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Detection of *Lr19/Sr25* in segregating populations of wheat (*Triticum aestivum* L.) using robust molecular markers

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Abstract

Marker assisted breeding (MAB) technology has been proved effective to transfer the genes of interest and also increased the accuracy of selection in wheat improvement programmes. The *Agropyron elongatum* derived *7D.7Ag* translocation carrying *Lr19/Sr25* is not only effective against leaf rust pathotypes but also effective against stem rust race *Ug99* and its variants. Therefore, wheat breeding lines carrying *Lr19/Sr25* translocation segment have been developed worldwide. The present study was aimed for developing wheat genotypes with *Lr19/Sr25* using *Xwmc221*, *PSY1-E1* and *Gb* DNA markers in segregating populations of wheat. We could infer that *Xwmc221*, *PSY1-E1* and *Gb* are effective molecular markers to tag *Lr19/Sr25* in segregating generations of wheat. The data also revealed consistency between host pathogen interaction (HPI) test and *Xwmc221*, *PSY1-E1* and *Gb* molecular markers for selecting lines with *Lr19/Sr25* in segregating generations. This work not only confirmed the robustness of the three *Lr19/Sr25* markers but also demonstrated the application of both genotyping and phenotyping in making full-proof selection of superior progenies with gene of interest in wheat.

Key words: rust resistance markers, MAB

1. Introduction

Among the three wheat rusts, leaf rust, caused by the *Puccinia triticina* is most common, widely distributed throughout the world (Kumar *et. al.*, 2021) and usually causes 10-15% yield loss and also decreases grain quality (Slikova *et al.*, 2003). Leaf rust (*Lr*) can inflict 50% loss in epidemic years (Anonymous, 1992). Among the various techniques to manage the wheat rusts, introgression of genes from novel sources of rust resistance is an effective and environment friendly strategy to combat leaf rust pathogen. As many as 80 *Lr* genes have been reported in wheat and its relatives (McIntosh *et al.*, 2020). Among the *Lr* genes, the *Agropyron elongatum* (Syn. *Thinopyrum ponticum or Lophopyrum elongatum*) derived *7D.7Ag* translocation carrying *Lr19/Sr25* provides protection against leaf rust in Northern India. A large number of recombinant lines using *7D.7Ag* translocation carrying *Lr19/Sr25* have been developed world wide. In India, two wheat varieties PBN142 (HD2189/NI917//Agatha) and WH533 (Agatha/Yacora17) have been released, probably carrying *Lr19/Sr25* due to the presence of Agatha in their pedigree lineage (Tomar *et al.*, 2014). Though virulence to *Lr19* designated as 77-8 has been reported from peninsular India (Bhardwaj *et al.*, 2005) but field population of leaf rust lacks virulence for *Lr19* during 2013-2016 surveys (Bhardwaj *et al.*, 2019). In recent surveys, virulence for *Lr19* has not been observed in India but it was identified in 0.27% of the samples from Nepal only (Bhardwaj *et al.*, 2021). The evolution of *Ug99*

and its variants, virulent to many of the *Sr* genes including *Sr31* has created a fear of stem rust epidemics worldwide. The *7D.7Ag* translocation carrying *Lr19/Sr25* not only provides leaf rust resistance but also effective against *Ug99* and its variants (Singh *et al.,* 2011). Besides, it has positive effects on grain yield under favourable conditions (Monneveux *et al.,* 2003). However, the use of *Sr25/Lr19* germplasm was limited until development of a mutant line Agatha-28 (Knott, 1980) through mutation in the linked gene *PSY1-E1* causing undesirable yellow flour (Zhang and Dubcovsky, 2008).

In different studies molecular markers viz., *Xwmc221* (Prins *et al.*, 2001; Somers *et al.,* 2004), *PSY1-E1* (Zhang and Dubcovsky, 2008), *Gb* (Liu *et al.,* 2010) and host-pathogen interaction (HPI) tests have been used for selecting plants with *Lr19/Sr25* in segregating generations. As an outcome of our breeding efforts for leaf and stem rust resistance, segregating and fixed populations of wheat with *Lr19/Sr25* have been developed.

2. Materials and Methods

2.1 Plant material

The wheat cultivar HS240 and rust resistant genetic stocks, FLW20 (*Lr19/Sr25*) and FLW13 (*Yr15*) were used to develop BC_2F_1s *viz*, HS240*2/FLW20, HS240*2/FLW13, separately. Both the BC_2F_1s were further inter crossed to generate F_1 (HS240*2/FLW20//HS240*2/FLW13) and subsequently selfed for developing F_2, F_3, F_4 generations. Based on rust phenotyping and marker linkage data, F_4 resistant progenies were advanced to F_5.

2.2 Seedling resistance tests

The material comprising parents, genetic stocks and segregating generations of the cross HS240*2/FLW20// HS240*2/FLW13 along with the sets of differentials were raised in a mixture of loam soil and farm yard manure using aluminium trays. Seven days old seedlings were inoculated with pure culture (5 mg uredospores per ml in light weight, non-phytotoxic isoparaffinic oil-Soltrol®) of virulent pathotypes *viz*, THTTM (121R63-1 or Ptr77-5), PHTTL (21R55 or Ptr104-2) of *P. triticina* and PTTSF (127G29 or Ptg40-3) of *P. graminis* of Indian sub-continent (Table 1). The inoculated seedlings were atomized with fine mist of water and placed in dew chambers for 48 hrs at 20±2^{0}C for initiation of infection. The seedlings were then shifted on to the green house benches, maintaining temperature 22±2°C for leaf rust and 25±2°C for stem rust with 80% RH till recording of infection types (IT's). The seedling reaction for IT's of rusts was recorded after a fortnight following Stakman *et al.,* (1962).

Table 1: SRT to rust pathotypes and validating wheat genotypes for *Lr19/Sr25* using molecular markers.

S.No	Genotype*	Infection Score to Rust pathotypes			Response to mol. Marker		
		Ptr 77-5	*Ptr 104-2*	*Pgt 40-3*	*Xwmc221*	*PSY1-E1*	*Gb*
1	HS240	3+	3+	;2-	-	-	-
2	FLW20	0;	0;	;2-	+	+	+
3	*Tc+Lr19*	0;	0;	;2-	+	+	+
4	PBW343	3+	2+	2-	-	-	-
5	WBM3617	0;	0;	;1	+	+	+
6	WBM3618	0;	0;	;1	+	+	+
7	WBM3619	0;	;	;2-	+	+	+
8	22	0;	;	;1	+	+	+
9	150	0;	0;	;2+	+	-	+
10	155	0;	;	;2+	+	+	+
11	156	0;	;	;2-	+	+	+
12	157	0;	;	;2-	+	+	+
13	158	0;	0;	;2	+	+	+
14	159	0;	;	;2	+	+	+
15	160	0;	;	;2	+	+	+
16	161	0;	;	;2	+	+	+

17	162	0;	;-	;2-	+	+	+
18	163	0;	;-	;2	+	+	+
19	164	0;	NG	;2-	+	+	+
20	165	0;	0;	;2-	+	+	+
21	169	0;	0;	;2-	+	+	+
22	170	0;	0;	;2-	+	+	+
23	171	0;	;-	;2-	+	+	+
24	172	0;	;-	;2-	+	+	+
25	WBM3617	0;	0;	;1	+	+	+
26	WBM3618	0;	0;	;1	+	+	+
27	WBM3619	0;	;	;2-	+	+	+
28	19	0;	;-	;2-	+	+	+
29	20	0;	;-	;2-	+	+	+
30	21	0;	;-	;2-	+	+	+
31	22-1	0;	0;	;2-	+	+	+
32	23	3+, 0;	;	;2, 3P3-	+/-	+	+
33	24	0;	;-	;2-	+	+	+
34	25	0;	;-	;2-	+	+	+
35	26	0;	0;	;2-	+	+	+
36	27	0;	;-	;2-	+	+	+
37	28	0;	;-	;2-	+	+	+
38	29	0;	0;	;2-	+	+	+
39	30	0;	;-	;2-	+	+	+
40	31	0;	0;	;2-	+	+	+
41	32	0;	;	;1	+	+	-
42	33	0;	;-	;1	+	+	+
43	34	0;	;-	;2-	+	+	+
44	35	0;	;-	;2-	+	+	+
45	36	0;	;-	;2-	+	+	+
46	37	0;	;-	;2-	+	+	+
47	38	0;	;-	;2-	+	+	+
48	39	0;	0;	;2-	+	+	+
49	40	0;	;-	;1	+	+	+
50	43	0;	0;	0;	+	+	+
51	44	0;	;-	;1	+	+	+
52	45	0;	;-	;2-	+	+	+
53	46	0;	;-	;2-	+	+	+
54	47	0;	;-	;2-	+	+	+
55	48	3+	3	3+	-	-	-
56	49	3+	;1	3+	-	-	-
57	50	3+	;2+	3	-	-	-
58	51	0;	0;	;2-	+	+	+
59	56	0;	0;	;1	+	+	+
60	57	0;	0;	;1	+	+	+
61	58	0;	0;	;2-	+	+	+
62	71	0;	;	;1	+	+	+

63	134	0;	0;	;2-	+	+	+
64	140	0;	0;	;2-	+	+	+
65	141	0;	;	;2-	+	+	+
66	142	0;	0;	;2-	+	+	+
67	143	0;	;-	;2-	+	+	+
68	146	0;	;-	;2-	+	+	+
69	147	0;	;-	;2-	+	+	+
70	148	0;	;-	;2-	+	+	+

*1-4 Parents & genetic stocks, 5-7 &25-27 F5 lines, 8-24 & 28-70F4 lines, Infection score of rust pathotypes recorded according to Stakman et al. 1962.
Infection score 0; (naught fleck) / ; (fleck) / ;1, ;2-, 2, 2+ =Resistant, 3-, 3, 3+=Susceptible

2.3 Molecular markers used and DNA isolation

Closely linked molecular markers *viz*, *Xwmc221*, *PSY1-E1* and *Gb* were used to tag *Lr19/Sr25* in segregating populations of cross HS240*2/FLW20//HS240*2/FLW13. DNA was extracted by CTAB method (Rogers and Bendich, 1985). PCR amplification with the primers for *Lr19* was performed in a 20 µl reaction mixture containing 10mM Tris-HCL (pH 8.8), 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM of each dNTPs, 0.75 U *Taq* DNA Polymerase, 22 ng of each of SSR primer and 40 ng genomic DNA. PCR for *Xwmc221* was performed in a Thermal Cycler programmed for 10 minutes at 95°C, 35 cycles [94 °C 1 min, 1 min at annealing temperature (Table 2), 72 °C 1 min] followed by final extension for 7 minutes at 72 °C. The amplified products were separated on 2.5 % high resolution agarose gel, stained with ethidium bromide and visualized in Vilber Lourmet gel documentation system. Allele scoring was performed using Gene Mapper v 4.0 software (Applied Biosystems). Negative controls DNAs were included for comparison in the marker analysis and observations were repeated to ascertain the accuracy of the results.

Table 2: Markers closely linked with rust resistance genes *Lr19/Sr25*, their primer sequences, amplicon size and PCR conditions or annealing temp.

Marker	Type	Sequence(5' -3')	Amplicon size (bp)	AT °C	Reference
Xwmc221	SSR	F ACGATAATGCAGCGGGGAAT R GCTGGGATCAAGGGATCAAT	190	61	Gupta *et al.*, 2006
Gb	STS	F CATCCTTGGGGACCTC R CCAGCTCGCATACATCCA	130	60	Liu *et al.*, 2010
PSY1- E1	STS	F CTACGTTGCGGGCACCGTT R AGAGAAAACCATTGCATCTGTA	191	60 TD	Zhang and Dubcovsky, 2008

AT-annealing temperature; TD-touch down.

3. Results and Discussion

The plants of the cross HS240*2/FLW20//HS240*2/FLW13 selected for *Lr19/Sr25* were carried forward through F_2, F_3, F_4 and F_5 generations. Poor performing plants were rejected and the selected F_1 plants were selfed to obtain F_2. Two hundred and ninety-seven F_2 plants were analysed for *Lr19* using *Xwmc221* and positive plants (Fig.1) were carried forward to develop F_3 generation. Twenty-three F_3 lines were selected and validated for presence of *Lr19* using *Xwmc221* and all were recorded positive for *Lr19* (Fig. 2). Seventy genotypes comprising parents, test stocks, ear to row progenies of F_4 and selected F_5 bulks were evaluated for seedling resistance to leaf rust pathotypes THTTM (Ptr77-5) and PHTTL (Ptr104-2) and stem rust pathotype PTTSF (Pgt40-3) through HPI test and validated for the presence of *Lr19/Sr25* using molecular markers *Xwmc221, PSY1-E1* and *Gb* (Table 1). Among 60 F_4 progenies, 56 lines showed resistant reaction (0; naught fleck), one line (F_4-23) showed segregating reaction and three lines (F_4-48, F_4-49, F_4-50) were observed as susceptible (3+) in HPI test to the Ptr77-5. F_5 bulks (WBM3617, WBM3618, WBM3619) and F_4 progenies except genotype F_4-48 were found to be resistant in HPI test to pathotype 104-2, whereas, among 60 F_4 progenies, 56 showed resistant reaction. Line F_4-23 showed

segregating reaction and three lines (F_4-48, F_4-49, F_4-50) were observed as susceptible to the Ptg40-3 (Table 1).

On marker analysis, 56 F_4 progenies were positive for *Xwmc221*, *PSY1-E1*, *Gb* and therefore, validated to carry *Lr19/Sr25* gene (Fig 3-5). Three genotypes (F_4-48, F_4-49, F_4-50) were negative for all the three markers and were also found to be susceptible in HPI test to pathotype Ptr104-2 and Pgt 40-3. One genotype F_4-23 at lane #32 showed two polymorphic fragments of size 190 bp and 209 bp, indicating its heterozygous status with *Xwmc221*. Similar results were also reported by Singh *et al.* (2017) for transferring *Lr19/Sr25* in wheat variety HD2733 using *Xwmc221*. Three F_5 bulks *viz*., WBM3617, WBM3618, WBM3619 which were replicated twice, showed a resistant reaction (;0) in HPI test to the pathotype Ptr77-5 and were also positive for microsatellite markers *Xwmc221*, *PSY1-E1* and *Gb,* indicated the presence of carry *Lr19/Sr25* linked genes.

Fig 1. Lane M – 100bp ladder, lanes 1-HS240; 2-FLW20 (*Lr*19); 3-12 representative F_2 population (*Plants positive for *Lr*19)

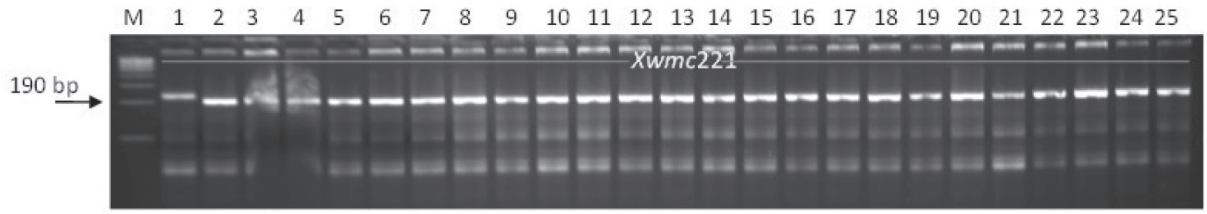

Fig 2. Lane M – 100bp ladder, lanes P1-HS240; P2-FLW20 (*Lr*19); 3-25 representative F3 lines positive for *Lr*19.

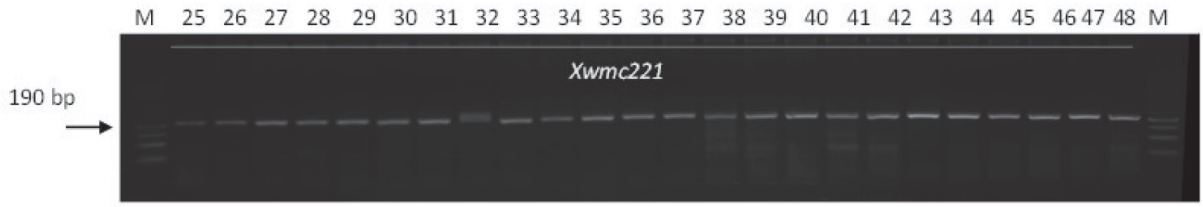

Fig 3. Lane M – 50 Kb ladder, lanes: 25-27 (F5), 28-48 (F4), *Lr*19 positive lines except lane 32 (Line F4-23).

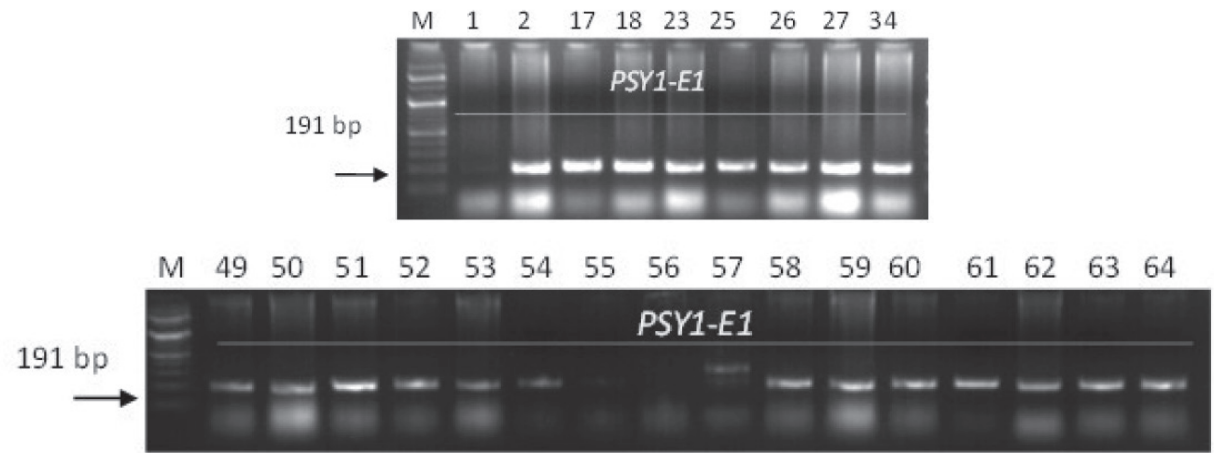

Fig 4a & b- Lane M – 100bp ladder, lanes 1-HS240; 2-FLW20 (Lr19/Sr25); 17, 18, 23, 25, 26, 27, 34, 49-64 representative F4 lines positive for Sr25.

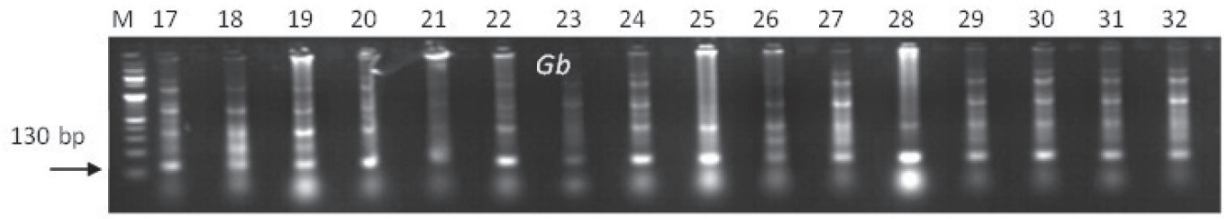

Fig 5- Lane M – 100 bp ladder, lanes: 17-32F4 Sr25 positive lines.

In the present study, we could identify, differentiate and substantiate homozygous and heterozygous resistant plants in different segregating generations for *Lr19/Sr25* using three highly effective microsatellite marker*s*. It has helped in selecting lines with confirmed *Lr19/Sr25* in segregating generations of wheat cross. Microsatellite markers were also used to confirm the presence of *Lr19* in winter wheat cultivars by Tomkowiak *et al.* (2016). In our study, one F_4 line #23 was found to be heterozygous with co-dominant marker *Xwmc221*.

The same line was recorded as positive carrier by two other markers, namely, *PSY1-E1* and *Gb* because of their dominant nature. A dominant SCAR marker $SCS265_{512}$ was used previously for validating *Lr19* (Pal *et al*., 2015) and microsatellite marker *Sr24*#12 for tagging *Sr24/Lr24* in back cross generations of wheat (Kumaran *et al*., 2021).

The role of co-dominant microsatellite marker *Xwmc221* was also advocated by Gupta *et al.* (2006) and Singh *et al.* (2017) for identifying heterozygotes and suggested it as an important tool for rapid transfer of *Lr19* into wheat cultivars. *Sr25*, tightly linked to *Lr19*, was counter validated using marker *PSY1-E1* and *Gb* giving an additional support for the validation of *Lr19*. Our results showed consistency between seedling resistance test and molecular marker assisted selection of genotypes for *Lr19/Sr25* using microsatellite markers, *Xwmc221*, *PSY1-E1* and *Gb*. The use of HPI test and molecular markers for validation has resulted in precise and perfect selection of the targeted rust resistance gene in the wheat breeding programme. It is concluded from the present study that *Xwmc221*, *PSY1-E1* and *Gb* are all robust markers to tag *Lr19/Sr25* in rust resistance wheat breeding programme.

A number of earlier workers have advocated the role of *Lr19/Sr25* in wheat improvement programmes. The *7D.7Ag* segment with *Lr19/Sr25* is also known to increase biomass and grain yield by 14% and 20% respectively, in wheat genotypes, Borlaug and Oasis (Singh *et al.,* 1998). A significant increase in yield, biomass and grain number was also reported by Reynolds *et al* (2001) with introgression of *Lr19* in all genetic backgrounds. In another study, *7D.7Ag* translocation increased grain yield potential by 10-15 per cent in a range of genotypes (Singh *et al.,* 2006). Breeding lines with *Lr19/Sr25* from Agatha and Sears' translocations were characterized by high levels of yellow pigment in the endosperm. Mutant lines carrying *Lr19/Sr25* with reduced level of yellow pigmentation in flour were obtained (Knott, 1980). The parent FLW20 involved in hybridization carry *Lr19/Sr25* from Agatha and therefore, the derived progenies do not possess undesirable trait yellow flour. This work not only confirmed the robustness of three *Lr19/Sr25* markers for developing leaf and stem rust resistant plants in segregating wheat material but also demonstrated the application of both genotyping and phenotyping in making full-proof selection of superior progenies in wheat.

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Conflict of Interest

Authors declares that they do not have any conflict of interest

Ethical Compliance Statement

NA

Authors Contribution

Conceptualization of research and designing of experiments (DP, SCB), Molecular marker validation (HK, SK, PS), Seedling resistance tests (SCB), Preparation of manuscript (DP, SCB, MP, RN).

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