Indian J. Genet., 69(1): 17-27 (2009)

Introgression of useful linked genes for resistance to stem rust, leaf rust and powdery mildew and their molecular validation in wheat (*Triticum aestivum* L.)

M. Sivasamy[1], Vinod[2], Sushma Tiwari[2], R. S. Tomar[2], Bhanwar Singh[2], J. B. Sharma[2], S. M. S. Tomar[2] and Suresh Chand[3]

[1]IARI Regional Station, Wellington, The Nilgiris 643 231; [2]Division of Genetics, IARI, New Delhi 110012
[3]Devi Ahilya Vishwa Vidyalaya, Indore

(Received: November 2008; Revised: January 2009; Accepted: February 2009)

Abstract

Two independent DNA segments with two linked genes each namely, *Lr*19/*Sr*25 and *Sr*36/*Pm*6 were introgressed by three cycles of backcrossing into fifteen bread wheat (*Triticum aestivum* L.) cultivars, susceptible to leaf rust (*Lr*), stem rust (*Sr*) and powdery mildew (*Pm*). Evaluation of the BC_3-F_5 along with their recurrent parents and controls carrying specific *Lr* and *Sr* genes under natural and artificial conditions indicated that conventionally improved newly constituted lines confer resistance to leaf rust, stem rust and powdery mildew in adult stage against the pathotypes prevailing in the Nilgiris. Some of these lines also displayed resistance to leaf rust pathotype 77-8 (253R 31) in adult stage. However, the improved lines, *viz*., C306*[3]/Cook*[6]/C80-1, HD2687*[3]/Cook*[6]/C80-1, MACS2496*[3]/Cook*6/C80-1, PBW226*[3]/Cook*[6]/C80-1, UP2338*[3]/Cook*[6]/C80-1 and UP262*[3]/Cook*[6]/C80-1 showed moderate susceptibility to 77-8. The leaf rust pathotype 77-8 was found avirulent on *Lr*26. The resistance in UP262 can be ascribed to *Lr*23 and some unknown gene(s) possessed by it. Since *Lr*19 is effective to all the pathotypes of leaf rust in India except 77-8, the differential response exhibited by BC_3-F_5 lines against a mixture of prevailing races at Wellington and against a new pathotype 77-8 at New Delhi indicated unambiguously the presence of *Lr*19 in the introgressed lines. High degree of resistance to stem rust and powdery mildew in all the BC_3-F_5 lines evidenced that these carry the genes *Sr*36 and *Pm*6. The conventional methodology proved successful in phenotype-based selection of resistance gene combinations in the absence of molecular markers. To ensure the correct identity of target genes, the BC_3-F_5 lines (2 to 9) were tested with molecular markers SCS265, SCS253 and Gb linked to leaf rust resistance gene *Lr*19 and SSR stm773 for stem rust resistance gene *Sr*36. The presence of genetically associated genes was verified by the amplification of translocated DNA segment in most of the backcross lines. With the detection and testing with a new *Lr*19 virulence, host-pathogen interaction test response against leaf rust and the molecular test response did not correspond in HW2004*[3]/Cook*[6]/C80-1 because of presence of an additional segment of *Lr*24/*Sr*24. The availability of a combination of major genetically diverse resistance genes in adapted wheat cultivars would facilitate the strategic deployment to achieve enhanced durable resistance. Also these lines could be used as donor and supplement the molecular mapping and tagging of genes.

Key words: Leaf rust, stem rust, powdery mildew, adult plant resistance, seedling test, back-crossing, *Triticum aestivum*

Introduction

Wheat is attacked by various fungal pathogens that pose a great threat to wheat production. Among them, leaf rust (*Puccinia triticina* Eriks.= *P. recondita* Rob. Ex Desm.), stem rust (*P. graminis f. sp. tritici* Pers. Eriks & Henn.) and powdery mildew (*Erysiphe graminis tritici* Em. Marchal) are destructive foliar pathogens of wheat occurring in India. Genetic potential of the cultivars is seldom realized due to damage caused by their appearance in epidemic proportions. Therefore, breeding varieties with multiple diseases resistance is essential and also most economical way to manage them. Conventional gene transfer offers a useful means of introgressing well characterized resistance genes into susceptible cultivars, provided a reliable donor of resistance is available. The method has been successfully employed to make genetic improvement

*Corresponding author: smstisgpb@gmail.com

in wheat [1-3]. Although many rust resistance gene(s) have been postulated in present day Indian wheat cultivars but most of them are ineffective to prevailing pathotypes of rust. Genes of alien origin exhibit resistance to leaf and stem rusts but some of them have been reported to have yield penalty [4]. However, not all the genes are disadvantageous, rather few of them enhances yield potential, e.g., 1BL.1RS (*Lr*26/*Sr*31/*Yr*9/*Pm*8) translocation from *Secale cereale*. The nexus between alien gene and yield can be broken [5], if selection strategy is carefully adopted in segregating populations. Singh *et al*. [6] observed that 7D.7Ag segment with *Lr*19/*Sr*25 increased grain yield potential by 10-15% in a range of genotypes. Similarly the alien segment *Lr*24/*Sr*24 do not impose any deleterious effect on yield [3]. The linked genes *Lr*19/*Sr*25 have been combined in an Australian line Cook with another DNA segment carrying *Sr*36/*Pm*6 from *Triticum timopheevi*. The present paper reports the result of successful introgression of two independent segments of linked genes *Sr*25/*Lr*19 and *Sr*36/*Pm*6 into the genetic background of high yielding commercially adapted wheat cultivars susceptible to rusts and powdery mildew in India. Conventional genetic transfer assisted by molecular technique either for the confirmation of gene introgressed or MAS has been instrumental in improving the cultivars. Hence gene specific SCAR, STS and SSR molecular markers *viz*., SCS265, SCS253, Gb and Stm773 linked closely with rust resistance genes were used to ensure the presence of target genes in the background of improved genotypes.

Materials and methods

The materials used in the present study comprised 15 indigenously developed Indian commercial bread wheat (*Tritictum aestivum* L.) cultivars, namely, C306, HD2285, HD2402, HD2687, HW2004, Kalyansona, Lok 1, MACS2496, NI5439, PBN52-1, Raj3077-1, UP2338, UP262 and WH147. Most of these varieties, except HW2004 and PBW226, showed susceptibility to different races of leaf rust, stem rust and powdery mildew. Cook*[6]/C80-1 is an Australian line carrying resistance genes *Sr*25/*Lr*19 from *Agropyron elongatum* and *Triticum timopheevi*-derived genes *Sr*36 and *Pm*6 was used as donor.

Backcrossing and subsequent selection technology

The key to successful introgression of disease resistance genes in to susceptible cultivars lies with the knowledge about pathogenic variability, availability of resistance sources, the knowledge of genetic relationship and the appropriate selection methodologies. Both phenotypic and genotypic selection technologies necessary for resistance gene(s) introgression into selected cultivars were utilized. Three backcrosses were administered to recurrent parents. IARI Regional station Wellington (altitude 1850 m a.m.s.l., longitude 77°N) in the Nilgiris, South India, is a hot spot providing conducive environment for perpetual occurrence and heavy incidence of rust(s) and powdery mildew. Segregating populations derived from each backcross were screened for selecting rust and powdery mildew resistant genotypes based on adult plant response. Genetic control and expression of resistance was used as a criterion for differentiation among the genotypes. The first step was the rejection of powdery mildew susceptible plants followed by the elimination of leaf and stem rusts susceptible plants. The leaf rust resistance gene *Lr*19 is linked to stem rust resistance gene *Sr*25, while *Sr*36 is linked to *Pm*6 and all these genes are dominant in nature. Therefore, known genetic associations were the main selection criterion for selection in segregating populations. Genotypes, phenotypically looking similar to the recurrent parent(s) with regard to general traits in addition to possessing resistance to leaf rust and powdery mildew were constituted in BC_3-F_5 generation.

Seedling test and field inoculations

Three weeks old seedlings in all the segregating generations were inoculated in the field with a mixture of locally available stem rust, leaf rust and powdery mildew pathotypes. The artificial inoculation procedure helps in establishing the rust infection in early stages of plant growth. All the genes chosen for introgression are effective in adult stage against different races of rust and powdery mildew pathogens prevailing in the Nilgiris. The adult plant reactions were recorded by combining severity (percent infection) and responses (type of infection). Seedling assays with 3 pathotypes of stem rust *viz*., 40A (62G29), 40-1(62G29-1) and 117-6 (37G19) and 4 of leaf rust, namely, 12-3 (49R37), 77-5 (121R63-1), 77-7 (121R127), 77-8 (253R31) and 104-2 (21R55) were conducted to postulate gene combinations. Only 9 backcross lines were tested under glasshouse conditions at temperatures ranging from 15 to 28 °C at New Delhi during the month of November to February 2006-07. All the BC_3-F_5 lines along with their recurrent parents were also tested in adult stage with a newly identified pathotype of leaf rust, 77-8 (253R31) at New Delhi during the same year. All the BC_3-F_5 lines were tested with powdery mildew inoculum prevailing

in the Nilgiris. Standard procedures were followed for screening against rusts [7] and powdery mildew [8, 9].

Molecular markers

Molecular markers of *Lr*19 and *Sr*36 were utilized (Table 1) to ensure the presence of introgressed gene(s) for leaf rust and stem rust resistance in the BC_3-F_5 lines. The number of each finally constituted backcross lines subjected to confirmation of the introgressed gene(s) through molecular markers is given in Table 3. The PCR conditions of the used molecular markers are described here as under.

DNA Extraction

Healthy leaves (2-3) were collected from each plant before inoculating them with rust. The lyophilized leaf samples were ground to a fine powder with liquid nitrogen in a pestle and mortar and 50 mg of each powdered sample was used for DNA extraction using micro-extraction method [10]. The DNA was diluted to a final concentration of 25 ng/µl for SCAR and 10 ng/µl for SSR analysis.

PCR Amplification with SCAR marker

The specific primer pair of the SCAR markers SCS265, SCS253 (within 10.2 ± 0.062cM on either side of the locus) and STS marker Gb described in Table 1 were used for amplification of *Lr*19, amplification was carried out in 25 µl reaction volume containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.0 mM $MgCl_2$, 100 µM of each dNTP (MBI Fermentas Germany), 0.3 unit *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., India), 10-20 ng of each primer and 20-40 ng of genomic DNA. Amplification reactions were performed in PTC-200 Thermal Cycler (MJ Research, USA) with the following thermal profile: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min (denaturation), 60°C for 1 min (primer annealing) and 72°C for 2 min (primer extension), with a final extension at 72°C for 7 min. PCR products were resolved on 2% agarose gel at 80 V for 3.5h.

PCR Amplification with SSR markers

The specific primer pair of the SSR marker Stm773 described in Table 1 was used for amplification of *Sr*36. PCR amplification was performed in 20 µl reaction volume containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTP (MBI Fermentas, Germany), 1 unit *Taq* DNA Polymerase (Bangalore Genei Pvt Ltd, India), 0.2 µM of primer and 10-15 ng of genomic DNA. PCR amplification was achieved in PTC-200 thermal cycler (MJ research, USA) with the following thermal profile: one cycle of 4 min at 95°C (initial denaturation), followed by 40 cycles of 30 sec. at 94^0C (denaturation), 30 sec. at 60°C (primer annealing) and 30 sec. at 72°C and 7 min at 72°C (final extension). PCR products were resolved on 3% metaphor gel at 80 V for 3.5h.

Results and discussion

Appropriate screening under artificial epiphytotic conditions in the field against a mixture of leaf rust and stem rust pathotypes and prevailing powdery mildew cultures enabled the selection of resistant segregants in each backcross populations as well as in BC_3-F_5. The data of seedling test of finally constituted BC_3-F_5 lines along with their recurrent parents with selected pathotypes of rusts supported the identification of resistance, while molecular markers assisted in confirmation of the introgressed resistance genes. The results of disease screening of newly constituted BC_3-F_5 lines are presented in Tables 1 & 2. The expression of leaf rust resistance in different lines constituted after three backcrosses and subsequent selection displayed consistency. The donor Cook*6/C80-1 also exhibited the similar pattern of adult plant reaction to leaf rust, stem

Table 1. Characters of molecular markers linked to the leaf rust resistance genes *Lr*19 and stem rust resistance gene *Sr*36.

Gene	Marker	Primer sequence (5'-3')	Product size (bp)	Reference
*Lr*19	SCS265 (coupling)	F: GGCGGATAAGCAGAGCAGAG R: GGCGGATAAGTGGGTTATGG	512	Gupta *et al.* [11]
*Lr*19	SCS253 (repulsion)	F: GCTGGTTCCACAAAGCAAA R: GGCTGGTTCCTTAGATAGGTG	736	Gupta *et al.* [11]
*Lr*19	Gb	F: CATCCTTGGGGACCTC R: CCAGCTCGCATACATCCA	152	Prins *et al.* [12]
*Sr*36	Stm773 Codominant	F: AAACGCCCCAACCACCTCTCTC R: ATGGTTTGTTGTGTTGTGTGTAGG	172	Bariana *et al.* [13]

Table 2. Adult plant reactions to rusts and powdery mildew in newly constituted lines (BC_3-F_5) developed through limited backcrossing

Stocks/Parents/ backcross lines	Postulated gene(s)[#]	Adult plant reaction to rusts and powderyb mildew			
		Stem rust	Leaf rust		Powdery mildew
		Well.	Well.	Delhi 77-8	Well.
Cook*[6]/C80-1 (donor)	*Lr*19/*Sr*25, *Sr*36/*Pm*6	F	F	40MS	1
C306	*Lr*34	80S	60MSS	40MS	4
C306*[3]/Cook*[6]/C80-1		5MR	F	40MS	2
HD2285	*Lr*13, *Lr*14a, *Lr*23, *Sr*2, *Sr*17, *Sr*30	30MS	50S	40S	3
HD2285*[3]/ Cook*[6]/C80-1		F	F	20MSS	1
HD2402	*Lr*13, *Lr*14a, *Lr*23, *Sr*2, *Sr*8a, *Sr*17, *Sr*30	30S	60S	50MSS	3
HD2402*[3]/ Cook*[6]/C80-1		5MRMS	F	50MSS	1
HD2687	*Lr*26/*Sr*31/*Yr*9/*Pm*8	10MRMS	70S	20MS	2
HD2687*[3]/ Cook*[6]/C80-1		F	F	20MS	1
HW2004	*Sr*24/*Lr*24	10MRMS	F	F	2
HW2004*[3]/ Cook*[6]/C80-1		F	F	F	1
Kalyansona (= KS)	*Lr*13, *Lr*14a, *Sr*17	60S	60S	50S	4
KS*[3]/ Cook*[6]/C80-1		F	F	40MSS	1
Lok 1	*Lr*13, *Sr*2	60MS	60S	30MSS	4
Lok 1*[3]/Cook*[6]/C80-1		5MRMS	F	30MSS	1
MACS2496	*Lr*26/*Sr*31/*Yr*9/*Pm*8, *Lr*23	10MRMS	60S	20MS	2
MACS2496*[3]/Cook*[6]/C80-1		F	F	20MS	1
NI5439	-	90S	90S	60S	4
NI5439*[3]/ Cook*[6]/C80-1		10MRMS	F	40S	2
PBW226	*Lr*23	20S	20S	10MS	2
PBW226*[3]/ Cook*[6]/C80-1		10MR	F	20MS	1
Raj3077-1	-	20S	60S	30S	2
Raj3077-1*[3]/ Cook*[6]/C80-1		10MRMS	F	20MSS	1
UP2338	*Lr*26/*Sr*31/*Yr*9/*Pm*8	5MS	60MSS	20MS	2
UP2338*[3]/Cook*[6]/C80-1		F	F	20MS	1
UP262	*Lr*13, *Lr*14a, *Lr*23, *Lr*34, *Sr*2, *Sr*8a, *Sr*11	40MSS	50S	10MS	3
UP262*[3]/ Cook*[6]/C80-1		5MRMS	F	10MS	1
PBN52-1	*Lr*13, *Sr*2	30S	60S	60S	4
PBN52-1*[3]/ Cook*[6]/C80-1		F	F	40S	1
WH147	*Lr*13, *Sr*2, *Sr*7a, *Sr*11	80S	70S	50S	4
WH147*[3]/ Cook*[6]/C80-1		10MRMS	F	20S	2
TR380-14*[7]/3Ag#14 (Check)	*Sr*24/*Lr*24	10MSS	F	F	3
CS2A/2M 4/2 (Check)	*Lr*28	60S	F	F	2
HP1633 (Check)	*Lr*9	40S	F	F	3

F = Resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; - = not tested; Well. = Wellington
[#] = Adopted after Nayar *et al.* (1997); Sharma, (1990) and Rao *et al.* (2001).

rust and powdery mildew. The pattern of rust reaction to stem rust, leaf rust and the degree of resistance to powdery mildew in BC_3-F_5 lines in comparison with donor and parental genotypes indicate that they presumably carry *Lr*19/*Sr*25 and *Sr*36/*Pm*6. The effectiveness of *Sr*25 (Infection Type (IT) 0; to 2^-), against 19 Indian pathotypes of stem rust in seedling stage [14, 15] but only moderate resistance (30MRMS) in adult stage has been reported earlier [16]. *Sr*25 is also effective against predominant stem rust pathotypes from USA [17] and Australia [13]. On the other hand, *Triticum timopheevi* derived gene *Sr*36 (*SrTt*1), which is linked with *Pm*6 gene displays high to moderate resistance to stem rust and powdery mildew in adult stage in India. Genotypes HW657 and HW888 involving Timgalen as one of the parents still exhibit resistance to stem rust in India [3], which is likely due to the presence of *Sr*36 bred into them inadvertently. This indicates that India is still free from the virulence attacking the gene *Sr*36. In Australia, several cultivars *viz*., Songlen, Timgalen, Mendos, Mengavi etc. carried *Sr*36 and some of them had *Sr*36 in combination with *Sr*5, *Sr*6, *Sr*8a which remained resistant for a very long period. Based on these observations, Johnson [18] proposed that durable resistance is due to combination of genes, which are known to be race specific and none of which provided durable resistance when used singly or in other combination. Detection of a stem rust virulence for *Sr*36 [19] in Australia resulted the withdrawal of Cook from northern wheat growing regions, however, the same pathotype could not be isolated from wheat belt in the last several years. It is often assumed that major resistance genes are worthless once matching virulences have been detected. But exceptions are also noticed. The *Sr*36 exhibited resistance in other countries too [20] and it is still being used in combination of race specific genes such as *Sr*24 and *Sr*38 in Australia [13]. The enhanced level of stem rust resistance in BC_3-F_5 lines appears to be due to *Sr*36 or may be due to its interaction with *Sr*25.

The gene *Sr*25 is linked with *Lr*19. All BC_3-F_5 lines exhibited adult plant resistance to the existing flora of leaf rust at Wellington indicating the presence of *Lr*19 in them. However, these lines when tested with pathotype 77-8 at Delhi, showed only moderate resistance. These observations suggest that all the tested BC_3-F_5 lines presumably carry *Lr*19. All the lines along with checks were also tested in seedling and adult stage under glass house and field conditions with newly identified [21] leaf rust pathotype 77-8 at New Delhi (Table 2). C306*^{3}/Cook*^{6}/C80-1, HD2285*^{3}/Cook*^{6}/C80-1, MACS2496*^{3}/Cook*^{6}/C80-1, WH147*^{3}/Cook*^{6}/C80-1, exhibited infection type 3C to 3^+, while Lok-1*^{3}/Cook*^{6}/C80-1, PBW226*^{3}/Cook*^{6}/C80-1, and UP262*^{3}/Cook*^{6}/C80-1 showed 2^{++} to 2C infection type against the pathotype 77-8 (Table 3). However, UP2338*^{3}/Cook*^{6}/C80-1 and HD2687*^{3}/Cook*^{6}/C80-1 produced IT ;1 and 0; respectively. The recurrent parents of backcross lines, HD2687*^{3}/Cook*^{6}/C80-1 MACS2496*^{3}/Cook*^{6}/C80-1 and UP2338*^{3}/Cook*^{6}/C80-1 are known to carry *Secale cereale*-derived resistance gene *Lr*26, and in addition UP2338 has also been identified to posses *Lr*34 [22]. The 20MS field response against 77-8 on varieties, HD2687, MACS2496 and UP2338 carrying *Lr*26 could be due to contamination from other nearby artificially inoculated plots. The parents *viz*., C306 (*Lr*34), HD2285 (*Lr*13, *Lr*14a, *Lr*23), PBW 226 (*Lr*23+), UP262 (*Lr*23, *Lr*34) and Lok-1 (*Lr*13) carried seedling resistance genes but most of them are ineffective against virulent pathotype 77-8, yet some of the backcross lines in genetic backgrounds of PBW226 and UP262 showed low to moderate infection type as well as low susceptible (10MS) response in adult stage, which may be due to the presence of *Lr*23. Some of the seedling genes such as *Lr*13, *Lr*23 and *Lr*26 in combination with *Lr*34 may enhance the degree of resistance but this may not be true in all the cases. The role of *Lr*34 in imparting adult plant resistance has been emphasized [23]. The adult plant reactions as exhibited by C306*3/Cook*6/C80-1, MACS2496*^{3}/Cook*^{6}/C80-1, PBW226*^{3}/Cook*^{6}/C80-1, UP2338*^{3}/Cook*^{6}/C80-1 and UP262*^{3}/Cook*^{6}/C80-1 (Table 2) may be due to interaction of *Lr*34 with other genes present in these backgrounds. Since 30MS reaction to 77-8 exhibited by UP262 and its backcross line with *Lr*19 displayed only 10MS reaction, it is likely that UP262 may carry some other unknown factor(s) for resistance to 77-8. It can also be presumed that the moderate rust reactions exhibited by these backcross lines is due to interaction of postulated genes with some unknown factors but the presence of *Lr*19 may not be disadvantageous. The pathotype 77-8 is avirulent on both *Lr*23 and *Lr*26. Seedling genes (Table 2) conferring resistance to leaf rust have been earlier postulated [22-25] in the genotypes selected for improvement. To combat leaf rust infection, the combination of seedling resistance with APR genes is likely to be the most effective alternative [26]. It has been observed earlier that interactive complimentary effects provide enhance resistance [27]. In the present study the backcross lines carrying *Lr*34, an APR gene exhibited desirable resistance in combination of *Lr*19, which may be durable.

Since Agatha equals its parent Thatcher in yield, milling and baking quality, the authors do not expect any kind of yield reduction in the backcross lines carrying *Lr*19, although yield reductions associated with alien genetic transfers have been reported in some cases in hexaploid wheat cultivars in Sweden, Mexico and Australia [4]. But release of feed wheat cultivar Currawong with an alien gene *Sr*26 gave 10% higher yield than its competitors, which suggested that the nexus between low yield and the alien gene can be broken [5]. Genotypes carrying *Sr*26, *Sr*32, *Sr*39 and *Sr*R on shortened alien segments have been produced [28]. In the present investigation the authors by choice selected the donor Cook*^{6}/C80-1, which is white grained wheat with highly reduced yellow pigmentation carrying genes for stem rust and powdery mildew resistance for the development of backcross lines. The donor was observed to be immune to stem rust in Wellington. The presence of *Sr*36 was further confirmed by its high resistance to powdery mildew score (0-1). The data in Table 4 indicate that most of the selected lines in BC_3-F_5 were verified for the presence of *Lr*19 and *Sr*36 with molecular markers *viz*., SCAR, STS and SSR which are closely linked with the target genes. Since SSR Stm773 did not amplify in the selected progeny from the cross PBW226*^{3}/Cook*^{6}/C80-1, it ruled out the presence of *Sr*36, but the resistance to stem rust displayed may be ascribed to the presence of *Sr*25, which is closely linked with *Lr*19. The presence of *Lr*19 was ensured by the amplification of all the three markers (Table 4).

All the BC_3-F_5 lines showed a high degree of adult plant resistance to powdery mildew and the seedling score of 1 (on 0-4 scale) indicating that the gene *Pm*6, which is associated with *Sr*36, is indeed introgressed in all the tested lines. In earlier studies, it was observed

Table 3. Seedling reactions of nine backcross lines to selected pathotypes of stem and leaf rusts

Parents/stocks/backcross lines	Reactions to pathotypes of							
	Stem rust			Leaf rust				
	40A	40-1	117-6	12-3	77-5	77-7	77-8	104-2
Cook*^{6}/C80-1 (*Lr*19)	;1	;1	;1	;1+	0;	;1	3C	;
TR380-14*^{7}/3Ag#14 (Lr24)	;1	3^{+}	;1	;	;1	0N	;1	0;
CS 2A/2M 4/2 (Lr28)	3^{+}	4	4	0	0	0	0;	0
HP1633 (Lr9)	-	-	-	0;	0;	3+	;1	0;
HD2285	2C	3^{+}	3C	3^{+}	3^{+}	3^{+}	3^{+}	3^{+}
HD2285*^{3}/Cook*^{6}/C80-1	;1	1-	0;1	;1	;1	;1	3	;1
HD2687	;1	;1	;1	3^{+}	3^{+}	3^{+}	;	2C
HD2687*^{3}/Cook*^{6}/C80-1	0;	0;	0;	;1	;1	;1	0;	;1
Lok-1	4	4	4	3^{+}	3^{+}	-	3^{+}	3^{+}
Lok-1*^{3}/Cook*^{6}/C80-1	;1	;1	0	;	;1	;1	2^{++}	1-
C306	4	4	4	3^{+}	3^{+}	2C	3C	3^{+}
C306*^{3}/Cook*^{6}/C80-1	;1	;1	;1	;1	;1	;	3	;1
PBW226	;	;1	;1	0	;	0;	2^{+}	;
PBW226*^{3}/Cook*^{6}/C80-1	;	;	;	;	;1	;	2C	;1
NI5439	4	4	3^{+}	3	3^{+}	3^{+}	3^{+}	3^{+}
NI5439*^{3}/Cook*^{6}/C80-1	0;	0;	;	0;	;	0;	3C	;
WH147	4	4	4	3^{+}	3^{+}	3^{+}	3^{+}	3^{+}
WH147*^{3}/Cook*^{6}/C80-1	;1	;1	;1	;1	;1	;1	3^{+}	;1
UP2338	;1	;1	0;	3^{+}	3^{+}	3^{+}	;1	3^{+}
UP2338*^{3}/Cook*^{6}/C80-1	1	;1	0;	;1	;1	;1	;1	;1
UP262	3^{+}	3^{+}	3^{+}	2C	3	2^{++}	3C	3^{+}
UP262*^{3}/Cook*^{6}/C80-1	;1	;1	;1	0;	0;	0;	2^{++}	;1
Agra Local	3^{+}	3^{+}	3^{++}	3^{+}	4	3^{+}	3^{+}	3^{+}

- = not tested; Ltn = leaf tip necrosis; C = chlorosis; N = necrosis

Table 4. Number of backcross lines selected with the help of molecular markers for the presence of *Lr*19 and *Sr*36

S. No.	Backcross lines	No. of lines tested	No. of lines having *Sr*36	No. of lines having *Lr*19		
			SSR marker stm773	Gb	SCS_{265} (Coupling)	SCS_{253} (Repulsion)
1	C306*[3]/Cook*[6]/C80-1	6	6	6	6	6
2	HD2285*[3]/Cook*[6]/C80-1	3	2	3	3	3
3	HD2402*[3]/Cook*[6]/C80-1	7	7	7	7	7
4	HD2687*[3]/Cook*[6]/C80-1	3	3	3	3	3
5	HW2004*[3]/Cook*[6]/C80-1	9	3	4	4	5
6	Lok 1*[3]/Cook*[6]/C80-1	2	2	2	2	2
7	KS*[3]/Cook*[6]/C80-1	2	2	2	2	2
8	MACS2496*[3]/Cook*[6]/C80-1	5	4	5	4	5
9	NI5439*[3]/Cook*[6]/C80-1	3	2	3	3	3
10	PBW226*[3]/Cook*[6]/C80-1	3	0	3	3	3
11	UP262*[3]/Cook*[6]/C80-1	2	1	1	1	2
12	PBN52-1*[3]/Cook*[6]/C80-1	7	6	6	6	7
13	WH147*[3]/Cook*[6]/C80-1	4	4	4	4	4
14	Raj3077-1*[3]/Cook*[6]/C80-	8	7	8	8	8
15	Cook*[6]/C80-1 (Check)	1	0	0	0	0
16	CS2A/2M 4/2 (Check)	1	0	0	0	0
17	HP1633 (Check)	1	0	0	0	0
18	TR380-14*7/3Ag#14 (Check)	1	0	0	0	0

that the association of *Sr*36 and *Pm*6 is not very strong and therefore, they poorly recombine in some of the genotypes, e.g., Maris Huntsman and Combination III a rare recombinant of *Sr*9e and *Sr*36 (20cM) [29]. However, during the present investigation, the selections made in BC_3-F_2 generations were carried forward and these resulted in successful recombination of stem rust and powdery mildew resistance.

The seedling data (Table 3) revealed that most of the tested BC_3-F_5 lines exhibit IT 3, 3+ or 3C to 77-8 in seedling stage except PBW226*3/Cook*6/C80-1 (IT:2C) and UP262*3/Cook*6/C80-1 (IT: 2^{++}). Since infection type observed in tested BC_3-F_5 with pathotypes 12-3, 77-5, 77-7 and 104-2 is ranging from 0-;1 and against 77-8, IT 2C to 3+ except HD2687 and UP2338 (both carrying *Lr*26), provided direct evidence that BC_3-F_5 lines carry *Lr*19. The expression of resistance or the reaction pattern against all the five pathotypes of leaf rust used in the investigation indicated that the resistance provided by *Lr*19 is different than that of *Lr*28 because this gene produces immune reaction. Newly identified virulence(s) 77-7 and 77-8 could discriminate the presence of *Lr*9 and *Lr*19 and provided unequivocal evidence of presence of genes because they are virulent on *Lr*9 and *Lr*19 respectively. Although on the basis of seedling tests in the absence of discriminatory virulence, it may be difficult to establish precisely the identity of the genes, *Lr*19/*Sr*25 and *Sr*36/*Pm*6 that these lines carry, but the assumption can be based on the use of donor, Cook*[6]/C80-1, which carries these linked genes. The postulation of the introgressed genes is well supported by both the reactions to rust and powdery mildew scored in adult stage. In present study, linkage between the genes has also been efficiently used to ensure the presence of a particular gene or genes in few cases. The use of molecular markers offer an opportunity to finally select the BC_3-F_5 lines on the basis of genotype rather than phenotype, particularly when two or more than two genes are combined in a single genetic background. In the present investigation the SSR (Stm773), STS (Gb) and SCAR (SCS 265, SCS 253) molecular markers were utilized and the amplification of these markers confirmed the introgressed genes (Figs.1-8). Although phenotypic selections under optimum rust infection created artificially in the field led to the constitution of resistant

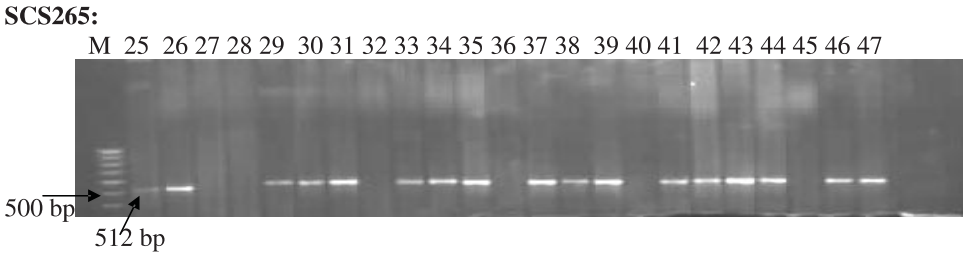

Fig. 1

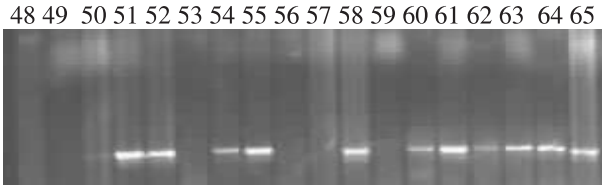

Fig. 2

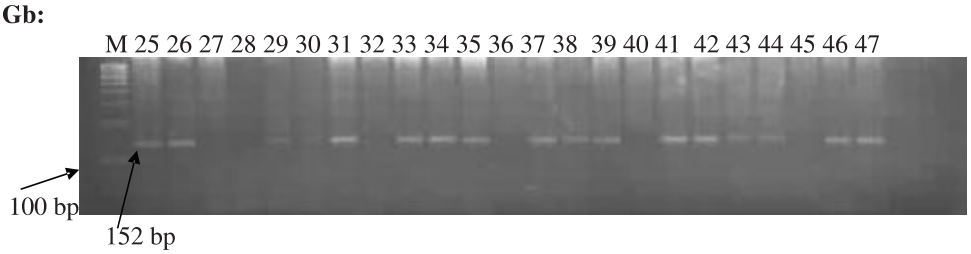

Fig. 3

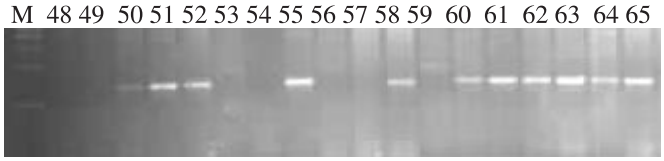

Fig. 4

Fig. 5

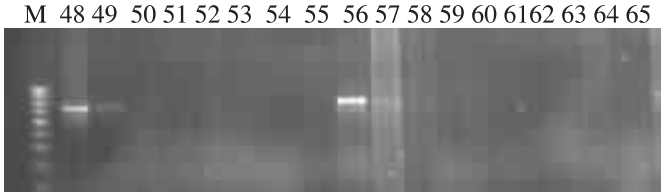

Fig. 6

Figs. 1-6: Screening of leaf rust resistant individuals with SCAR markers SCS265, SCS253 and STS marker Gb, linked to the gene *Lr*19 in different BC_3-F_5 lines: (M: Molecular weight marker, Lane 25, 26: Agatha, Cook*^{6}/ C80-1 (*Lr*19), 27, 28: Agra Local, HD2285, 29, 30, 31: HD2285*^{3}/Cook*^{6}/C80-1, 32: PBW226, 33, 34, 35: PBW226*^{3}/ Cook*^{6}/C80-1, 36: HD2687, 37, 38, 39: HD2687*^{3}/Cook*^{6}/C80-1, 40: WH147, 41, 42, 43, 44: WH147*^{3}/Cook*^{6}/ C80-1, 45: Kalyansona, 46, 47: KS*^{3}/Cook*^{6}/C80-1, 48, 49: Lok-1, NI5439, 50, 51, 52: NI5439*^{3}/Cook*^{6}/C80-1, 53: Lok-1, 54, 55: Lok-1*^{3}/Cook*^{6}/C80-1, 56: UP262, 57, 58: UP262*^{3}/Cook*^{6}/C80-1, 59: C306, 60, 61, 62, 63, 64, 65: C306*^{3}/Cook*^{6}/C80-1).

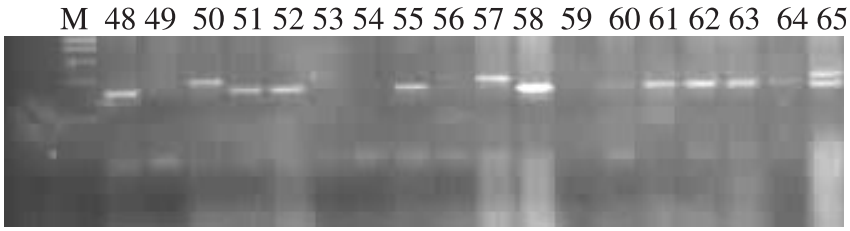

Fig. 7

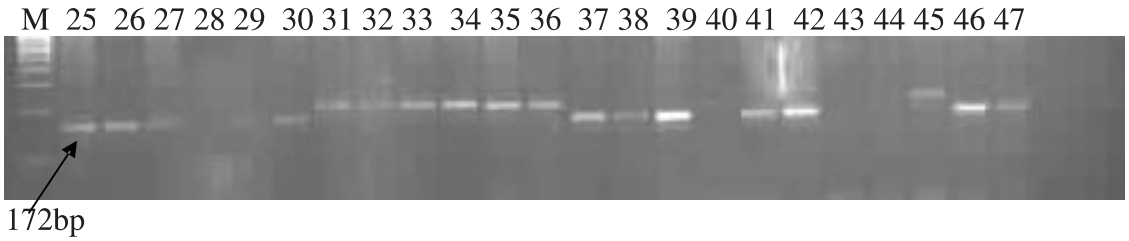

Fig. 8

Figs. 7-8. Screening of Stem rust (Black rust) resistant individuals with SSR marker Stm773 linked to the gene *Sr*36 in different backcross lines: (M: Molecular weight marker, Lane 25, 26, 27: *SrTt*1, Cook*^{6}/C80-1 & Timgalen (*Sr*36), 28: HD2285, 29, 30, 31: HD2285*^{3}/Cook*^{6}/C80-1, 32: PBW226, 33, 34, 35: PBW226*^{3}/Cook*^{6}/C80-1, 36: HD2687, 37, 38, 39: HD2687*^{3}/Cook*^{6}/C80-1, 40: WH147, 41, 42, 43, 44: WH147*^{3}/Cook*^{6}/C80-1, 45: Kalyansona, 46, 47: KS*^{3}/Cook*^{6}/C80-1, 48, 49: Lok-1, NI5439, 50, 51, 52: NI5439*^{3}/Cook*^{6}/C80-1, 53: Lok-1, 54, 55: Lok-1*^{3}/Cook*^{6}/C80-1, 56: UP262, 57, 58: UP262*^{3}/Cook*^{6}/C80-1, 59: C306, 60, 61, 62, 63, 64, 65: C306*^{3}/Cook*^{6}/C80-1).

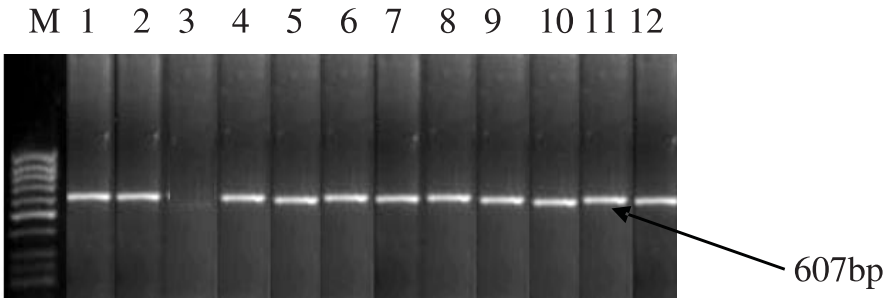

Fig. 9. Amplification of SCAR marker SCS1302$_{607}$ linked to the gene *Lr*24 in 9 backcross lines of HW2004*^{3}/ Cook*^{6}/ C80-1: (M: Molecular weight marker, 1 & 2: Presence of the linked marker fragment in TR380-14*7/3Ag#14 *Lr*24 and HD2687*5/*Lr*24 (confirm sources of *Lr*24), 3: Absence of the linked marker in C306, Lane 4-12: Presence of the linked marker fragment).

backcross lines in BC_3-F_5 generation, the molecular markers SCAR, STS and SSRs substantiated the introgression of linked genes by conventional approach. Amplification of marker fragment (SCS1302$_{607}$) discriminated HW2004*3/Cook*6/C80-1 which is carrying *Lr*24 (Fig. 9) in addition to genes *Lr*19/*Sr*25 and *Sr*36/*Pm*6. The use of gene specific molecular markers provided unambiguous evidence that the above mentioned backcross lines are a pyramid of three segments of linked genes. It can therefore be emphasized that conventional gene transfer can successfully be employed for gene pyramiding. The advantage in conventional breeding is enormous because the simultaneous field testing supported the recovery of genotypes having desirable yield contributing traits. Although single backcross approach to capture the genetic variation in BC_1-F_1 or BC_1-F_2 has been useful [30] in achieving gene combination but it may not be effective to select the assembly of different traits of recurrent parent(s) to release it as a cultivar. Therefore, for recovering all the desirable traits of a recurrent parent(s), administering more number of backcrosses is a worthwhile exercise.

Results of this investigation show the usefulness of conventional introgression assisted by molecular markers for authentication of the presence of introgressed genes for leaf rust and stem rust resistance.

The availability of newly constituted lines (BC_3-F_5) will serve as donor for leaf rust, stem rust and powdery mildew resistance. The gene combinations available in these commercial genetic backgrounds with enhanced durability of resistance can be strategically deployed after testing their yield potential and also these lines can be used as a material for molecular mapping and tagging.

References

1. **Friebe B., Jiang J., Raupp W. L., McIntosh R. A. and Gill B. S.** 1996. Characterization of wheat-alien translocations conferring resistance to diseases and pests: Current status. Euphytica, **91**: 59-87.
2. **Marais G. F., McCallum B., Suyman J. E., Pretorius Z. A. and Marais A. S.** 2005. Leaf rust and stripe rust resistance genes *Lr*54 and *Yr*37 transferred to wheat from *Aegilops kotschyi*. Plant Breeding, **124**: 538-541.
3. **Tomar S. M. S. and Menon M. K.** 2001. Genes for disease resistance in wheat. Indian Agricultural Research Institute, New Delhi, pp. 152.
4. **The T. T., Latter B. D. H., McIntosh R. A., Ellison F. W., Breman P. S., Fisher J., Hollamby G. L., Rathjen A. J. and Wilson R. E.** 1988. Grain yields of near-isogenic lines with added genes for stem resistance. In: Proc. 7th Intern. Wheat Genet. Symp. T.E. Miller and R. M. D. Koebner (eds.) pp. 901-906, IPSR, Cambridge, England.
5. **Penrose L. D. J., Walsh K., Clark K.** 1998. Characters contributing to high yield in Currawong, an Australian winter wheat. Aust. J. Agril. Res., **49**: 853-866.
6. **Singh R. P., Huerta-Espino J., Sharma R. and Joshi A. K**. 2006. High yielding spring bread wheat germplasm for irrigated agro-ecosystem. Challanges to International Wheat Breeding. International symposium on Wheat Yield Potential, 20-24 March 2006. Ciudad Obregon, Sonora, Maxico Abst, pp16.
7. **Stackman E. C., Stewart D. M. and Loegeing W. Q.** 1962. Identification of physiologic races of *Puccinia graminis tritici* Bull. USDA, ARS pp.53.
8. **Nayar S. K., Prashar M. and Bhardwaj S. C.** 1997. Manual of Current Techniques in Wheat Rust. Research Bulletin No. 2, Pp 32. Regional Station, D.W.R., Flowerdale , Shimla.
9. **Joshi L. M., Singh D. V. and Srivastava K. D.** 1988. Manual of wheat Diseases. Malhotra Publishin House, New Delhi. Pp. 40-45.
10. **Prabhu K. V., Somers D. J., Rakow G. and Gugel R. K.** 1998. Molecular markers linked to white rust resistance in mustard *Brassica juncea*. Theor. Appl. Genet., **97**: 865-870.
11. **Gupta S. K., Charpe A., Prabhu K. V., Haque Q. M. R.** 2006. Indentification and validation of molecular markers linked to the leaf rust resistance gene *Lr*19 in wheat. Theor. Appl. Genet., **113**: 1027-1036.
12. **Prins R., Groenewald J. Z., Marais G. F., Snape J. W., Koebner R. M. D.** 2001 AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theor. Appl. Genet., **103**: 618–624.
13. **Bariana H. S., Brown G. N., Bansal U. K., Miah H., Standen G. E., Lu M.** 2007. Breeding triple rust resistant wheat cultivars for Australia using conventional and marker-assisted selection technologies. Aust. J. Agric. Res., **58**: 576-587.
14. **Sawhney R. N. and Goel L. B.** 1981. Race specific interaction between wheat genotypes and Indian culture of stem rust. Theor. Appl. Genet., **60**: 161-166.
15. **Patil V. V. and Deokar A. B.** 1996. Host parasite interactions between lines and varieties of wheat with known Sr genes and races of stem rust. Cereal Rusts and Powdery Mildew Bull., **24**: 91-97.
16. **Tomar S. M. S. and Menon M. K.** 2000. Evaluation of stem rust resistance genes in bread wheat and introgression of *Sr26* into adapted cultivars. Proc. Intern. Conf. on Integrated Plant Disease Management for Sustainable Agriculture, Indian Phytopathological Society, New Delhi, pp. 650-653.
17. **Roelf A. P. and McVey D. V.** 1979. Low infection types produced by *Puccinia graminis* f. sp. *tritici* and wheat lines with designated genes for resistance. Phytopathology, **69**: 722-730.
18. **Johnson R.** 1984. A critical analysis of durable resistance. Annu. Rev. Phytopath., **22**: 309-330.
19. **Zwer P., Park R. F., McIntosh R. A.** 1992. Wheat stem rust in Australia-1965-1969. Australian Journal of Agricultural Research, **43**: 399-431.
20. **Bartos P., Vaclov S. I. P., Charpova J., Vacke J., Stuchlikova E., sarova J. and Hanzlova A.** 2002. Achievements and prospects of wheat breeding for disease resistance. Czech. J. Genet. and plant breed., **38**: 16-28.
21. **Bhardwaj S. C., Prashar M., Kumar S., Jain S. K. and Dutta D.** 2005. *Lr19* Resistance in wheat becomes susceptible to *Puccinia triticina* in India. Plant Disease, **89**: 1360.
22. **Nayar S. K., Tandon J. P., Kumar J., Prashar M., Bhardwaj S. C., Goel L. B. and Nagarajan S.** 1994. Basis of rust resistance in Indian wheats. Research Bulletin No.1: 33, Pp. Regional Station, Directorate of Wheat Research, Flowerdale, Shimla-171002 (India).
23. **Singh, Ravi P., Rajaram S., Saini R. G., Huerta-Espino J. and Willium M.** 2004. Genetics of Host-Pathogen Interaction and Breeding for Durable

Resistance. *In*: Plant Breeding-Mendelian to Molecular Approaches, H.K. Jain and M.C. Kharkwal (eds.), Pp. 145-166, Narosa Publishing House, New Delhi 110 033.

24. **Rao S., Singh G. and Chatterjee M.** 2001. Indian Wheat Pool. *In*: A.P. Bonjean and W.J. Angus (ed.), The World Wheat Book, A History of Wheat Breeding, pp. 773-816. Lavoisier Publishers, Paris, France.

25. **Sharma J. B.** 1990. Visiting scholar report. Plant Breeding Institute, University of Sydney, Castle Hill, N.S.W., Australia, Pp. 50.

26. **Dyck P. L. and Samborski D. J. and Anderson R. G.** 1966. Inheritance of adult plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana. Can. J. Genet. Cytol., **8**: 665-671.

27. **Wamishe Y. A. and Milus E. A.** 2004. Genes for Adult-Plant Resistance to Leaf Rust in Soft Red Winter Wheat. Plant Dis., **88**: 1107-1114.

28. **Dundas I. S., Verlin D. C., Park R. F., Bariana H. S., Anugrahwati D., Shepherd K. W., McIntosh R. A. and Islam A. K. M. R.** 2004. Progress in development of new rust resistant wheat using chromosomes from uncultivated relatives. In Proc. 54th Australian Cereal Chemistry Conference and 11th Wheat Assembly. Canberra, ACT, C.K. Black, J.F. Panozzo, G.J. Rebetzke (eds.), pp. 122-124.

29. **McIntosh R. A. and Luig N. H.** 1973. Recombination between genes for reaction to Puccinia graminis at or near the Sr9 locus. In Proc. 4th Intrn. Wheat Genet. Symp. Paestum, Italy, N.E. Pogna, M. Romano, A. Pogna, G. Galterio (eds.).

30. **Bariana H. S., Willey N. J., Venkata B. P., Lehmenseik A., Standen G. E. and Lu M.** 2004. Breeding methodology to achieve durability for rust resistance in wheat. In Proc. 54th Australian Cereal Chemistry Conference and 11th Wheat Assembly. Canberra, ACT, C.K. Black, J.F. Panozzo, G.J. Rebetzke (eds.), pp. 8-12.