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Marker assisted gene pyramiding of leaf rust resistance genes *Lr24, Lr28* along with stripe rust resistance gene *Yr15* in wheat (*Triticum aestivum* L.)

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Abstract

Two highly effective genes for leaf rust resistance *viz*., *Lr*24, *Lr*28 and a stripe rust resistance gene *Yr15* were selected for pyramiding in the background of a susceptible but high yielding bread wheat variety HD2877. The screening against most virulent pathotypes of leaf rust 77-5 (121R63-1) and stripe rust, 46S119 and 78S84 indicated that all the three genes confer a high degree of seedling and adult plant resistance. The use of molecular markers, namely, $SCS1302_{607}$, $SCS421_{570}$ and Xgwm273 validated the presence of resistance genes, *Lr24* and *Yr15* in Sunstar*[6]/C80-1//V763-2312 and *Lr*28 in HW2033 both being donors. The application of molecular markers facilitated identification of individual plants in three-way cross (HD2877 x Sunstar*[6]/C80-1//V763-2312) x HW2033, BC_1 -F_1 and BC_2 -F_1 generations possessing the targeted genes. Finally eight plants were selected in BC_2 -F_2 generation carrying the desired resistance genes, *Lr24*, *Lr28* and *Yr15* in different combinations in the background of HD2877. The availability of combination of major rust resistance genes in desirable background would facilitate the strategic deployment of wheat varieties to achieve durable resistance.

Key words: *Triticum aestivum* L., pyramiding, leaf rust, stripe rust, resistance genes, molecular markers

Introduction

Among several constraints towards realizing the potential yield in wheat, the rust diseases pose major threat to wheat production worldwide including India. All the three rusts of wheat, stem rust caused by *Puccinia graminis* Pers.f.sp.*tritici* Eriks.& Henn., leaf rust incited by *Puccinia triticina* Eriks. (Syn: *Puccinia recondita*) and stripe rust caused by *Puccinia striiformis* Westend. are occurring in designated wheat zones in India and cause significant losses in wheat production. Chemical control of rust pathogens is inefficient, expensive and can not be adopted by small and marginal farmers. Hence the development of genetic resistance to rusts in host is advocated which is economical, effective and eco-friendly to prevent the losses caused by rust epidemics [1]. Although conventional gene transfer offer useful means of introgressing or pyramiding more than one well characterized resistance gene into susceptible genetic background, however, when no distinguishing virulence available for pyramiding two effective major genes, conventional technique is not useful for precisely pyramiding these genes in single genetic background. In such situations, gene transfers assisted by molecular techniques will be instrumental. Several rust resistance genes are available in the common wheat background originating from *Triticum* and its wild relatives like, *Agropyron*, *Aegilops* and *Secale*. 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* and *Yr* genes providing resistance to leaf rust and stripe rust pathogens world over have been documented [2]. Among these, the alien leaf rust resistance genes, *Lr24* derived from *Agropyran elongatum* and *Lr28* originating from *Aegilops speltoides* provide effective resistance against all the Indian leaf rust pathotypes [3]. 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 [4]. Although, *Lr28* has not yet been commercially deployed on large scale, but a cultivar MACS6145 (=HW2034) carrying this gene has

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been released for North Eastern zone in India [5]. The stripe rust resistance gene *Yr15* derived from *Triticum turgidum* var. *dicoccoides* is also effective against all the virulent stripe rust pathotypes prevailing in India [6].

Gene pyramiding is a breeding strategy where two or more genes are combined together within one genotype. The combination of two or more resistance genes is often difficult or impossible due to lack of specific pathogen race(s) necessary for detection and confirmation of specific resistance genes. Available molecular markers, tightly linked to desired genes can help in selection of individuals with introduced genes within segregating populations. This approach is used in different crops including wheat [7]. With the availability of PCR based markers linked to leaf rust resistance genes *Lr24* [8], *Lr28* [9] and stripe rust resistance gene *Yr15* [10], marker assisted selection can be practiced for pyramiding these resistance genes into a single genetic background. This communication reports the genetic transfer of above mentioned rust resistance genes into susceptible genotype using SCS and SSR molecular markers linked closely with resistance genes to ensure presence of targeted genes.

Materials and methods

The bread wheat genotype HD2877, which is susceptible to leaf rust, was selected as recipient parent for pyramiding the leaf rust resistance genes, *Lr24, Lr28* and stripe rust resistance gene *Yr15*. HD2877, a high yielding genotype, stood first in non-significant group in National Initial Evaluation and Advanced Varietal Trials of All India Coordinated Wheat Improvement Project in North Western Plains as well as in North Eastern Plains Zones conducted during 2002-03 and 2003-04 respectively. Genetic stock, Sunstar*^{6}/C80-1//V763-2312, which carries rust resistance genes, *Lr24* and *Yr15,* and HW2033 a backcross line (WH 147*^{6}/ CS 2A/2M 4/2) in the background of WH147 carrying *Lr28* were utilized as donors. The desired crosses were made in the net house and in field in the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi and alternate segregating generations were grown in off season at IARI Regional Station, Wellington, The Nilgiris during the years 2006-2008. Normal agronomical practices were followed for raising 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) and stripe rust, 46S119 and 78S84. The pure inoculum of rust pathotypes was obtained from the Division of Plant Pathology, IARI, New Delhi. Rust severity was recorded according to the modified Cobb's scale described and was estimated on the basis of percentage area covered with pustules [11]. Seedling assays with above selected pathotypes were also conducted in parental lines at temperature ranging from 15 to 25ºC under greenhouse conditions at New Delhi. Standard procedures were followed for screening against rust [12].

PCR analysis

DNA was isolated from young leaves by a modified CTAB method [13]. PCR reactions were performed in a total volume of 25 µl, containing 1× PCR buffer, 200µM of each dNTP, 20 ng of each primer, 1 U of *Taq* DNA polymerase (Banglore Genei Pvt. Ltd., India) and 100 ng of genomic DNA in a PTC-200 thermal cycler (MJ Research). 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 genotypes HD2877, donor Sunstar*^{6}/C80-1//V763-2312, and HW2033, control genotypes HW1042+*Lr24,* HD2329 +*Lr24* and a susceptible check Agra local were screened with the help of SCAR markers linked to leaf rust resistance genes *Lr24* and *Lr28* and

Table 1. Molecular markers for the targeted rust resistance genes used in the study

Molecular markers	Gene tagged	Primer sequence (5'——3')	Amplification product size (bp)	AT (^{o}C)
SCAR:SCS1302	*Lr24*	F:CGC AGG TTC CAA TAC TTT TC R: CGC AGG TTC TAC CTA ATG CAA	607	60
SCAR:SCS421	*Lr28*	F: ACA AGG TAA GTC TCC AAC CA R: AGT CGA CCG AGA TTT TAA CC	570	60
SSR:Xgwm273	*Yr15*	F: ATT GGA CGG ACA GAT GCT TT R: AGC AGT GAG GAA GGG GAT C	165	55

AT-Annealing Temperature

SSR marker linked to stripe rust resistance gene *Yr15.* Theses markers consistently amplified their specific marker fragment size of 607bp of *Lr24*, 165bp of *Yr15* in the donor, namely, Sunstar*^{6}/C80-1//V763-2312 and 570bp of *Lr28* in HW2033 confirming the presence of resistance genes in the corresponding donors (Fig. 1). One of the essential steps in marker assisted selection is the validation of the molecular markers linked to the gene of interest between the donor and recurrent parents. Thus these markers can be effectively utilized in marker assisted selection programme.

The test with leaf rust pathotype 77-5 revealed a high level of seedling resistance in Sunstar*^{6}/C80-1//V763-2312 and HW2033 exhibiting infection type (IT) '0;' and the recipient genotype HD2877 showed susceptible reaction with the infection score of '3^{+}' (Table 2). The donor Sunstar*^{6}/C80-1//V763-2312 screened against stripe rust pathotypes, 46S119 and 78S84 exhibited high level of seedling resistance with infection type (IT) ';'. However, both HW2033 and HD2877 were susceptible with IT '3^{+}'. At adult plant stage, the donor HW2033 remained resistant with no infection against leaf rust (F) but showed susceptibility to stripe rust (80S). Sunstar*^{6}/C80-1//V763-2312 exhibited resistant reaction against leaf rust as well as stripe rust with leaf score at adult stage 5R and TR respectively. The recipient genotype HD2877 was susceptible to both leaf and stripe rusts exhibiting 80S reaction (Table 2).

The F_1 hybrid between HD2877 and Sunstar*^{6}/C80-1//V763-2312, (*Lr24* and *Yr15*) was crossed with HW2033 (*Lr28*) and the three way F_1 plants were screened for the presence of resistance genes, *Lr24*,

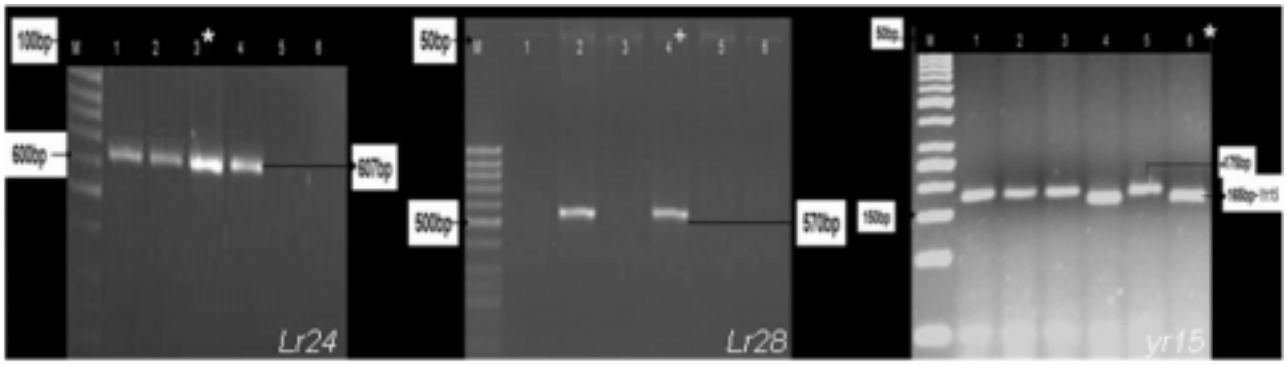

Fig. 1. Validation of molecular markers for leaf rust resistance gene *Lr24*, *Lr28* and stripe rust resistance gene *Yr15*

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

Parental lines	Reaction to			
	Leaf rust		Stripe rust	
	Seedling score	APR	Seedling score	APR
HD 2877	3^{+}	80 S	3^{+}	70 S
Sunstar*6/C80-1//V763-2312 (*Lr24*,*Yr15*)	0;	5R	;=	TR
HW 2033(*Lr28*)	0;	F	3	80 S

APR= Adult plant response; R= Resistant (hypersensitive flecks and small uredia with necrosis); S=Susceptible (Large uredia with or without necrosis or chlorosis); T= Traces and F= No infection

Table 3. Number of plants screened for adult plant response in different generations against leaf rust (LR) and stripe rust (YR) pathotypes

Cross	Generation	Rust	Total no. plants	Resistant	Susceptible
HD2877/Sunstar*^{6}/C80-1//V763-2312	Three way F_1	LR	60	56	4
		YR	60	37	53
HD2877/Sunstar*^{6}/C80-1//V763-2312//HD2877	$BC_2 - F_1$	LR	95	56	53
		YR	95	32	43
HD2877*^{2}/Sunstar*^{6}/C80-1//V763-2312	$BC_1 - F_1$	LR	100	57	43
		YR	100	34	56

Table 4. Association of phenotypic characters between HD2877 and identified plants from BC_2 - F_2 population

Character	Mean ± SE BC_2 -F_2	Mean ± SE HD2877	't' value
Plant height	81.47 ± 1.39	83.76 ± 0.17	2.086
Number of tillers	8.71 ± 0.47	14.90 ± 0.18	2.086
Spike length	12.14 ± 0.34	11.19 ± 0.16	2.086
Number of spikelets	20.76 ± 0.54	20.71 ± 0.12	2.086
Thousand grain weight	38.14 ± 0.72	37.90 ± 0.27	2.086

Lr28 and *Yr15* with the help of linked molecular markers. Out of 60 plants, 26 showed the presence of *Lr24* gene by amplifying 607bp size band (Fig. 2), 58 showed the presence of *Lr28* gene by amplifying 570bp band, while 30 plants showed the presence of *Yr15* gene by amplifying 156bp band. The marker $SCS1302_{607}$ and Xgwm273 are expected to segregate in the ratio of 1:1. Heterozygous F_1 plants for *Lr24* and *Yr15* were crossed with HW2033, which carries *Lr28*. The marker that linked to *Lr28* expected to be segregated in the ratio of 1: 0. A simple Chi-square test was applied to test the goodness of fit for appropriate genetic ratio at one degree of freedom [$\chi^2_{1:1= 0.53, 0.00}$ $\chi^2_{1:0 =0.06}$, $P_{= 0.46, 0.00, 0.80}$) (P-value; the probability of this result not being due to chance)]. Fourteen plants were identified to carry the targeted resistance genes, *Lr24, Lr28* and *Yr15* and these were backcrossed with recipient parent HD2877 to produce BC_1F_1 seeds. Sharp *et al*. [15] and Babu *et al*. [16] described that molecular markers linked to resistance genes were the best option for achieving selection for different gene combinations.

Out of 95 BC_1F_1 plants screened, 42 showed the presence of *Lr24* gene, 44 showed the presence of *Lr28*, whereas, 19 plants exhibited the presence of both *Lr24* and *Lr28* genes. These 19 plants were further subjected to molecular analysis for the presence of *Yr15* gene. Out of 19 plants, eight showed the presence of *Yr15* gene. BC_1F_1 progenies segregated as expected in the ratio of 1:1 for resistance and susceptibility at each segregating locus. ($\chi^2_{1:1 = 0.64, 0.26, 0.23;}$ $P_{= 0.42, 0.61, 0.97}$). A total of 100 BC_2F_1 plants were subjected to molecular analysis. Out of these, 43 showed the presence of *Lr24* gene by amplifying specific fragment of 607bp, 47 showed the presence of *Lr28* (Fig. 3) and 17 plants showed the presence of both the genes *Lr24* and *Lr28*. Out of 17 plants, eight plants showed the presence of *Yr15* gene. Out of these selected plants with all the three genes, six were identified to be in heterozygous condition and two were in homozygous condition. The markers linked to resistance genes segregated into 1:1 ratio with slight deviation.

The homozygosity and heterozygosity of both the effective genes for leaf rust resistance *Lr24* and *Lr28* could not be confirmed because the molecular markers are dominant in nature. It is therefore, suggested that progeny test for selecting homozygous plants should be carried out. Progenies in BC_2F_3 derived from BC_2F_2 plants can be rejected employing seedling test with the leaf rust pathotypes or creating rust epiphytotics in BC_2F_3 generation. It is therefore, imperative to develop co-dominant markers that are linked to disease resistance genes for pyramiding the resistance genes precisely.

The successful marker-assisted pyramiding of disease resistance genes has already been reported in wheat with respect to three leaf rust genes *Lr13, Lr34* and *Lr37* [17] and three powdery mildew genes *Pm3, Pm4a* and *Pm21* [7]. Sivasamy *et al*. [18] successfully achieved multi gene combination in BC_3-F_5 generation by conventional breeding utilizing the advantage of linkage and confirmed the presence of genes involved in their study. They had used the linked genes (*Lr19*/*Sr25* and *Sr36*/*Pm6*), which facilitated easy transfer of targeted genes in different genetic backgrounds. Attempts have been made to pyramid resistance genes in rice with the objective of developing more robust genetic resistance [19-21].

The populations obtained in different generations such as three way F_1, BC_1-F_1 and BC_2-F_1 were also screened under artificial epiphytotic conditions to study their rust reaction and to study 1:1 segregation for resistance *vs.* susceptibility (Table 3). The adult plant rust reactions of individual plants were in correspondence with molecular data. Plants were also identified which carried single resistance gene as well as two genes in combination either for leaf rust resistance or leaf and stripe rusts resistance. All these plants were selfed to produce BC_2F_2 progenies for further selection. The plants carrying alleles of *Lr24*, *Lr28* and *Yr15* resistance genes (based on their marker genotypes) were analyzed for their phenotypic traits and

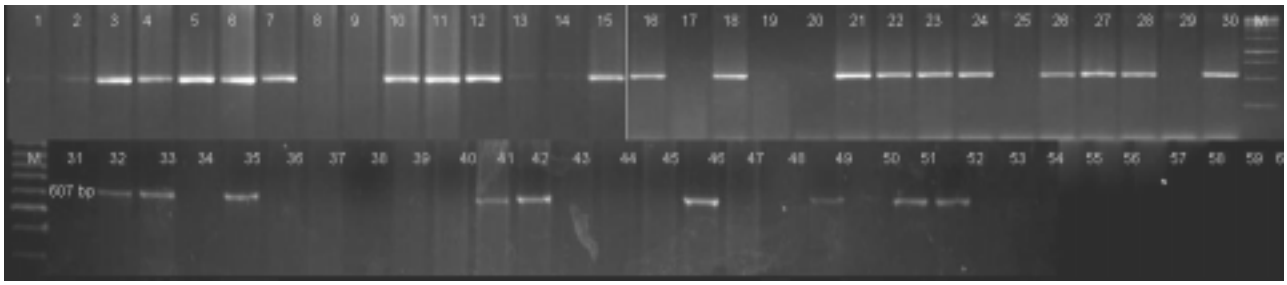

Fig. 2. Segregation of molecular marker SCS1302$_{607}$ linked to *Lr24* in the three- way cross

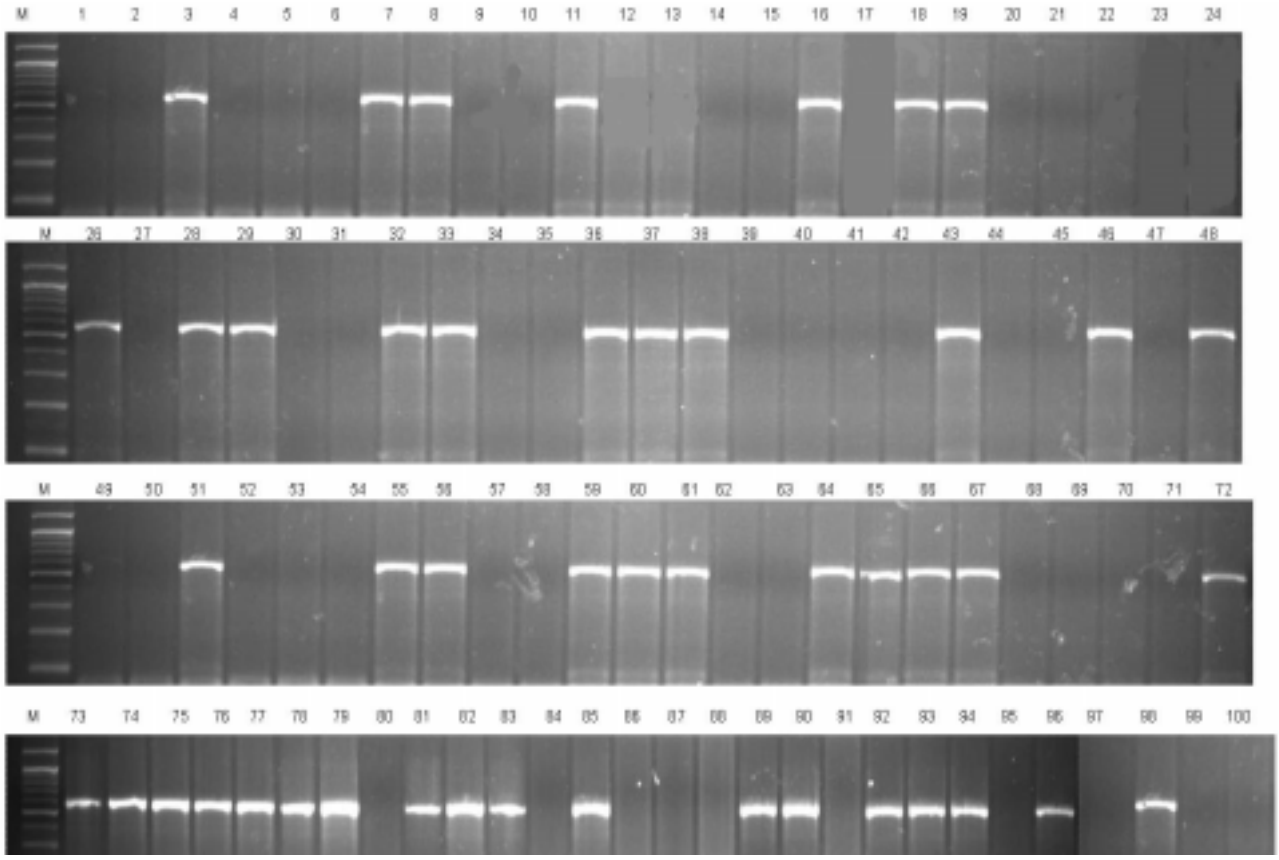

Fig. 3. Segregation of molecular marker SCS 421$_{570}$ linked to *Lr28* in the BC$_2$-F$_1$ population

indicated that BC_2F_2 population segregating for important agronomic traits (Table 4) and have recovered the genetic background of HD2877 to the satisfactory level. However, selected plants with three gene combination showed significantly poor tillering indicating some amount of drag in some of the DNA segment. Additional back crossing is needed to recover the number of tillers. The present study highlighted the usefulness of DNA markers linked to resistance genes minimizing the need of disease development methods in selecting for resistance to the diseases and revealed the importance of MAS in pyramiding the gene combinations of *Lr24, Lr28* for leaf rust and *Yr15* for stripe 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*. [22] 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 HD2877 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 material can also be used for molecular studies.

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References

1. **Kolmer J. A.** 1996. Genetics of resistance to wheat leaf rust, Annu. Rev. Phytopath., **34**: 435-455.

2. **McIntosh R. A., Devos K. M., Dubcovsky J., Rogers W. J., Morris C. F., Appels R., Somers D. J. and Anderson O. A.** 2007. Catalogue of gene symbols for wheat: 2007 Supplement. GrainGenes.Website: *http://wheat.pw.usda.gov*.

3. **Tomar S. M. S. and Menon M. K.** 2001. Genes for disease resistance in wheat. IARI, New Delhi, pp. 152.

4. **Singh Bhanwar, Tomar S. M. S., Vinod and Singh Rajendra.** 2007. Notification of crop varieties: A bread wheat variety HD2851. Indian J. Genet., **67**: 305-306.

5. **Rao V. S., Misra S. C., Bhagwat M. D., Dixit R. N., Honrao B. K., Chavan A. M., Surve V. D., Khade V. M., Menon M. K., Brahma R. N., Sivasamy M. and Tomar S. M. S.** 2007. Notification of crop varieties: A bread wheat variety MACS6145. Indian J. Genet., **67**: 305.

6. **Vinod M., Sivasamy M., Prashar M. K., Menon V. C., Sinha and Tomar S. M. S.** 2006. Evaluation of stripe rust resistance genes and transfer of *Yr15* into Indian wheats (*Triticum* species). Indian J. agric. Sci., **76**: 362-366.

7. **Liu J., Liu D., Tao W., Li W., Wang S., Chen P., Cheng S. and Gao D.** 2000. Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. Plant Breed., **119**: 16-22.

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

9. **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 *Lr*28 in wheat. Euphytica, **143**: 19-26.

10. **Roder M. S., Korzun V., Wendehake K., Plaschke J., Tixier M. H. and Leroy P.** 1998. A microsatellite map of wheat. Genetics, **149**: 2007-2023.

11. **Peterson R. F., Campbell A. B. and Hannah A. E.** 1948. A diagrammatic scale for rust intensity on leaves and stems of cereals. Can. J. Res., **26**: 496-500.

12. **Nayar S. K., Parashar M. and Bhardwaj S. C.** 1997. Manual of Current techniques in Wheat Rust. Research Bulletin No. 2, Regional Station D.W.R., Flowerdale, Shimla, pp. 32.

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

14. **Prabhu K. V., Singh A. K., Basavaraj S. H., Cherukuri D. P., Charpe A., Gopala Krishnan S., Gupta S. K., Joseph M., Koul S., Mohapatra T., Pallavi J. K., Samsampour D., Singh A., Vikas K. Singh., Singh A. and Singh V. P.** 2009. Marker assisted selection for biotic stress resistance in wheat and Rice. Indian J. Genet., **69**: 305-314.

15. **Sharp P. J., Johnston S., Brown G., McIntosh R. A., Pallotta M., Carter M., Bariana H. S., Khartkar S., Lagudah E. S., Singh R. P., Khairallah M., Potter R. and Jones M. G. K.** 2001. Validation of molecular markers for wheat breeding. Aust. J. Agric. Res., **52**: 1357-1366.

16. **Babu R., Nair S. K., Prasanna B. M. and Gupta H. S.** 2004.Integrating marker-assisted selection in crop breeding—prospects and challenges. Curr. Sci., **87**: 607-619.

17. **Kloppers F. J., Pretorious Z. A.** 1997. Effects of combinations amongst genes *Lr13*, *Lr34* and *Lr37* on components of resistance in wheat to leaf rust. Plant Pathol., **46**: 737-750.

18. **Sivasamy M., Vinod , Tiwari Sushama, Tomar R. S., Singh Bhanwar, Sharma J. B., Tomar S. M. S. and Chand Suresh.** 2009. Introgression of useful linked genes for resistance to stem rust, leaf rust and powdery mildew and their molecular validation in wheat. Indian J. Genet., **69**: 17-27.

19. **Huang N., Angeles E. R., Domingo J., Magpantay G., Singh S., Zhang G., Kumaravadivel N Bennett J. and Khush G. S.** 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor. Appl. Genet., **95**: 313-320.

20. **Singh S., Sidhu J. S., Huang N., Vikal Y., Li Z., Brar D. S., Dhaliwal H. S., Khush G. S.** 2001. Pyramiding three bacterial blight resistance genes (*Xa5, Xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. Theor. Appl. Genet., **102**: 1011-1015.

21. **Sharma P. N., Torii A., Takumi S., Mori N., Nakamura C**. 2004. Marker-assisted pyramiding of brown plant hopper (*Nilaparvata lugens* Stal.) resistance genes *Bph1* and *Bph2* on rice chromosome 12. Hereditas, **140**: 61-69.

22. **Bhardwaj S. C., Parashar M., Jain S. K., Kumar S., Sharma Y. P., Sivasamy M. and Kalappanavar I. K.** 2010. Virulence of *Puccinia triticina* on *Lr28* and its evolutionary relation to prevalent pathotypes in India. Cereal Res. Commun., **38**: 83-89.