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Short Communication

Validation of molecular markers linked to the stem rust resistance genes effective in India

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Abstract

An investigation was carried out to validate the already known molecular markers Xgwm533, Xcfa2019 & Xcfa2123, *Sr24*#12 & *Sr24*#50, *Sr26*#43 and BE518379, SCSS30.2 and *Sr39*-I & *Sr39*-II for stem rust resistance genes *Sr2, Sr22, Sr24, Sr26, Sr31* and *Sr39*, respectively in wheat cultivars Kundan and UP 2338 carrying unknown stem rust resistance gene(s). The *Sr2* and *Sr22* gene specific SSR markers amplified the gene specific or expected allele in both *Sr2* and *Sr22* carrying and non-carrying cultivars. *Sr24* associated STS markers (Sr24#12 & Sr24#50) amplified their respective expected fragments (500 bp and 200 bp) only in known *Sr24* gene carrying cultivar, HD 2851. Two DNA markers (Sr26#43 & BE518379) known for *Sr26* gene were screened. Marker, Sr26#43 amplified the specific fragment of 207 bp only in known source carrying *Sr26* gene, while other marker, BE518379 functioned as null allele marker by not amplifying the critical band in *Sr26* lines. SCAR marker, SCSS30.2 specific to *Sr31* gene amplified the gene specific fragment in wheat cultivar UP 2338 confirming the presence of *Sr31*. Out of 14 entries tested for *Sr39* gene including Kundan and UP 2338, the expected PCR product was not amplified in any of the entries indicating absence of *Sr39* gene.

Key words: Wheat, stem rust resistance genes, molecular markers, validation

Three rusts, namely stem rust (*Puccinia graminis* Pers f. sp. *tritici* Eriks and Henn.), leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* West) attack wheat and cause significant losses worldwide [1]. Damage caused by stem rust is quite significant [2] and can be as high as 100% [1]. Host resistance is an effective control measure that has been used in numerous wheat breeding programs [3]. Forty five stem rust resistance genes (*Sr*) have been designated in wheat [4], however, molecular markers for only a few genes are reported [5]. Virulence for stem rust resistance genes, *Sr26, Sr27, Sr31, Sr32, Sr35, Sr37, Sr39, Sr40* and *Sr43* does not exist in India [6].

Resistance gene postulation is a rapid method to hypothesize the likely presence of resistance gene in a host genotype [7], which is a relative postulation and entirely dependent on phenotyping and environment dependent host-pathogen interaction. This is not feasible option at all for wheat breeding institutions, as all the required virulences cannot be maintained by all stations as this can pose an imminent risk of spreading the races in the fields by escape. Resistance genes could however be detected unambiguously by testing host genotypes with DNA based markers linked to resistance genes irrespective of the location and season. This alternative approach can overcome some of the problems associated with traditional gene postulation, such as gene interactions and the plant stage of gene expression [8]. Molecular markers associated with disease resistance enable effective and early selection of resistant genotypes, despite lacking favourable environment [9]. Moreover, several resistance genes can be tracked simultaneously by testing for the presence of multiple molecular markers (multiplexing) and the markers can be used at an early developmental stage (seed/seedling) [10]. The present study was conducted with the objective to validate the molecular markers already

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available for stem rust resistance genes *Sr2, Sr22, Sr24, Sr26, Sr31* and *Sr39* to know the status (presence or absence) of these genes in Ug99 resistant wheat varieties Kundan and UP 2338.

Seeds of Kundan, UP 2338, Agra Local and the donor of the genes, *Sr2*, *Sr11*, *Sr22*, *Sr24* (HD 2851), *Sr26* (Eagle) and *Sr31*, PBW 343, Chinese spring and Thatcher collected from Division of Genetics, IARI, New Delhi were used for this investigation. Total genomic DNA from all plant material was extracted after collecting and lyophilizing the leaf samples by employing the microextraction method described by Prabhu *et al.* [11]. The concentration of the extracted DNA was estimated with Unicoâ UV-2100 Spectrophotometer (SIA Global Inc., USA) at 260 nm wavelength. The DNA was diluted to final working concentration of 10 ng/µl.

The primers sequences used to amplify molecular markers for stem rust resistance genes are given in Table 1. PCR amplification of stem rust resistance genes specific markers Xgwm533 (*Sr2*), Xcfa2019 & Xcfa2123 (*Sr22*) and BE518379 (*Sr26*) was conducted on a PTC 200 PCR system (MJ Research, USA). PCR amplifications were carried out in 1x PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$], 0.2 mM of each dNTPs, 40 ng each of forward and reverse primer, 0.9 unit of Taq polymerase and 50 ng of genomic DNA in a final 20 µl reaction volume. PCR conditions followed were presented in Table 2. PCR products were separated for 4 h on 3% Metaphore® agarose gel at a constant voltage of 80 V and viewed on a UV transilluminator and photographed on a digital gel documentation system (Alpha Innotech, San Leandro, CA, USA) after staining with 10 mg/ml ethidium bromide.

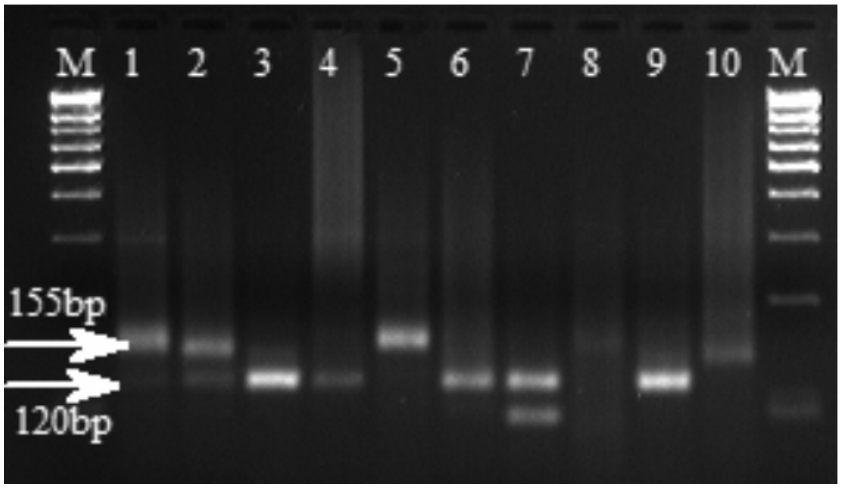

Fig. 1. Validation of microsatellite marker Xgwm533 linked to stem rust resistance gene *Sr2*. M: Marker, 1: *Sr2* donor, 2: *Sr22* donor, 3: *Sr24* donor, 4: *Sr24* (HD 2851), 5: Chinese spring, 6: Thatcher, 7: PBW 343, 8: Kundan, 9: UP 2338, 10: Agra Local

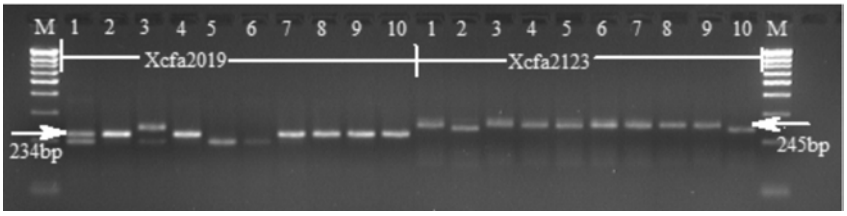

Fig. 2. Validation of microsatellite markers Xcfa2019 & Xcfa2123 specific to stem rust resistance gene *Sr22*. M: Marker, 1: *Sr2* donor, 2: *Sr22* donor, 3: *Sr24* donor, 4: *Sr24* (HD 2851), 5: Chinese spring, 6: Thatcher, 7: PBW 343, 8: Kundan, 9: UP 2338, 10: Agra Local

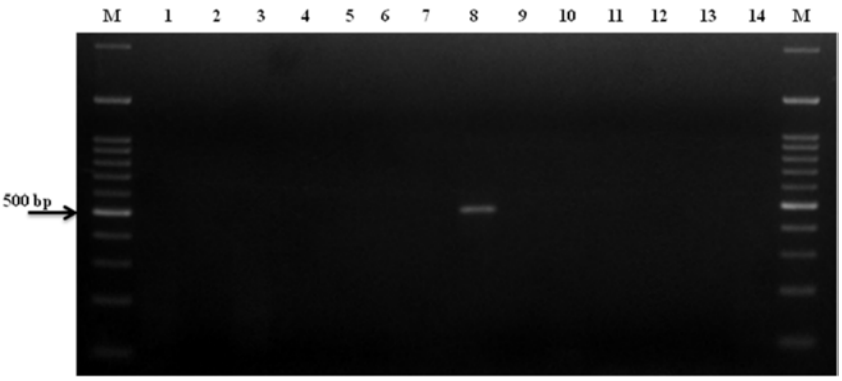

Fig. 3. Validation of *Sr24* gene specific marker Sr24#12. M: Marker, 1: Kundan, 2: UP 2338, 3: Agra Local, 4: Sr2 donor, 5: *Sr11* donor, 6: *Sr22* donor, 7: *Sr24* donor, 8: Sr24 (HD2851), 9: *Sr26* donor, 10: *Sr26* (Eagle), 11: *Sr31* donor, 12: *Sr31* donor, 13: PBW 343, 14: Thatcher

Validation of molecular markers already known for stem rust resistance genes *Sr24, Sr26, Sr31* and *Sr39* was also done on a PTC 200 PCR system (MJ Research, USA). Polymerase Chain Reaction amplifications were carried out in 25 µl reaction volume with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2

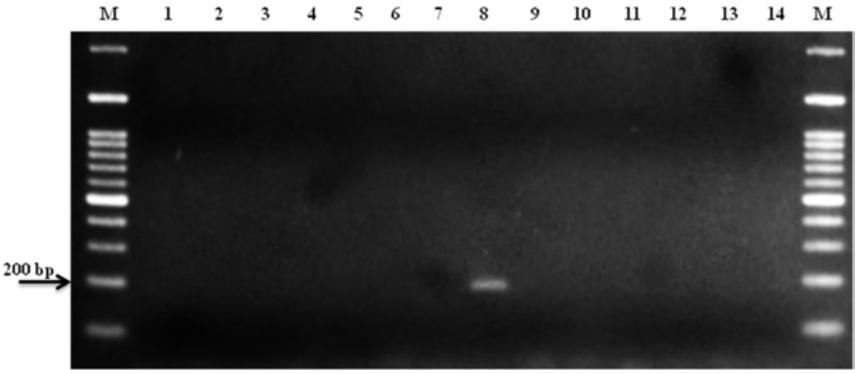

Fig. 4. Validation of *Sr24* gene specific marker Sr24#50. M: Marker, 1: Kundan, 2: UP 2338, 3: Agra Local, 4: *Sr2* donor, 5: *Sr11* donor, 6: *Sr22* donor, 7: *Sr24* donor, 8: *Sr24* (HD2851), 9: *Sr26* donor, 10: *Sr26* (Eagle), 11: *Sr31* donor, 12: *Sr31* donor, 13: PBW 343, 14: Thatcher

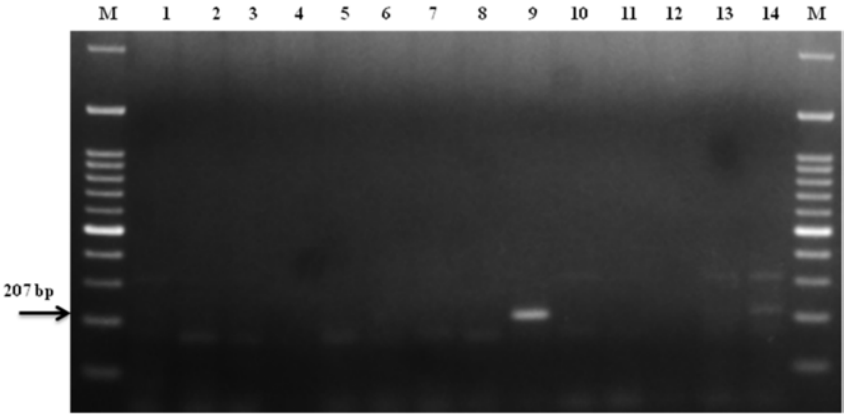

Fig. 5. PCR amplification of Sr26#43 marker specific to *Sr26* gene. M: Marker, 1: Kundan, 2: UP 2338, 3: Agra Local, 4: Sr2 donor, 5: *Sr11* donor, 6: *Sr22* donor, 7: *Sr24* donor, 8: *Sr24* (HD2851), 9: *Sr26* donor, 10: *Sr26* (Eagle), 11: *Sr31* donor, 12: *Sr31* donor, 13: PBW 343, 14: Chinese spring

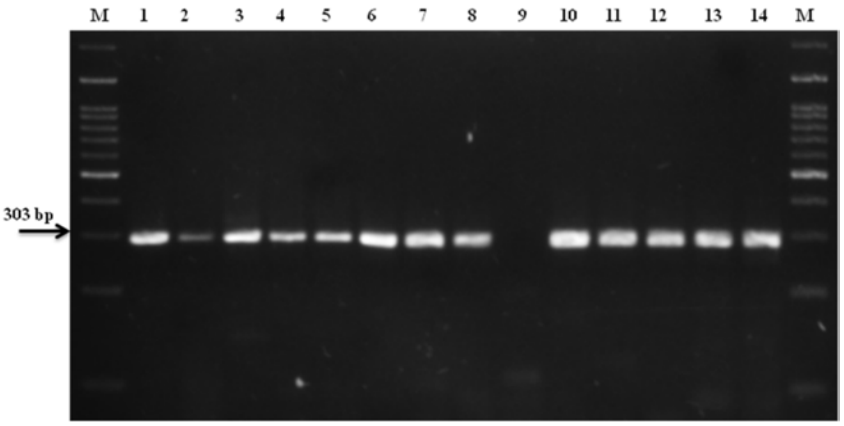

Fig. 6. PCR amplification of BE518379 marker specific to *Sr26* gene. M: Marker, 1: Kundan, 2: UP 2338, 3: Agra Local, 4: Sr2 donor, 5: *Sr11* donor, 6: *Sr22* donor, 7: *Sr24* donor, 8: *Sr24* (HD2851), 9: *Sr26* donor, 10: *Sr26* (Eagle), 11: *Sr31* donor, 12: *Sr31* donor, 13: PBW 343, 14: Chinese spring

mM $MgCl_2$, 0.2 mM of each dNTPs, 22 ng each of forward and reverse primer, 0.75 unit of *Taq* DNA polymerase and 40 ng of genomic DNA. PCR conditions followed were presented in Table 2. PCR products were separated for 3 h on 2% (*Sr24, Sr26, Sr31)* and 1.4% (*Sr39*) agarose gel at a constant voltage of 80 V and viewed on a UV transilluminator and photographed on a digital gel documentation system (Alpha Innotech, San Leandro, CA, USA) after staining with 10 mg/ml ethidium bromide.

Sr2 gene specific SSR marker (Xgwm533) amplified the expected fragment of 120 bp in UP 2338 and 120 bp fragment also amplified in non *Sr2* gene carriers (Fig. 1.). Speilmeyer *et al*. [13] reported that SSR marker Xgwm533 for *Sr2* gene was amplified a PCR product of 120 base pairs (bp) from all of the wheat lines that carried *Sr2*. However, the 120 bp allele was also amplified from a small number of wheat lines that were assessed not to carry *Sr2*. DNA sequence analysis of the 120 bp product cloned from *Sr2* carrying cultivars revealed 100% sequence identity, but when compared with cultivars which lacked this stem rust resistance gene, four base pairs were polymorphic within the 120 bp sequence. These results indicated that apart from recombination between the marker and *Sr2,* the microsatellite marker Xgwm533 amplified at least two different 120 bp alleles of which one appeared to be diagnostic of *Sr2* gene [13].

The *Sr22* linked SSR markers Xcfa2019 and Xcfa2123, amplified the specific fragments of 234 bp and 245 bp respectively, in both carriers and presumably non *Sr22* carrying cultivars including

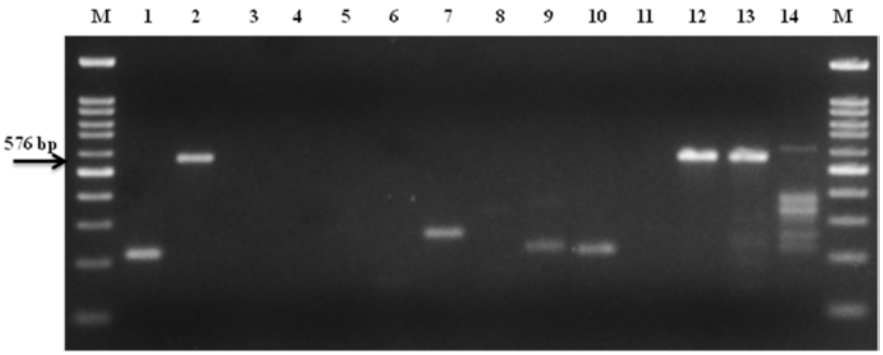

Fig. 7. Amplification of SCAR marker SCSS30.2 specific to *Sr31* gene. M: Marker, 1: Kundan, 2: UP 2338, 3: Agra Local, 4: Sr2 donor, 5: *Sr11* donor, 6: *Sr22* donor, 7: *Sr24* donor, 8: *Sr24* (HD2851), 9: *Sr26* donor, 10: *Sr26* (Eagle), 11: *Sr31* donor, 12: *Sr31* donor, 13: PBW 343, 14: Thatcher.

varieties Kundan and UP 2338 (Fig. 2). Thus, *Sr22* gene associated SSR markers could not be used to precisely diagnose the status of *Sr22* in Kundan and UP 2338.

Two STS markers Sr24#12 and Sr24#50 used to identify the stem rust resistance gene *Sr24* amplified specific fragments of 500 bp (Fig. 3) and 200 bp (Fig. 4) respectively, only in the known *Sr24* gene carrying cultivar HD 2851, while the fragments were absent in all other lines including the *Sr24* donor source. This indicated that the line being maintained as *Sr24* donor could actually be a non-*Sr24* carrier with stem rust resistance.

Screening of entries with DNA markers *Sr26*#43 and BE518379 associated with *Sr26* gene was carried out. *Sr26*#43 marker amplified the expected 207 bp fragment (Fig. 5) only in known *Sr26* donor and BE518379 marker did not amplify 303 bp fragment functioning as a null allele (Fig. 6) in same source. In all other materials, including Eagle expected to carry *Sr26,* the 207 bp fragment was absent but 303 bp fragment was present. These results are same as the results obtained by Liu *et al*. [15] *i.e.* these two STS markers; Sr26#43 and BE518379 in combinations can be used as codominant marker to differentiate heterozygote from homozygote *Sr26* gene carriers.

SCAR marker, SCSS30.2 used to validate the *Sr31* gene, amplified the gene specific fragment in wheat cultivars UP 2338, PBW 343 and known *Sr31* gene carrying source confirming the presence of *Sr31* gene in UP 2338 (Fig. 7). Earlier studies also reported the presence of *Sr31* in UP 2338 and absence of same gene in Kundan and Agra Local [5]. Out of the 14 entries validated for *Sr39* gene using two SCAR markers in the present study, none of the lines amplified the critical

Table 1. Molecular markers with their primer sequences used for validation of stem rust resistance genes.

Gene	Marker	Forward primer sequence	Reverse primer sequence	Expected fragment size (bp)	Reference
Sr2	Xgwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	120	Spielmeyer *et al*., [13]
Sr22	Xcfa2019	GACGAGCTAACTGCAGACCC	CTCAATCCTGATGCGGAGAT	234	Khan *et al*., [16]
	Xcfa2123		CGGTCTTTGTTTGCTCTAAACC		
			ACCGGCCATCTATGATGAAG	245	
Sr24	Sr24#12	CACCCGTGACATGCTCGTA	AACAGGAAATGAGCAACGATGT	500	Mago *et al*., [17]
Sr24	Sr24#50	CCCAGCATCGGTGAAAGAA	ATGCGGAGCCTTCACATTTT	200	Mago *et al*., [17]
Sr26	Sr26#43	AATCGTCCACAT TGGCTTCT	CGCAACAAAATCATGCACTA	207	Mago *et al*., [17]
Sr26	BE518379	AGCCGCGAAATCTACTTTGA	TTAAACGGACAGAGCACACG	303	Liu *et al*., [15]
Sr31	SCSS30.2	GTCCGACAATACGAACGATT	CCGACAATACGAACGCCTTG	576	Das *et al*., [15]
Sr39	Sr39-I	AGAGAGAGTAGAAGAGCTGC	AGAGAGAGCATCCACGA	900	Gold *et al*., [10]
Sr39	Sr39-II	GAGAGAGAGTAGAAGAGC	AGAGAGAGAGCATCCACC	900	Gold *et al*., [10]

Table 2. PCR conditions for primer pairs used to validate molecular markers of *Sr2, Sr22, Sr24, Sr26, Sr31* and *Sr39* stem rust resistance genes

Gene	Marker	PCR conditions	Dominant/ codominant	Gel conc.
Sr2	Xgwm533	94°C /3 min.; 45 cycles (94°C /1 min; 60°C /1 min; 72°C /2 min); 72°C /10 min	Codominant	3% Metaphore
Sr22	Xcfa2019	94°C /5 min.; 30 cycles (94°C /30 s; 60°C /30 s; 72°C /30 s); 72°C /10 min	Codominant	3% Metaphore
	Xcfa2123	94°C /5 min.; 30 cycles (94°C /30 s; 60°C /30 s; 72°C /30 s); 72°C /10 min	Codominant	3% Metaphore
Sr24	Sr24#12	94°C /3 min.; 30 cycles (94°C /30 s; 65°C /30 s; 72°C /40 s); 20°C /1 min	Dominant	2% Agarose
Sr24	Sr24#50	94°C /3 min.; 30 cycles (94°C /30 s; 57°C /30 s; 72°C /40 s); 20°C /1 min	Dominant	2% Agarose
Sr26	Sr26#43	94°C /3 min.; 30 cycles (94°C /30 s; 56°C /30 s; 72°C /40 s); 20°C /1 min	Dominant	2% Agarose
Sr26	BE518379	94°C /3 min.; 45 cycles (94°C /1 min; 60°C /1 min; 72°C /2 min); 72°C /10 min	Dominant	3% Metaphore
Sr31	SCSS30.2	95°C/5 min,; 35 cycles (95°C/1 min; 60°C/1 min, 72°C/30 s); 72°C/10 min	Dominant	2% Agarose
Sr39	Sr39-I	94°C /3 min.; 35 cycles (94°C /1 min; 60°C /1 min; 72°C /2 min); 72°C /10 min	Dominant	1.4% Agarose
Sr39	Sr39-II	94°C /3 min.; 35 cycles (94°C /1 min; 60°C /1 min; 72°C /2 min); 72°C /10 min	Dominant	1.4% Agarose

PCR product of 900 bp, indicating the absence of *Sr39* gene.

In the present study for validation of already known molecular markers resulted in the detection of the presence of *Sr22* in Kundan and *Sr2, Sr22* and *Sr31* in UP 2338 with some ambiguity due possibly to the loose linkage. However, only the presence of *Sr31* in UP 2338 can be considered as confirmation of earlier reports. Presence of *Sr22* in Kundan and *Sr2* and *Sr22* in UP 2338 is only speculative in view of their detection in the non-carriers as well. Both Kundan and UP 2338 showed negative for the remaining *Sr24, Sr26* and *Sr39* genes known markers.

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