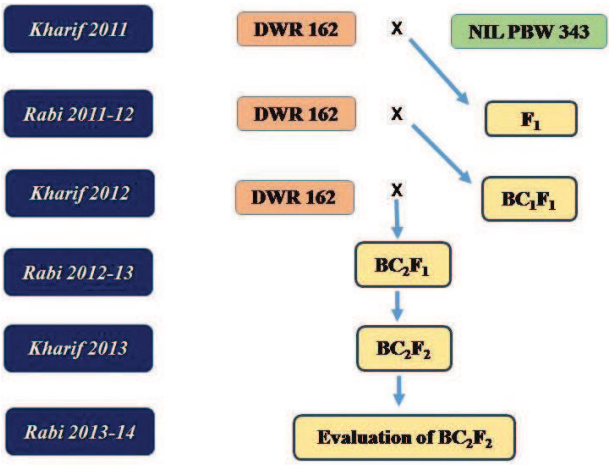

Fig. 1. Breeding scheme for pyramiding *Lr24* and *Lr28* genes into DWR 162

DNA extraction and PCR amplification

DNA was extracted from one month old seedlings of parental genotypes and backcross progenies by following CTAB method (Dellaporta et al. 1983). The PCR ampliûcation was performed in a reaction mixture of 20 µl containing 200M dNTPs (MBI; Fermentas, Lithuania, USA), 0.75 U Taq DNA polymerase (MBI; Fermentas, Lithuania, USA), 5 pmole of each primer, 20-30 ng template DNA and 10 X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM MgCl2). Details of the primer sequence and amplification product is presented in Table 1. The amplified products were resolved on 2.5 percent agarose gel for the foreground selection and on 10% PAGE (followed by silver staining for visualization) for the background selection.

Marker assisted selection

Marker-assisted foreground and background selection technique was used to incorporate leaf rust resistance genes into DWR 162. Validated molecular markers linked to targeted rust resistance genes (Table 1) were used for foreground selection. A total of 136 SSR markers covering all the chromosomes and chromosome arms in a genetic and physical consensus SSR map of wheat (Somers et al. 2004) were surveyed for polymorphism. Markers which were polymorphic between parents, DWR 162 and NIL PBW 343 were used for background selection in BC_2F_2

Table 1. DNA markers used for marker assisted selection

Genes tagged	Molecular markers	Primer sequence 5'-3'	Amplification product size(bp)	References
Lr24	SCAR:SCS719	F: TCG TCC AGA TCA GAA TGT GR: CTC GTC GAT TAG CAG TGA G	719	Prabhu et al. 2004
Lr24	SCAR:SCS1302	F: CGC AGG TTC CAA TAC TTT TCR: CGC AGG TTC TAC CTA ATG CAA	607	Gupta et al. 2006
Lr28	SCAR:SCS421	F: ACA AGG TAA GTC TCC AAC CAR: AGT CGA CCG AGA TTT TAA CC	570	Cherukuri et al. 2005
Lr28	SSR:XWMC313	F: GCA GTC TAA TTA TCT GCT GGC GR: GGG TCC TTG TCT ACT ATG TCT	320	Bipinraj et al. 2011

population. Percent genomic recovery was estimated by using GGT2.1 (Graphycal GenoTyping2.1) software as number of homozygous loci corresponding to recurrent parent allele + half the number of heterozygous loci divided by the total number of polymorphic SSR markers used.

Recording of phenotypic data

Phenotypic data was recorded on eight quantitative traits like grain yield (YPP) and its attributing traits *viz*., days to fifty percent flowering, number of productive tillers per plant, plant height, spike length, number of spikelets per spike and 1000-grain weight (TGW) and protein content (%). For identification of plants having high resemblance with the recurrent parent DWR 162 observations were recorded on nine DUS (Distinctiveness Uniformity Stability) characters that distinguishes the parents.

Results and discussion

DWR 162 is a well adopted popular bread wheat variety of Peninsular Zone of India. However it is highly susceptibility to leaf rust disease. The combination of *Lr24*/*Sr24* and *Lr28* confers resistance to all the prevalent races of leaf and stem rust in India (Tomar and Menon 1998). *Lr24* does not seem to impose yield penalty as demonstrated by the fact that several cultivars with *Lr24* have been released for cultivation in India (Tomar et al. 2014). Although virulence for *Lr28* has been reported in India (Bhardwaj et al. 2010) and Australia (McIntosh et al. 1995), these pathotypes were not identified in recent annual pathogen surveys in these countries, therefore, represents a good candidate for gene pyramiding (Sohail et al. 2014). The present study aimed to incorporate *Lr24* and *Lr28* genes into an elite variety DWR 162 using MABC combined with phenotypic selection. A judicious use of phenotypic and marker assisted selection was made to rapidly recover the genotypic background of DWR 162.

Validation of the molecular markers linked to leaf rust resistance genes

To test the adoptability of markers reported to be linked with leaf rust resistance genes *Lr24* and *Lr28*, these markers were analyzed for parental polymorphism between donor and recurrent parents so as to utilize these markers in marker assisted selection for incorporating leaf rust résistance. The presence of both *Lr24* and *Lr28 genes* in the donor parent, NIL PBW 343, F1, BC2F1 and BC2F2 generations was confirmed with the respective SSR and SCAR markers validated the efficiency of these markers in marker assisted selection.

Leaf rust screening of parents

Field resistance of parents was tested at rust screening nursery with most virulent leaf rust pathotypes of 77 exhibited a very high level of seedling resistance exhibiting infection type ';0' in NIL PBW 343, whereas the recipient genotype DWR 162 showed highly susceptible reaction with the infection score of ';3+'. At adult plant stage also the donor parent NIL PBW 343 recorded a very high level of adult plant resistance, while DWR 162 exhibited susceptible reaction of 80S. This indicated that both *Lr24* and *Lr28* still provide excellent protection against prevailing leaf rust pathotypes (Chhuneja et al. 2011; Revathi et al. 2010).

Marker assisted selection

Using DNA markers linked to *Lr24* and *Lr28* genes, foreground selection was exercised in F_1 and backcross generations. One hundred and twenty

BC2F2 plants were subjected for foreground selection with respective SSR and SCAR markers (Fig. 2). Results indicated that 62 plants carried both *Lr24* and

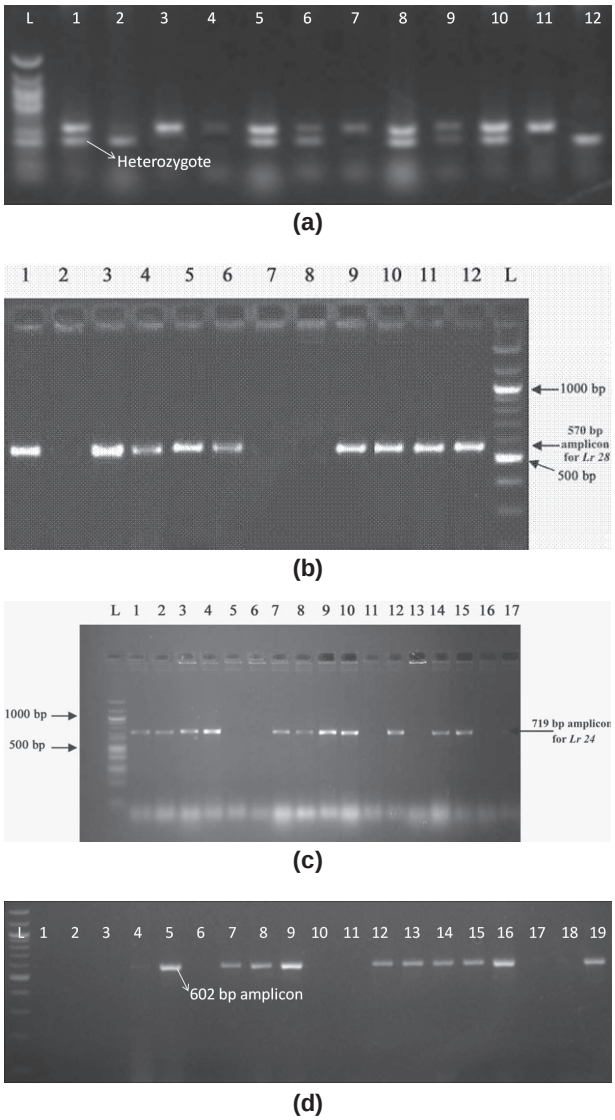

Fig. 2. Foreground selection for *Lr24 and Lr28* genes in BC_2F_2 population involving DWR 162 as recurrent parent. (a) *Lr28* with SSR marker Xcmc 313; (b) *Lr28* with SCAR marker SCS 421; (c) *Lr24* with SCAR marker SCS 719 and (d) *Lr28* with SCAR marker SCS 1302

Lr28, 24 plants carried only *Lr24*, 23 plants carried only *Lr28* and 11 plants showed absence of both the genes (Table 2). The expected segregation ratio of 9:3:3:1 in BC_2F_2 generation indicated the goodness of fit with a calculated chi square value of 2.76 (table chi square value 7.82). Segregation for leaf rust resistance genes in the ratio of 9:3:3:1 in F2 has been reported (Kaur et al. 2012).

Table 2. Observed and expected number of plants with *Lr24*, *Lr28* and both in BC_2F_2 population based on molecular studies

Genotypic class	Number of plants		χ^2
	Observed	Expected	
*Lr24_ Lr28_*(9)	62	67.5	0.45
Lr24_ lr28lr28 (3)	24	22.5	0.10
*lr24 lr24 Lr28_*(3)	23	22.5	0.01
lr24 lr24 lr28 lr28 (1)	11	7.5	1.63
Total	120	120	Calculated χ^2 value 2.19

*Table χ^2 value at 3 degrees of freedom = 2.60

Joint segregation analysis of two markers i.e., one co-dominant SSR marker Xwmc313 for *Lr28* and a dominant SCAR marker SCS1302 for *Lr24* revealed six categories of genotypic classes (Table 3). The

Table 3. Joint segregation of linked SSR marker Xwmc313 (*Lr28*) and SCAR marker SCS1302 (*Lr24*) in BC_2F_2

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		12	105	5.96	11.07

Cal. = Calculated; Tab. = Tabulated; E = Expected; O = observed

observed values of the six genotypic classes fit well with the expected ratio of 3:1:6:2:3:1. The joint segregation of Xwmc313 linked to *Lr28* and SCS1302 linked to *Lr24* in 3:1:6:2:3:1 ratio of genotypic classes has been reported (Bhawar et al. 2011).

Marker assisted background selection in BC_2F_2 generation with 136 chromosome specific SSR markers spanning all the chromosomal regions revealed 42 markers as polymorphic between the parents DWR 162 and NIL PBW 343 (Table 4). The 46 BC_2F_2 generation plants confirmed to have both *Lr24* and *Lr28* leaf rust resistance genes through foreground selection out of sixty (that were surviving up to flowering

Table 4. List of polymorphic SSR markers used for background selection

Marker	Chromosome	Marker	Chromosome
Xgwm33	1A	Xgwm113	4B
Xwmc9	1A	Xwmc617	4D
Xwmc619	1B	Xwmc74	4D
Xgwm124	1B	Xwmc47	5A
Xwmc432	1D	Xwmc492	5A
Xgwm232	1D	Xwmc386	5B
Xwmc122	2A	Xgwm408	5B
Xgwm312	2A	Xwmc215	5D
Xgwm265	2B	Xgwm190	5D
Xwmc317	2B	Xcfd190	6A
Xgwm484	2D	Xwmc179	6A
Xwmc41	2D	Xwmc398	6B
Xwmc532	3A	Xgwm70	6B
Xwmc169	3A	Xgwm325	6D
Xgwm389	3B	Xwmc469	6D
Xwmc307	3B	Xgwm60	7A
Xwmc552	3D	Xwmc182	7A
Xwmc533	3D	Xwmc276	7B
Xwmc313	4A	Xgwm112	7B
Xwmc262	4A	Xgwm46	7D
Xwmc149	4B	Xgwm428	7D

and grain formation) were subjected to background selection with two SSR markers per linkage group. Randhawa et al. (2009) suggested that 100 markers (about two per arm) are sufficient for marker assisted background selection although additional markers will further increase accuracy of the RPG recovery estimates for the selected plants. The recurrent parent genome percentage estimated by using GGT2.1 software revealed 67.5 to 94.1% RPG in selected BC_2F_2 plants with an average of 83.1%. This demonstrated the effectiveness of background selection in reducing the number of backcross generations. Randhawa et al. 2009 obtained more than 97% of the recurrent parent genome in just two backcross generations. Bhawar et al. (2011) reported recurrent parent genome recovery of 88.4% to 94.55% with an average of 91.12% in F_2 generation screening with 149 polymorphic SSR markers through background selection.

Evaluation of backcross generation plants progenies for resistance genes

Individual plants of BC_2F_2 generation were studied for their performance in the presence of individual leaf rust resistant genes *Lr24* or *Lr28* and in combination of both *Lr24* and *Lr28* (Table 5). Results indicated that the plants carrying *Lr24* gene recorded very low coefficient of infection as compared to plants with *Lr28.* Plants carrying *Lr28* gene were superior for all agronomic traits which could be attributed to the presence of *Lr28* gene which contributes for superior agronomic traits along with rust resistance. Increased grain yield, thousand grain weight and number of tillers per plant due to presence of *Lr28* gene has been reported by Kumar and Raghavaiah (2004). Plants pyramided with both leaf rust resistance genes *Lr24* and *Lr28* were superior in performance for all the characters along with zero coefficient of infection for leaf rust as compared to plants with individual leaf rust resistance genes, *Lr24* or *Lr28*. Similar results

Table 5. Performance of individual plants in BC_2F_2 generation with different gene combinations

Character	*Lr24*	*Lr28*	*Lr24* and *Lr28*	Without *Lr24* or *Lr28*	DWR 162	NIL PBW 343
	Mean± SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Days to 50 % flowering	72.68±4.46	71.67±2.40	70.70±2.89	71.05±0.55	64.50±2.12	83.8±1.6
Plant height (cm)	76.67±3.67	74.86±4.58	88.25±8.56	69.90±0.60	94.5±2.29	86.4±3.37
No. of tillers/plant	18.67±2.68	20.43±4.28	20.00±5.14	11.93±2.67	19.50±0.96	11.7±0.83
Spike length (cm)	9.03±0.44	11.04±0.81	11.75±0.23	9.11±0.07	12.60±0.50	10.4±1.02
Number of spikelets/spike	17.53±0.58	19.23±0.88	22.40±0.60	16.44±0.43	21.90±0.36	17.9±0.22
Grain yield/plant (g)	16.90±2.67	21.32±2.15	23.55±1.67	12.26±0.72	20.21±2.06	16.3±1.23
Thousand grain weight (g)	36.55±2.04	39.87±2.66	42.17±1.96	34.04±2.78	38.02±1.62	41.2±1.62
Average coefficient of infection of leaf rust	0.12±0.10	4.55±3.73	0.07±0.23	65.00±1.52	31.5±1.58	5.33±1.32

of PBW 343 lines pyramided with *Lr24* and *Lr28* showing broad spectrum rust resistance with newly introduced leaf rust resistance genes has been reported (Chhuneja et al. 2011).

DUS characterization of BC_1F_2 and BC_2F_2 plants

Phenotypic evaluation of BC_1F_2 and BC_2F_2 populations resulted in the isolation of agronomically superior and rust free segregants with *Lr24* and *Lr28* genes. To see whether the plants of BC_1F_2 and BC_2F_2 have resemblance with the recurrent parent DWR 162 DUS characterization was done. DUS characterization studies indicated 20 to 93 and 70 to 90 percent recovery of DWR 162 type of plants in BC_1F_2 and BC_2F_2, respectively (Table 6). Recovery of 20 to 93%

Table 6. Leaf rust resistant plants in BC_1F_2 and BC_2F_2 exhibiting various levels of recurrent parent DWR-162 recovery deduced by different DUS characters

Characters	BC_1F_2		BC_2F_2	
	No. of plants resembling DWR162	%age recovery of DWR 162 back-ground	No. of plants resembling DWR162	%age recovery of DWR 162 back-ground
Plant growth	13	45	42	70
Flag leaf	6	20	48	80
Ear head	20	67	54	90
Type of Awn	22	74	60	100
Glume	26	86	54	90
Grain	28	93	54	90

of the characters of the recurrent parent through the use of DUS characterization has been reported in BC_1F_2 by Yadawad et al. (2015). In the present study, high leaf rust incidence facilitated the successful introgression of two leaf rust resistance genes and identification of superior backcross lines. BC_2F_2 plants incorporated with two leaf rust resistance genes *Lr24* and *Lr28* enhanced resistance without much deviation in the traits of adaptation in the background of recurrent parent 'DWR162 were developed using marker assisted selection. These lines may serve as useful genetic resources for developing leaf rust resistant genotypes. The practical utility of MABC has been demonstrated for developing rust resistant lines in the background of any elite and popular wheat cultivar with relatively higher speed and precision.

Authors' contribution

Conceptualization of research (RRH, SAD); Designing of the experiments (HLN, SB, VRN); Contribution of experimental materials (AY, AG); Execution of field/ lab experiments and data collection (AY, AG); Analysis of data and interpretation (AY, AG, SAD); Preparation of manuscript (AY, SAD).

Declaration

The authors declare no conflict of interest.

References

Anonymous 2015. http://www.fao.org/worldfoodsituation/wfs-home/csdb/en.

Bhawar K. B., Vinod, Sharma J. B., Singh A. K., Sivasamy M., Mona Singh, Prabhu K. V., Tomar S. M. S., Sharma T. R. and Singh B. 2011. Molecular marker assisted pyramiding of leaf rust resistance genes *Lr19* and *Lr28* in bread wheat (*Triticum aestivum* L.) variety HD2687. Indian J. Genet., **71**(4): 304-311.

Bipinraj A., Honrao B., Prashar M., Bhardwaj S., Rao S. and Tamhankar S. 2011. Validation and identification of molecular markers linked to the leaf rust resistance gene *Lr28* in wheat. J. Appl. Genet., **52**: 171-175.

Cherukuri D. P., Gupta S. K., Charpe A., Koul S., Prabhu K. V., Singh R. B. and Haque Q. M. R. 2005. Molecular mapping of *Aegilops speltoides* derived leaf rust resistance gene *Lr28* in wheat. Euphytica, **143**: 19-26.

Chhuneja P., Vikal Y., Kaur S., Singh R., Juneja S., Bains N. S., Berry O., Sharma A., Gupta S. K., Charpe A., Prabhu K. V. and Dhaliwal H. S. 2011. Marker-assisted pyramiding of leaf rust resistance genes *Lr24* and *Lr28* in wheat (*Triticum aestivum*). Indian J. agric. Sci., **81**(3): 214-218.

Dellaporta S., Wood J. and Hicks J. B. 1983. A plant DNA mini-preparation: version II. Plant Mol. Biol. Rep., **1**: 19-21.

Gupta S. K., Charpe A., Koul S. and Prabhu K. V. 2006. Development and validation of SCAR markers co-segragating with an *Agropyron elongatum* derived leaf rust resistance gene *Lr24* in wheat. Euphytica, **150**: 233-240.

Gupta S., Charpe A., Koul S., Prabhu K. V. and Haq Q. M. 2005. Development and validation of molecular markers linked to an Aegilops umbellulata-derived leaf rust resistance gene, *Lr9*, for marker-assisted selection in bread wheat. Genome, **48**: 823-830.

Hanchinal R. R., Patil B. N, Kalappanavar I. K., Math K. K., Lohitaswa H. C., Desai S. A. and Nirmala Y. 2005. Fifty years of wheat Research in Karnataka. University of Agricultural Science, Dharwad.

Kaur A., Riar R., Kaur S., Dhaliwal H. S., Singh and

Chhuneja P. 2012. Introgression of a leaf rust resistance gene from *Aegilops caudata* to bread wheat. J. Genet., **91**: 155-161.

Knott D. R. 1989. The wheat rust breeding for resis-tance. *In*: Monographs on theoretical and applied genetics. No. **12**.

Kolmer J. A. 2005. Tracking wheat rust on a continental scale. Curr. Opin. Plant. Biol., **8**: 441-449.

Kumar A. A. and Raghavaiah P. 2004. Effect of the leaf rust resistance gene *Lr28* on grain yield and bread-making quality of wheat. Plant Breed., **123**: 35-38.

Kurupathy V., Sood S., Guedira G. B. and Gill B. S. 2011. Development of a PCR assay and marker assisted transfer of leaf rust gene *Lr58* into adapted winter wheats. Euphytica, **180**: 227-234.

McIntosh R. A., DubcovskyJ., Rogers W. J., Morris C., Appels R. and Xia X. C. 2016. Catalogue of gene symbols for wheat: 2015-16 Supplement. https:// shigen. nig.ac.jp/wheat/komugi/genes/symbol ClassList.jsp

Nayar S. K., Prashar M., Kumar J., Bhardwaj S. C., Verma L. R. and Basandrai A. K. 1993. Two new pathotypes of *Puccinia recondita tritici* with combined virulence for *Lr23* and *Lr26*. Pl. Dis. Res., **9**: 122-126.

Nocente F., Gazza L. and Pasquini M. 2007. Evaluation of leaf rust resistance genes *Lr1, Lr9, Lr24, Lr47* and their introgression into common wheat cultivars by marker-assisted selection. Euphytica, **155**: 329-336.

Peterson R. F., Campbell A. B. and Hannah A. E. 1948. A diagram scale for estimating rust severity on leaves and stems of cereals. Can. J. Res. Sect. C., **26**: 496-500.

Prabhu K. V., Gupta S. K., Charpe A. and Koul S. 2004. SCAR marker tagged to the alien leaf rust resistance gene *Lr19* uniquely marking the Agropyron elongatum-derived gene *Lr24* in wheat: a revision. Plant Breed., **123**: 417-420.

Randhawa H. S., Mutti J. S., Kidwell K., Morris C. F. and Chen X. 2009. Rapid and targeted introgression of genes into popular wheat cultivars using marker-assisted background selection. PLoS One, **4**: e5752.

Revathi P., Tomar S. M. S., Vinod and Singh N. K. 2010. Marker assisted gene pyramiding of leaf rust resistance genes *Lr24*, *Lr28* along with stripe rust resistance gene *Yr15* in wheat (*Triticum aestivum* L.). Indian J. Genet., **70**: 349-354.

Ribaut J. M., Jiang C. and Hoisington D. 2002. Efficiency of a gene introgression experiment by backcrossing. Crop Sci., **42**: 557-565.

Singh R., Datta D., Priyamvada, Singh S., Tiwari R. 2004. Marker-assisted selection for leaf rust resistance genes *Lr19* and *Lr24* in wheat (*Triticum aestivum* L.). J. Appl. Genet., **45**(4): 399-403.

Sohail Y., Bansal U., Bariana H., Chhuneja P., Mumtaz A., Rattu A. and Trethowan R. 2014. Identification of a co-dominant eSTS marker linked with leaf rust resistance gene *Lr28* in wheat (*Triticum aestivum* L.). Aust. J. Crop Sci., **8**(8):1210-1215.

Somers D. J., Isaac P. and Edwards K. 2004. A high-density microsatellite consensus map for bread wheat. Theor. Appl. Genet., **109**: 1105-1114.

Tomar S. M. S. and Menon M. K. 1998. Introgression of alien genes for leaf rust (*Puccinia recondita*) resistance in to bread wheat (*Triticum aestivum* L.) cultivars. Indian J. agric. Sci., **68**: 675-681.

Tomar S. M. S. and Menon M. K. 2001. Genes for disease resistance in wheat. pp. 152. Indian Agricultural Research Institute, New Delhi.

Tomar S. M. S., Singh S. K., Sivasamy M. and Vinod. 2014. Wheat rusts in India: Resistance breeding and gene deployment - A review. Indian J. Genet., **74**: 129-156.

Tyagi S., Mir R. R., Kaur H., Chhuneja P., Ramesh B., Balyan H. S. and Gupta P. K. 2014. Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.). Mol. Breed., **34**: 167-175.

Yadawad A., Hanchinal R. R., Nadaf H. L., Desai S. A., Biradar S. and Rudranaik V. 2015. Improvement of leaf rust resistance in bread wheat variety DWR162 (*Triticum aestivum* L.) through marker assisted selection. Indian J. Genet., **75**(2): 256-259.