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Research Article

Marker assisted pyramiding of stem rust, leaf rust and powdery mildew resistance genes for durable resistance in wheat (*Triticum aestivum* L.)

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Abstract

Wheat production is essential for food security. Stem and leaf rust diseases continually pose threat to wheat production. In recent years, climate change and intensive crop cultivation practices are making powdery mildew as a potential threat to wheat production. Deploying resistant cultivars are the most economic, reliable and sustainable way to manage the stem rust, leaf rust and powdery mildew of wheat. Using conventional selection system, it is difficult to select two or more genes in a single genotype. In such a situation, phenotype neutral selection based on marker-trait association along with seedling and adult plant reaction become inevitable. Stem rust, leaf rust and powdery mildew resistance genes, *Sr24/Lr24*, *Sr26* and *Sr36/Pm6* were pyramided in the background of nine Indian wheat cultivars through marker assisted backcross approach. The presence of the rust and powdery mildew resistance genes were confirmed using microsatellite markers such as *Sr24#12* (*Sr24/Lr24*), *Sr26#43* (*Sr26*) and *Stm773-2* (*Sr36/Pm6*) in the pyramided lines. Stable lines were selected at BC_3F_4 generation. Seedling and adult plant reaction of pyramided lines showed resistance to most of the stem and leaf rust pathotypes prevailing in India. Using gene pyramids (*Sr24/Lr24*, *Sr26* and *Sr36/Pm6*) that confer resistance to the predominant pathotypes of stem rust, leaf rust and powdery mildew could impart durability to the cultivars than single gene deployment.

Key words: Stem rust, leaf rust, powdery mildew, molecular markers

1. Introduction

Wheat is one of the most widely consumed cereal crops worldwide that provides 20% of dietary calories and protein (Shiferaw *et al.*, 2013). In India, wheat is the most important cereal crop after rice with a production of 107.18 MT during 2019-20 (Third Advanced Estimates, 2020). But with the rapidly increasing population, India will need more than 140 million tons of wheat by 2050. Among many factors, intensified agricultural practices and climate change have increased the incidence of pathogens in wheat in the recent years. Wheat is affected by various pathogens, amongst which the rusts caused by *Puccinia* spp. are the most devastating (Chaves *et al.*, 2013). Stem rust caused by *Puccinia graminis* f. sp. *tritici* can cause as much as 100% yield loss (Leonard and Szabo, 2005). Stem rust became a serious concern with the emergence of Ug99 race in East Africa and its migration to middle east, Iran etc. (Singh *et al.*, 2008). This race was able to overcome the widely used stem rust resistance gene *Sr31* (Pretorius *et al.*, 2000) which had protected wheat crop from stem rust for more than two decades. Since 1999,

pathogen is evolving rapidly resulting in thirteen variant within the Ug99 lineage of wheat stem rust (Rusttracker, 2019). In India, seven million hectares area covering Central and Peninsular India is considered to be stem rust prone (Bhardwaj *et al.*, 2019). Leaf rust caused by *Puccinia triticina* Eriks is prevalent in all the wheat growing regions of India (Bhardwaj *et al.*, 2019). Yield loss due to leaf rust varies from 15% to 60% (McIntosh, 1998). In recent years, powdery mildew of wheat caused by *Blumeria graminis* f.sp. *tritici* is gaining attention due to the changing climatic situations and modern cultivation practices. Powdery mildew can result in yield reductions of upto 40% under humid conditions (Bennett, 1984). In India, powdery mildew is cause of concern for wheat in cooler regions falling under the northwestern plain zone, northern and southern hill zone (Singh *et al.*, 2009). It has been estimated that PM can cause yield losses upto 35% (Sharma *et al.*, 1996).

Nearly 60, 80 and 58 stem rust, leaf rust and powdery mildew resistance genes, respectively have been cataloged in wheat (Prasad *et al.*, 2020; McIntosh *et al.*, 2017). But the rapid evolution of new virulent races makes most of the genes ineffective (Singh *et al.*, 2015). Hence, deployment of single resistance gene should be avoided as long-term and large-scale cultivation of such resistant varieties result breakdown of resistance and significant shifts in the virulence pattern of the pathogen population. To prevent or delay the breakdown of resistance, pyramiding multiple resistance genes in a single variety is a viable and vital strategy. However, gene pyramiding is difficult using conventional breeding methods. Such limitations of conventional breeding can be overcome using marker assisted pyramiding of rust resistance genes along with seedling and adult plant reaction. Availability and accessibility to closely linked molecular markers of the target genes makes the identification of genotypes with two or three genes possible (Gupta *et al.*, 2009), which can further assist in their pyramiding in single genotype.

Stem rust resistance gene (*Sr*), *Sr36*, derived from *Triticum timopheevii* is located in the 2BS chromosome provides resistance against stem rust pathotypes in India (Tomar and Menon, 2001). *Sr36* is also tightly linked to effective powdery mildew resistance gene, *Pm6* (Jorgensen and Jensen, 1973). Another *Sr* gene, *Sr*26 derived from *Agropyron elongatum* (Knott, 1961) translocated to chromosome 6AL remains effective inspite of its large scale utilisation in the 1970s and 1980s (McIntosh *et al.*, 1988). *Sr26* is effective gene against *Sr31*-virulent race Ug99 (TTKSK) and its derivatives (Singh *et al.*, 2011). The *Agropyron elongatum* derived rust resistance genes *Sr24/Lr24* conferred resistance to leaf and stem rusts for many years against the prevailing pathotypes in India (Tomar *et al.*, 2014). In India, the virulence for *Sr24* was first time detected from Wellington (Tamil Nadu) and named 40-1 (62G29-1) (Bhardwaj *et al.*, 1990). This pathotype remained mostly confined to Wellington (Tomar *et al.*, 2014). However, leaf rust resistant gene, *Lr24* linked to *Sr24* is still effective against the prevailing leaf rust pathotypes in Indian subcontinent (Prasad *et al.*, 2017).

In the present study, marker assisted pyramiding of stem rust, leaf rust and powdery mildew resistance genes viz., *Sr24/Lr24, Sr26* and *Sr36/Pm6* in the background of Indian wheat cultivars and their seedling and adult plant reaction are reported.

2. Materials and methods

2.1 Pyramiding of the target genes

Stem rust resistance gene, *Sr36/Pm6* introgressed in the background of nine Indian wheat cultivars viz., HD 2009, HD 2285, HD 2329, HS 240, J24, Kalyansona, Lok 1, MACS 2496 and NI 5439 through backcross breeding method at ICAR- Indian Agricultural Research Institute**,** (IARI), Regional Station, Wellington, were used as recipient parent (Fig 1). Cook (Cook*6/C80-1), an Australian line reported to carry *Triticum timopheevi* derived gene *Sr36/Pm6* was used as a donor to transfer *Sr36* gene. Darf Kite (Darf*6/3AG3/Kite), an Australian line known to carry *Sr26* and *Sr24/Lr24* was used as donor for transferring this gene into the *Sr36/Pm6* introgressed lines from 2016 onwards. In each generation, agronomic superior and resistance lines were selected and forwarded based on the reaction to stem rust, leaf rust and powdery mildew in the field.

Stable pyramided lines were selected at BC_3F_4 generation on which marker assisted selection was performed to select lines containing either (*Sr36/Pm6* or *Sr26* or *Sr24/Lr24*) or all the resistance genes (*Sr36/Pm6, Sr26* and *Sr24/Lr24*). Pyramided lines were assigned numbers as HW 5061, HW 5062, HW 5063, HW 5064, HW 5065, HW 5066, HW 5067, HW 5068 and HW 5069.

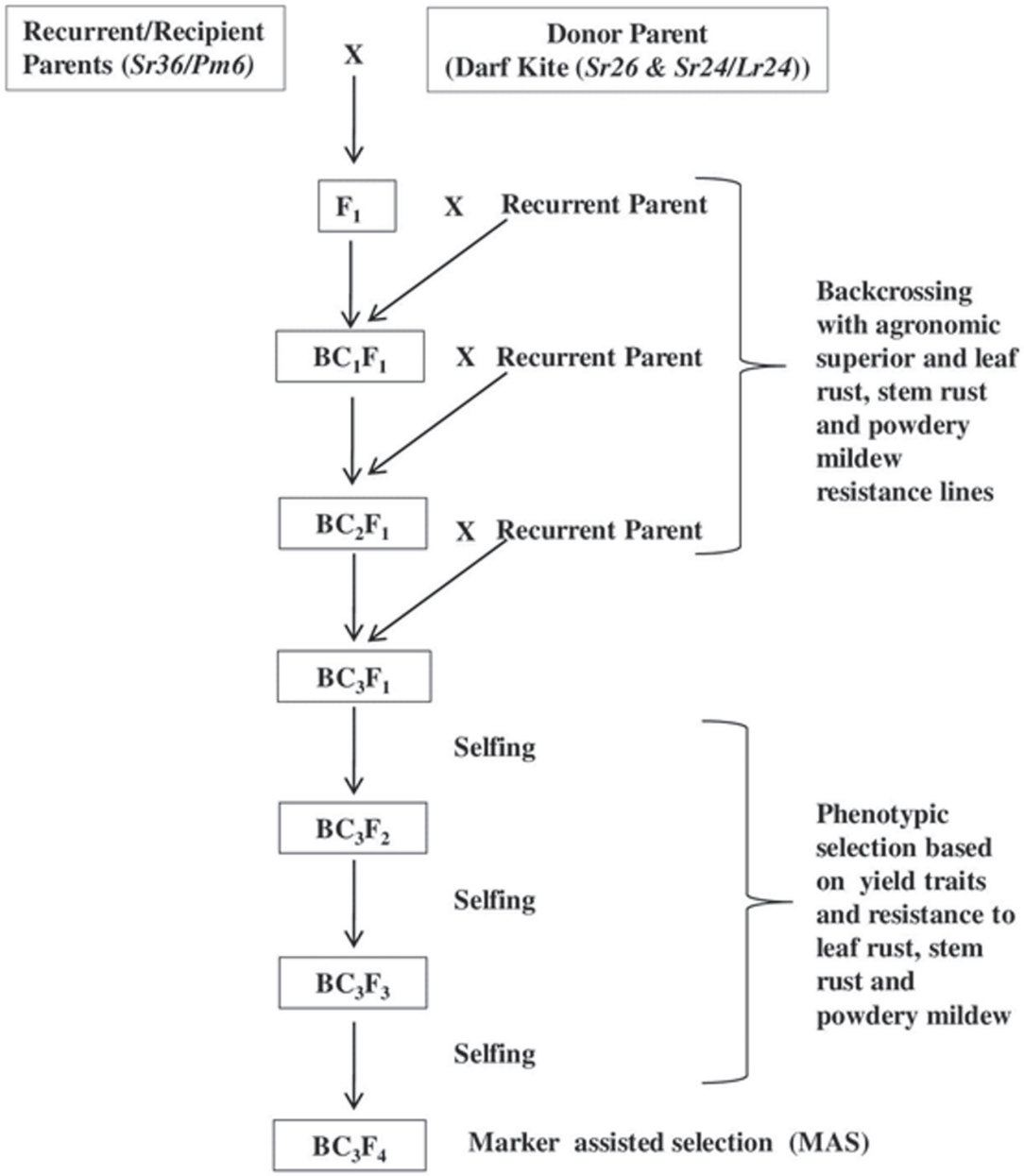


Fig. 1. Schematic representation of transfer of stem rust, leaf rust and powdery mildew resistance genes

2.2 Screening for stem rust, leaf rust and powdery mildew

Stable pyramided lines were evaluated for resistance to stem rust, leaf rust and powdery mildew diseases at ICAR- Indian Agricultural Research Institute**,** (IARI), Regional Station, Wellington in four seasons (Kharif and Rabi) during 2018-19 and 2019-20. Wellington is a natural hotspot for stem rust, leaf rust and powdery mildew diseases. Pyramided lines were planted in one-meter rows of six line each with a spacing of 23 cm between the rows. Spreader rows of mixture of susceptible cultivars were planted on four sides of the plot and one row of spreader was planted in between the pyramided lines after every 20 rows. All the recommended agronomic practices were followed throughout the crop duration.

Response of pyramided lines to stem and leaf rust diseases was recorded following the modified Cobb scale (Peterson *et al.*, 1948) at three crop growth stage corresponding to Zadoks growth stage (Zadoks *et al.*, 1974) of Z-60 (Begining of anthesis), Z-73 (Early milk stage) and Z-85 to 87 (Soft dough stage). Disease scores were determined by taking into account the severity of disease on plant parts,

recorded as percentage of area covered (5%, 10%, 20%, 40%, 60%, 80% and 100%) and kind of host response as described by Loegering (1959) as 0- No visible infection, R- Resistant: necrotic areas with or without minute uredia, MR- Moderately resistant: small uredia, surrounded by necrotic areas, MX- Intermediate: variable sized uredia, some with necrosis or chlorosis, MS- Moderately susceptible: medium uredia with no chlorosis present, S- Susceptible: large uredia, no necrosis or chlorosis.

Stable pyramided lines were also subjected to screening at seedling stage for stem and leaf rust resistance under controlled conditions in the glasshouse at ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Regional Station, Flowerdale, Shimla where the wheat genotypes were tested with four predominant pathotypes of stem rust viz., 40A (62G29), 40-1(62G29-1), 40-3 (127G29) and 117-6 (37G19) and three pathotypes of leaf rust viz., 77-5 (121R63-1), 77-9 (121R60-1) and 104-2 (21R55). The avirulence/virulence formulae for the pathotypes are provided in Table 1. Seedling tests were performed as per Bhardwaj (2011). Infection types (ITs) on the seedlings were recorded 14 days post inoculation using Stakman scale (Stakman *et al.*, 1962). The Infection types (ITs) 3, 3^+ were considered susceptible, whereas lower ITs ('0', '1', '2' and 'X') were considered as resistant.

Table 1: Avirulence and virulence profile of stem and leaf rust pathotypes

Sl. No.	Pathotypes	Avirulence on genes	Virulence on genes
Stem rust			
1	40A (62G29)	*Sr7a, 13, 21, 22, 24, 25, 26, 27, 30, 31, 32, 33, 35, 36, 37, 39, 40, 43, Tmp* and *Tt3*	*Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9f, 9g, 10, 14, 15, 16, 17, 18, 19, 20, 23, 28, 29, 34* and *McN*
2	40-1(62G29-1)	*Sr7a, 13, 21, 22, 25, 26, 27, 30, 31, 32, 33, 35, 36, 37, 39, 40, 43, Tmp* and *Tt3*	*Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9f, 9g, 10, 14, 15, 16, 17, 18, 19, 20, 23, 24, 28, 29, 34* and *McN*
3	40-3 (127G29)	*Sr 21, 22, 24,25, 26, 27, 30, 31, 32, 33, 35, 36, 37, 39, 40, 42, 43, Tmp* and *Tt3*	*Sr5, 6, 7a, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9f, 9g, 10, 11, 14, 15, 16, 17, 18, 19, 20, 23, 28, 29, 30, 34, 38, 44, McN* and *Gt*
4	117-6 (37G19)	*Sr5, 8a, 8b, 9b, 22, 24, 25, 26, 27, 28, 30, 31, 32, 33, 35, 36, 37* and *Tmp*	*Sr 6, 7a, 7b, 9e, 9f, 9g, 10, 11, 12, 13, 14, 15, 16, 17, 19, 21, 23, 29, 34* and *McN*
Leaf rust			
5	77-5(121R63-1)	*Lr9, 19, 24, 25, 28, 29, 32, 39, 42, 43, 45* and *47*	*Lr1,2a, 2b, 2c, 3,10, 11, 12,13, 14a, 14b, 15, 16, 17a, 17b, 18, 20, 21, 22a, 22b, 23, 26, 27, 30, 33, 35, 36, 37, 38, 40, 44,48* and *49*
6	77-9(121R60-1)	*Lr2a, 2b, 2c, 9, 19, 24, 25, 28, 32, 39, 42, 45* and *47*	*Lr1, 3, 10, 11, 12, 13, 14a, 14b, 15, 16, 17a, 17b, 18, 20, 21, 22a, 22b, 23, 26, 27+31, 30, 33, 35, 36, 37, 38, 44, 46, 48* and *49*
7	104-2(21R55)	*Lr9, 10, 13, 15, 19, 20, 24, 25, 28, 29, 32, 36, 39, 42, 43, 45* and *47*	*Lr1,2a, 2b, 2c, 3, 11,12, 14a, 14b, 16, 17a, 17b, 18, 21, 22a, 22b, 23, 26, 27+31, 30, 33, 34, 35, 37, 38, 40, 44, 48* and *49*

Powdery mildew reaction was recorded as per the scale (0-9) devised by Saari and Prescott (1975) for scoring the foliar disease intensity. Lines were scored for powdery mildew at dough stage (Zadoks growth stage of 83-87) (Zadoks *et al.*, 1974). Disease reactions of 10 randomly selected lines were averaged to get the final score. Disease score upto 5 was considered as resistant while > 5 as susceptible.

2.4 DNA extraction, molecular markers analyses

DNA was extracted from approximately 100 mg of fresh leaves collected from 20 days old seedlings. DNA isolation was carried out using CTAB method of Doyle and Doyle (1990). DNA was quantified on 1% agarose gel. The diluted DNA samples were used for PCR analysis.

Microsatellite markers s*tm773-2* linked to *Sr36* (Tasilo *et al.*, 2008), *Sr26#43* (Mago *et al.*, 2005) linked to *Sr26* and *Sr24#12* (Mago *et al.*, 2005) linked to *Sr24/Lr24* were used to perform marker assisted selection. The sequences of the primers with the amplicon are given in Table 2.

Table 2: Details of Primer sequences for DNA markers linked to rust resistance genes in wheat

Gene	Marker	Sequence of primer	Size of the amplicon (bp)	Reference
Sr36/ Pm6	*stm773-2F*	ATG GTT TGT TGT GTT GTG TGT AGG	155 bp	Tsilo *et al.*, 2008
	stm773-2R	AAA CGC CCC AAC CAC CTC TCT C		
Sr26	*Sr26#43-F*	AAT CGT CCA CAT TGG CTT CT	207 bp	Mago *et al.*, 2005
	Sr26#43-R	CGC AAC AAA ATC ATG CAC TA		
Sr24/ Lr24	*Sr24#12-F*	CAC CCG TGA CAT GCT CGT A	500 bp	Mago *et al.*, 2005
	Sr24#12-R	AAC AGG AAA TGA GCA ACG ATG T		

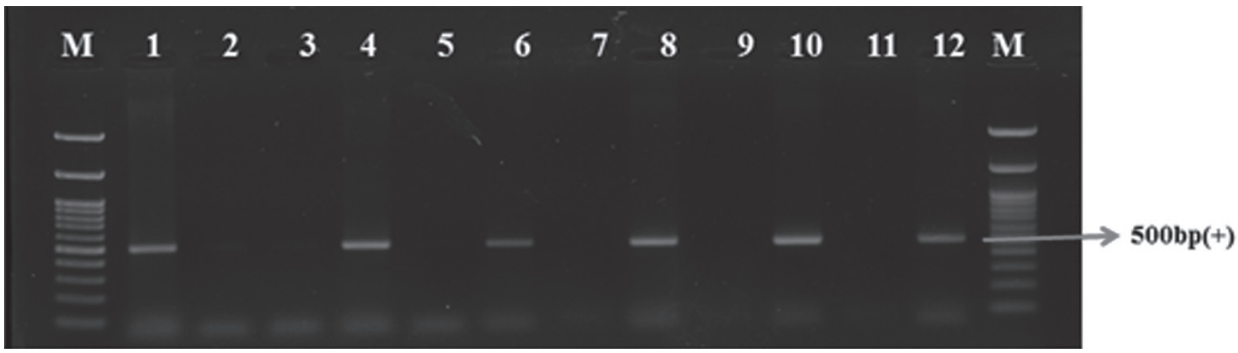
Polymerase chain reaction was performed in 20µl reaction volume containing 50-75 ng of template DNA, 250 nM of each primer (forward and reverse), 0.2 nM of each dNTPs, 2 µl of 10X Taq buffer and 1 unit of Taq DNA polymerase. PCR reaction was performed in Applied Biosciences (Veriti) thermo cycler using the following PCR conditions with little modifications. PCR condition for *Sr24#12*: one 3-min cycle at 94°C (initial denaturation), followed by 35 cycles of 45 s at 94°C (denaturation), 45 s at 65°C (annealing) and 1 min at 72°C and a final extension at 72°C for 10 min. *Sr26#43*: one 3-min cycle at 94°C (initial denaturation), followed by 30 cycles of 30 s at 94°C (denaturation), 30 s at 55°C (annealing) and 45 s at 72°C and a final extension at 72°C for 10 min. *stm773-2*: one 1-min cycle at 95°C (initial denaturation), followed by 35 cycles of 30 s at 95°C (denaturation), 30 s at 55°C (annealing) and 1 min at 72°C and a final extension at 72°C for 10 min. The amplified products were resolved in 2% agarose gel and visualized under the gel documentation system (Syngene, Gene Genius bioimaging system, UK).

3. Results and Discussion

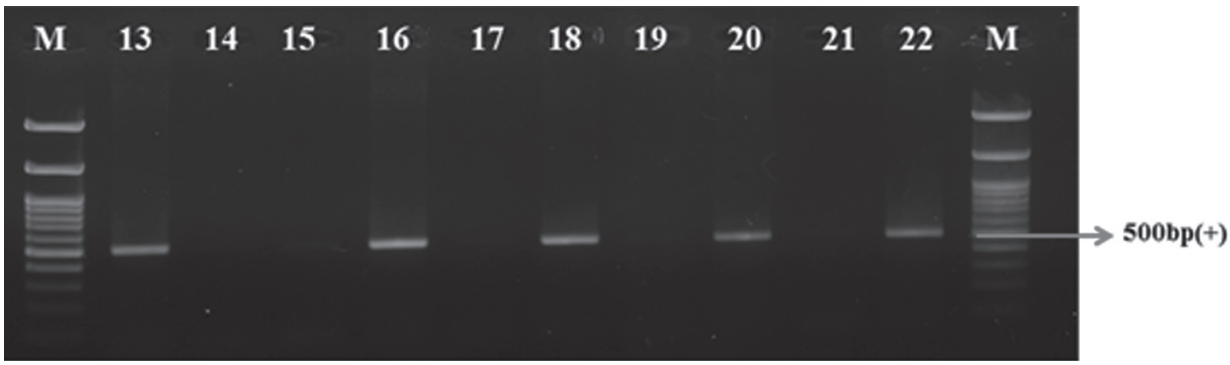
Pyramiding of rust resistance genes in the elite genetic background is one of the most important strategies to develop durable rust resistant wheat varieties. Providing genetic/host resistance through pyramiding of stem rust, leaf rust and powdery mildew (*Sr36/Pm6*, *Sr26*, *Sr24/Lr24*) resistance genes will be an effective strategy to combat the threats from stem rust, leaf rust and powdery mildew in India. Pyramiding of two or more resistance genes into a single background has been prominently assisted by the development of molecular markers and confirmed through the host pathogen interaction at seedling adult plant stage, which otherwise will be time consuming through conventional breeding approaches.

Lines carrying *Sr36/Pm6* gene were pyramided with stem and leaf rust resistance genes, *Sr26* and *Sr24/Lr24* and the F_1s were backcrossed for three generations with the respective recurrent parents and subsequent selfing for four generations. In F_2 and subsequent generations, phenotypic selection of lines resistant to stem rust, leaf rust and powdery mildew along with high yielding traits (number of productive tillers, panicle length, number of spikelets and grains per panicle etc.) were followed. High yielding and disease resistant lines were forwarded to select stable pyramided lines at BC_3F_4 generation. Molecular marker confirmation of stem and leaf rust and powdery mildew (*Sr36/Pm6*, *Sr26, Sr24/Lr24*) resistance genes were performed in the stable pyramided lines.

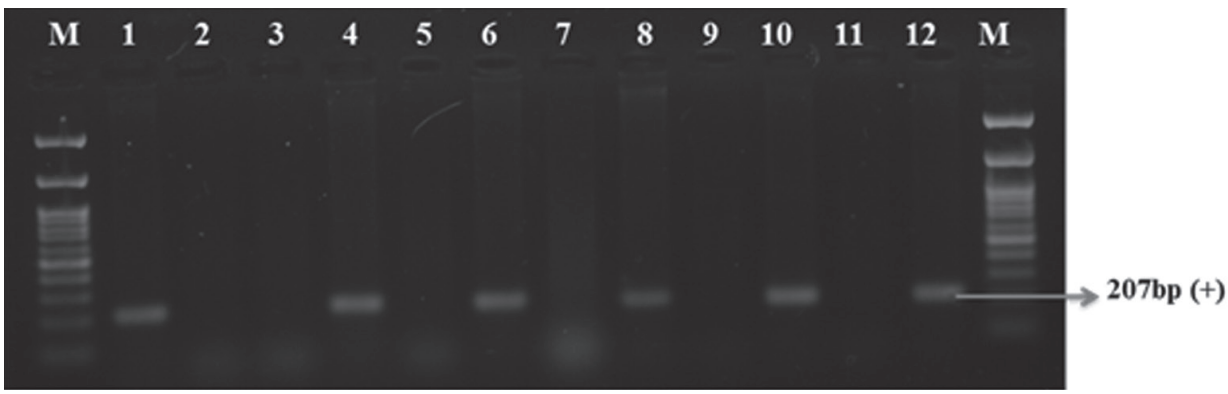
Molecular marker analysis using the microsatellite markers for stem/leaf rust resistance gene, *Sr24#12* displayed 500 bp amplicon in all the backgrounds, indicating the presence of *Sr24/Lr24* gene (Fig 2 (A1-A2)). In case of stem rust resistance gene, *Sr26*, all the pyramided lines showed the presence of the gene with 207 bp amplicon (Fig 2 (B1-B2)). Whereas, *stm773-2,* a co-dominant marker linked to stem rust and powdery mildew resistance gene, *Sr36/Pm6* revealed a 155bp amplicon in the homozygous lines positive for *Sr36* and a 190 bp amplicon in the homozygous lines negative for *Sr36*. Both the amplicons were present in the heterozygous lines. Among the nine pyramided lines, two lines (HW 5067 and HW 5068) showed a clear homozygous band of 155 bp indicating the presence of *Sr36/Pm6* gene, while three lines (HW 5062, HW 5063 and HW 5069) were heterozygous showing both 155 and 190 bp product (Fig 3 (C1-C2)) and remaining lines were negative for *Sr36/Pm6* gene. The marker score of the pyramided lines along with the donor and recurrent parents are presented in Table 3.


M 1 2 3 4 5 6 7 8 9 10 11 12 M
500bp(+)

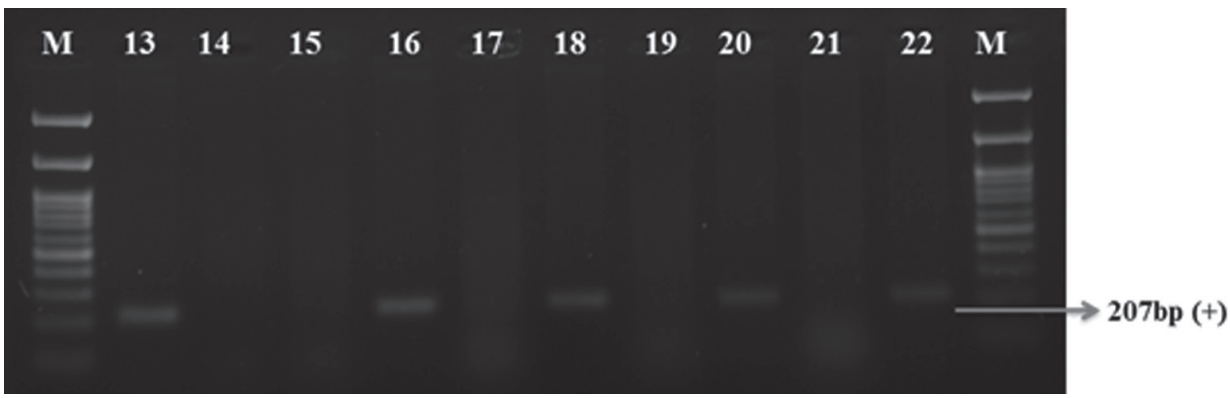
(**A1**)


M 13 14 15 16 17 18 19 20 21 22 M
500bp(+)

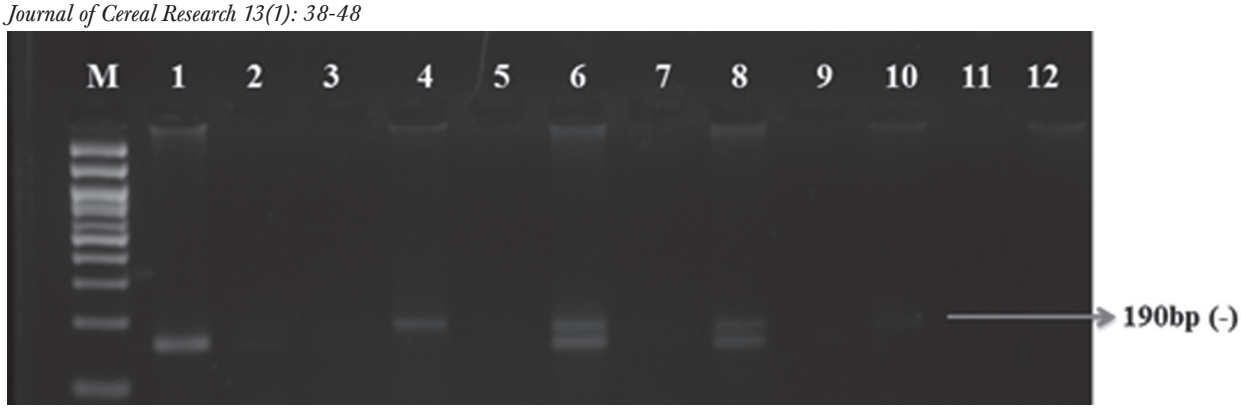
(**A2**)


M 1 2 3 4 5 6 7 8 9 10 11 12 M
207bp (+)

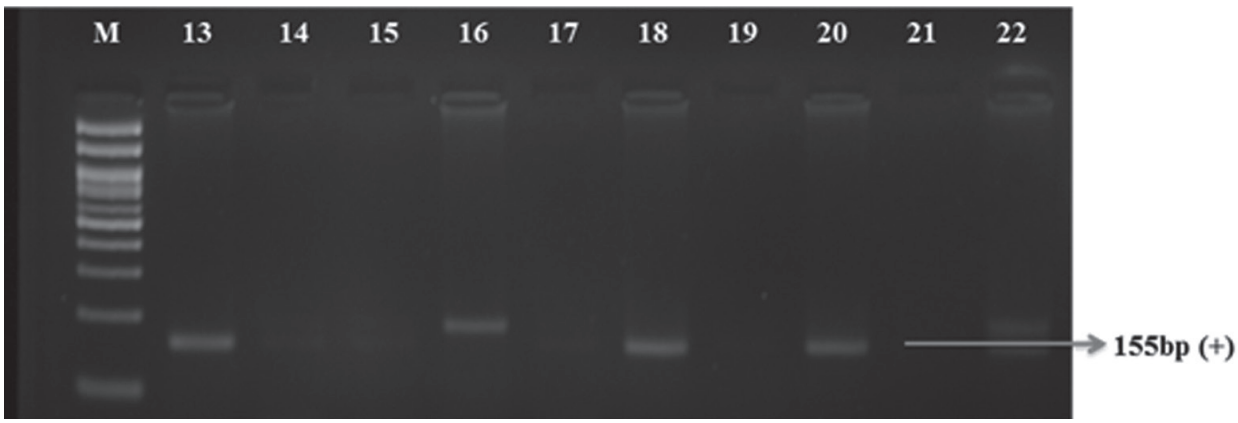
(**B1**)


M 13 14 15 16 17 18 19 20 21 22 M
207bp (+)

(**B2**)



(C1)



(C2)

Fig 2 Molecular marker confirmation of stem rust/leaf rust/powdery mildew resistance gene, *Sr24* (*Sr24#12*) (A1-A2), *Sr26* (*Sr26#43*) (B1-B2), *Sr36/Pm6* (*stm773-2*) (C1-C2) in pyramided lines

A1-A2:1&13- Darf Kite (Positive Control); 2&14-Lok1 (Negative Control); B1-B2: 1&13-Darf Kite (Positive Control); 2&14-Sonalika (Negative Control); C1-C2:1&13- Cook (Positive Control); 2&14- Sonalika (Negative Control); AI-A2, B1-B2 and C1-C2: M (100bp Ladder); 3-HD 2009; 4-HW 5061; 5-HD 2285; 6-HW 5062; 7-HD2329; 8-HW 5063; 9-HS 240; 10-HW 5064; 11-J 24; 12-HW 5065; 15-Kalyansona; 16-HW 5066; 17-Lok-1; 18-HW 5067; 19-MACS 2496; 20-HW 5068; 21-NI 5439; 22-HW 5069.

Table 3: Presence/absence of stem rust, leaf rust and powdery mildew resistance genes in pyramided lines using respective linked markers

Sl. No.	Donors/ Recurrent parent/ Pyramided lines	*Sr24/Lr24 (Sr24#12)*	*Sr26 (Sr26#43)*	*Sr36/Pm6 (Stm733-2)*
1	HD 2009	-	-	-
2	HW 5061	+	+	-
3	HD 2285	-	-	-
4	HW 5062	+	+	+/-
5	HD 2329	-	-	-
6	HW 5063	+	+	+/-
7	HS 240	-	-	-
8	HW 5064	+	+	-
9	J 24	-	-	-
10	HW5065	+	+	-
11	Kalyansona	-	-	-

12	HW 5066	+	+	-
13	Lok-1	-	-	-
14	HW 5067	+	+	+
15	MACS 2496	-	-	-
16	HW 5068	+	+	+
17	NI 5439	-	-	-
18	HW 5069	+	+	+/-
19	Darf Kite (Positive control) (Donor for *Sr26 & Sr24/Lr24*)	+	+	-
20	Cook (Positive control) (Donor for *Sr36*)	-	-	+
21	Sonalika (Negative control)	-	-	-
22	Lok1(Negative control)	-	-	-

Among the nine genotypes used for pyramiding, only two pyramided lines, HW 5067 and HW 5068 in the background of Lok 1 and MACS 2496 respectively were confirmed to carry all the stem rust, leaf rust (*Sr36/Pm6*, *Sr26* and *Sr24/Lr24*) and powdery mildew (*Sr36/Pm6*) resistance genes, while the rest of the pyramided lines showed the presence of stem and leaf rust (*Sr26* and *Sr24/Lr24*) and absence of stem and powdery mildew resistance genes (*Sr36/Pm6*). However, all the stable lines negative for stem rust and powdery mildew (*Sr36/Pm6*) gene showed resistance reaction to powdery mildew compared to their respective recurrent parent. This may be due to the partial/incomplete expression of the gene in those genetic backgrounds.

Stable pyramided lines were subjected to field screening for four seasons in two years. Pyramided lines carrying either *Sr26, Sr36/Pm6* and *Sr24/Lr24* or *Sr26* and *Sr24/Lr24* were resistant to both stem and leaf rusts in comparison to the recurrent parents which were susceptible with a stem rust score ranging from 20S to 60S and leaf rust from 40S to 80S (Table 4). Stem and leaf rust resistance genes have imparted resistance which got reflected in the resistance reaction of the pyramided lines. Pyramided lines (HW 5067 and HW 5068) with powdery mildew resistance gene (*Sr36/Pm6*) showed immune reaction, to powdery mildew, while rest of the lines without *Sr36/Pm6* also remained resistant (score ranging from 3 to 4) compared to their recurrent parent (Table 4). Partial/incomplete expression of the gene in those genetic backgrounds might have caused the discrepancies between the phenotype and genotype.

Table 4: Stem and leaf rust response of the parental and pyramided lines at seedling and adult plant stage and powdery mildew at adult plant stage

Sl. No.	Donors/ Recurrent parent/ Pyramided lines	Seedling response to stem rust pathotypes				Seedling response to leaf rust pathotypes			Adult plant response		
		40A	40-1	40-3	117-6	77-5	77-9	104-2	Stem rust	Leaf rust	Powdery mildew
1	HD 2009	3	3	3+	;	3+	3+	3+	40S*	40S	5
2	HW 5061	1	;1	1+	;	0;	0;	0;	F	F	3
3	HD 2285	3	3	2-	;	3+	3+	3+	40S	60S	6
4	HW 5062	;1	;1	1	0;	0	0	0	F	F	3
5	HD 2329	3+	3	0;	;	3+	3+	3+	40S	60S	6
6	HW 5063	2	;1	1+	;	0	0	0	F	F	4
7	HS 240	2-	2	0;	2-	2	3+	3+	20S	60S	6

8	HW 5064	1	;1	;1	1	0;	0;	0;	F	F	4
9	J 24	2-	3	2	2	3+	3+	3+	40S	40S	6
10	HW 5065	;	0	0;	;	0	0	0	F	F	4
11	Kalyansona	3+	3+	2+	;	3+	2	3+	60S	80S	6
12	HW 5066	2	;1	1	;	;	0	;	F	F	4
13	Lok 1	3+	3+	3+	3	3+	3+	3+	60S	60S	6
14	HW 5067	;	0	0;	;	0	0	;	F	F	0
15	MACS 2496	2-	2-	;1	;	3+	3+	3+	20S	40S	5
16	HW 5068	0	0;	0;	;	0	0	0	F	F	0
17	NI 5439	3+	3+	0;	3+	3+	3+	3+	60S	80S	7
18	HW 5069	2	1	1	;1	0;	0;	;-	F	F	3
19	Darf Kite (Donor for *Sr26 & Sr24/Lr24*)	;	0	;1	1	0;	0	0;	F	F	2
20	Cook (Donor for *Sr36/ Pm6*)	0	0;	;	;	-	-	-	F	-	0

*S-Susceptible; F-Free

Pyramided lines were also evaluated at seedling stage under glass house condition using prevalent pathotypes of stem (40A, 40-1, 40-3 and 117-6) and leaf rust (77-5, 77-9 and 104-2). These lines irrespective of the presence of two (*Sr26* and *Sr24/Lr24*) or three (*Sr26, Sr36/Pm6* and *Sr24/Lr24*) stem rust resistance genes displayed resistant reaction to all the pathotypes relative to recurrent parent which showed susceptible reaction to most of the pathotypes. Similar was the case for leaf rust pathotypes wherein all the pyramided lines were resistant due to the presence of leaf rust resistant gene, *Lr24* and the recurrent parent exhibited susceptible reaction. Seedling response of the pyramided lines to stem and leaf rust are presented in Table 4.

Pyramiding of resistance genes is one of the most promising approaches to enhance the effectiveness and durability of a gene and cultivars (Brown, 2015). Many of the resistance genes derived from alien sources have linkage drag in the form of yield penalty, quality traits etc., a common phenomenon in resistance breeding (Tomar and Menon, 2001). However, use of genetic stocks in elite background, growing large number of segregating lines in F_2 and use of closely linked/functional marker could limit the linkage drag to a greater extent. Stem rust resistance gene, *Sr36/Pm6* had been used in the development of cultivars and improved germplasm in North America, Australia, South Africa, Kenya and Ethiopia (McIntosh *et al.*, 1995). Similarly, *Sr36/Pm6* transferred to durum wheat cultivar, HI8498 provided near immune reaction to many pathotypes belonging to 117 group (Sai Prasad *et al.*, 2014). Stem/leaf rust resistance gene, *Sr24/Lr24* has been used extensively in the development and release of several wheat varieties in India. Although, pathotype (40-1) virulent on *Sr24* has been reported from India which is mostly confined to Wellington, *Sr24/Lr24* continues to provide resistance and does not affect yield (Tomar *et al.*, 2014).

Stem rust resistance gene, *Sr26* used in Australia for the development of stem rust resistance cultivars, although it had yield penalty (The *et al.*, 1988, McIntosh *et al.*, 1995). Yield reduction was not observed in the pyramided lines with *Sr26*. In our study, linkage drag in the form of yield penalty was not observed in the pyramided lines either in two gene (*Sr26* and *Sr24/Lr24*) or three gene (*Sr26, Sr36/Pm6* and *Sr24/Lr24*) combinations as selections were carried out for both yield traits (data not shown) and rust resistance in each generation.

Combining multiple resistance genes in a single genotype is more time consuming and laborious through conventional breeding approach because the dominant nature of resistance genes makes it is difficult to distinguish between the presence of single and multiple genes. But this problem is solved to great extend using molecular marker assays (Gupta *et al.*, 2010). Pyramided lines provided resistance to the predominant stem and leaf rust

pathotypes prevailing in India. Combination of resistance genes (*Sr24/Lr24, Sr26* and *Sr36/Pm6*) in the genetic background of cultivars will serve as a good source of resistance which can be strategically deployed to provide durability to resistance.

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Conflict of Interest

Authors declare that they have no conflict of interest.

Ethical Compliance Statement

NA

Author's Contribution

Conceptualization of research (VVK); Designing of the experiments (VVK, SM, JP); Contribution of experimental materials (VVK, SM, JP); Execution of field/lab experiments and data collection (VVK, PP, KS, SCB, GM, NR, SP, JP); Analysis of data and interpretation (VVK, SM, JP, SCB); Preparation of the manuscript (VVK, MS, JP, SCB).

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