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Characterization of stem rust resistance gene *Sr*2 in Indian wheat varieties using polymerase chain reaction (PCR) based molecular markers

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Stem rust or black rust is one of the most important diseases of wheat worldwide. In India, central, peninsular and southern hill zones are particularly prone to stem rust where favourable environmental conditions exist. The recent emergence of wheat stem rust race Ug99 (TTKSK) and related strains threatens global wheat production as Ug99 overcome resistance gene *Sr*31 effective for many years. Resistance gene *Sr2*, derived from cultivar 'Hope' is responsible for slow rusting and providing partial but durable resistance against stem rust of wheat. In addition to other unknown minor genes (*Sr*2 complex), this gene tends to be non-specific and is currently effective against all isolates of *Puccinia graminis tritici* throughout wheat-growing regions of the world. A set of 135 bread wheat varieties developed in the last forty years for prominent northern, central, peninsular and southern hill regions of India was screened with molecular markers, CsSr2 and GWM533, developed and identified for *Sr*2 gene. Out of 135 varieties screened, 92 confirmed the presence of *Sr*2 gene at molecular level. The molecular information of *Sr*2 gene was corroborated with the available morphological marker data for selected varieties to evaluate the efficacy of these molecular markers in Indian wheat germplasm. It was observed that these two molecular markers in combination provide accuracy in 92% lines for this gene at molecular level with presumed *Sr*2 status in Indian wheat varieties. It is proposed that the use of CsSr2 and GWM533 will help in gene pyramiding of *Sr*2 along with other stem rust resistance genes in future wheat varieties to accelerate Indian breeding program for rust resistance.

Key words: Wheat, stem rust, *Puccinia graminis* f.sp. *tritici* (Pgt), *Sr*2 gene, molecular marker.

INTRODUCTION

Wheat stem rust (*Puccinia graminis* f. sp. *tritici,* Pgt) is the most feared disease of wheat inflicting severe yield losses on periodic basis due to its wide distribution and tendency to form new races. The disease has been effectively controlled through the deployment of diverse sources of resistance, based primarily on major genes. In the last decade, a new race commonly known as Ug99 and designated as "TTKSK" erupted in Uganda, and was found virulent on most of the widely used stem rust resistance (*Sr*) genes (Pretorius et al., 2000). In India, central and peninsular regions are particularly prone to stem rust disease and this necessitates the deployment

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of resistance against stem rust including Ug99 in wheat breeding program to ensure durable resistance to this disease. The *Sr*2 gene is located on short arm of wheat chromosome 3B which has provided broad-spectrum protection against stem rust since its introgression from Yaroslav (*Triticum turgidum* var. *dicoccum*) to Hope genotype. This gene has been widely deployed in Indian wheat breeding program along with *Sr*9, *Sr*11 and *Sr*31 genes to ensure durable resistance against stem rust, especially in varieties released for central and southern regions of India (Prashar et al., 2008). Traditionally, Pseudo black chaff (PBC) has been used for screening *Sr*2 during rust breeding programmes but use of this morphological marker for the presence of *Sr*2 is limited due to its partial dominance and variable level of expression in different genetic and environmental backgrounds (Bhowal and Norkhede, 1981).

In recent years, molecular markers assisted pyramiding of several stem rust resistance genes into commercial cultivar resulted in the development of new varieties carrying multiple genes for stem rust resistance (Rouse et al., 2011). Simple sequence repeat (SSR) based GWM533 and CAPS based CsSr2 markers reported for *Sr*2 gene were found promising for molecular confirmation of gene and have been used in stem rust breeding programmes in Australian, US and CIMMYT, Mexico (Spielmyer et al., 2003; Mago et al., 2011). Therefore, application of these markers is essentially needed to accelerate stem rust breeding program in Indian conditions. More than 300 wheat varieties have been developed for high yield and rust resistance under wheat breeding program for six agro-climatic zones in India. These genotypes have played important role for accelerating wheat production in the last forty years and ensured food security of the Nation. In the present investigation, *Sr*2 specific molecular markers were used to assess their efficacy for assessing the deployment of *Sr*2 gene in Indian wheat cultivars of highly productive north-west plains and stem rust prone central-southern regions.

MATERIALS AND METHODS

A set of 135 bread wheat varieties developed in the last forty years and representing prominent northwest, central, peninsular and southern hill regions of India, were procured from Genetic Resource Unit, Directorate of Wheat Research, Karnal. An equal number of fresh, young leaves (ten days old) of at least five plants from each genotype were bulked for DNA extraction using modified CTAB method (Saghai-Maroof et al., 1984).

Molecular marker analysis

Molecular markers CsSr2 (CAPS) and GWM 533 (SSR) developed and identified for *Sr*2 gene were used in the present study for characterization of selected varieties. PCR reaction was conducted in reaction volume of 20 µl containing 1X PCR buffer, 200 mM dNTPs, 0.25 µM of primer, 2 mM $MgCl_2$, 1 unit Taq polymerase and 50 ng template DNA using BIORAD S1000 thermocycler for respective markers as reported in previous studies (Speilmeyer et al., 2003; Mago et al., 2011). For CsSr2 marker analysis, PCR products were restricted with 0.1 µl of BspHI (10 U/µl; NEB) in 2.5 µl NEB buffer 4 at 37°C for 60 min. Amplified PCR products were separated by electrophoresis in 2 to 3% agarose gels according to the product size (HiMedia) at 4 v/cm in 0.5 X TBE buffer. Fragment sizes were approximately calculated by interpolation from the migration distance of marker fragments of 100-bp DNA ladder (Invitrogen, USA). The occurrence of 'null' alleles was verified by re-amplification using the same primer pair in the same conditions. DNA banding patterns were visualized with UV light and recorded by imagining system (Syngene Synoptics Ltd. USA).

Wheat genotypes, Hope and Chinese Spring were used as positive and negative controls for *Sr*2 gene in the present study. Marquis genotype was used as negative control for BspH1 restriction for CsSr2 marker based *Sr*2 gene confirmation. The molecular confirmation of this gene in a given wheat variety was corroborated with the morphological marker based on presumed status of wheat varieties for *Sr*2 gene (Bhardwaj et al., 2011).

RESULTS

Two molecular markers, *Sr*2 gene specific CsSr2 (CAPS marker) and closely linked SSR marker GWM533 were used in the present study to screen 135 released Indian bread wheat varieties and corroborated with available gene matching data for precise characterization of this gene (Table 1). Diagnostic marker, CsSr2, amplified characteristic band of 337 bp (base pair) in 95 varieties and null allele in 40 varieties out of the selected 135 varieties in the present study. The amplified bands of 337 bp gave two different restriction profiles after BspHI enzymatic restriction as shown in Figure 1. In type one profile, two cleaved fragments of 112 and 225 bp were obtained from 337 bp amplified band representing restriction pattern of Marquis genotype that was used as negative control for BspHI restriction site. In the second type of restriction profile, 337 bp amplified band was cleaved in three fragments of 172, 112 and 53 bp that was obtained for Hope genotype (positive control for BspHI restriction site and *Sr*2 gene). Among the 95 varieties that amplified 337 bp bands with CsSr2, only 17 were confirmed for the presence and 78 for the absence of *Sr*2 gene at molecular level. These results suggested that CsSr2 marker alone could not be sufficient enough to characterize *Sr*2 gene at molecular level.

Another SSR marker GWM533 that was reported to be closely linked to *Sr*2 gene was used in this study for molecular screening of selected lines. GWM533 amplified band fragment of 120 bp in 88 varieties for the presence of *Sr*2 gene and the rest 47 varieties gave either 155 bp or null allele for absence of this gene (Figure 2). In total, out of 135 varieties, 92 lines were confirmed for *Sr*2 gene with two molecular markers screened in this study. Gene matching data scored for Indian wheat varieties for *Sr*2 gene was compared with molecular characterization of these varieties with CsSr2 and GWM533 for accurate confirmation of *Sr*2. Out of 135 Indian varieties,

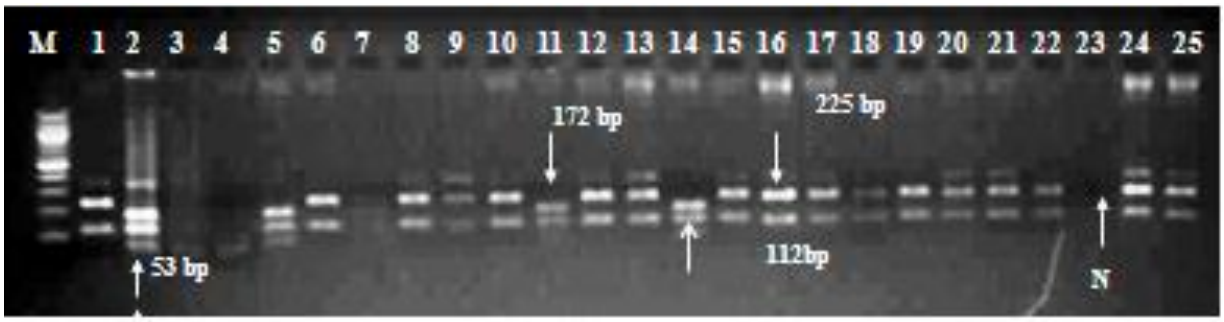

Figure 1. Amplification profiles of selected Indian wheat varieties for *Sr*2 gene with CAPS molecular marker CsSr2. M 100 bp ladder; N, Null allele. 1, Marquis; 2, Hope; 3, Chinese Spring; 4, Durgapura 65; 5, AKW1071; 6, DL788-2; 7, DL803-3; 8, GW40; 9, GW173; 10, GW273; 11, GW190; 12, GW496; 13, GW503; 14, HD2278; 15, HD2327; 16, HI385; 17, HI1077; 18, HI1418; 19, HI1454; 20, HW2004; 21, AKAW3722; 22, DWR39; 23, J24; 24, Lerma Rajo; 25, Narbada4.

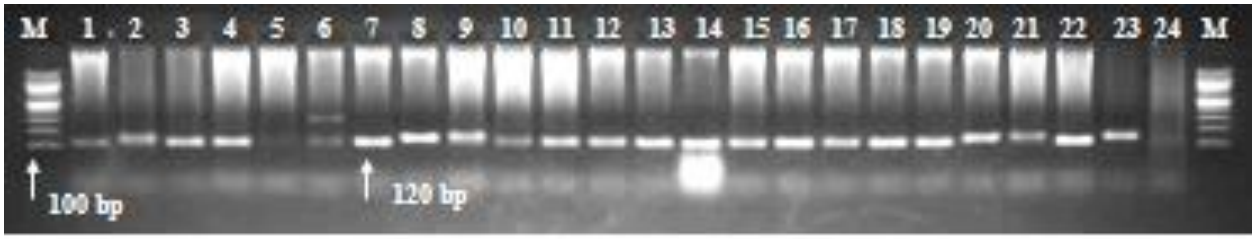

Figure 2. Amplification profiles of selected Indian wheat varieties for *Sr*2 gene with molecular marker GWM533. M, 100 bp ladder; 1, Hope; 2, Durgapura65; 3, DL803-3; 4, GW18; 5, GW10; 6, GW89; 7, GW190; 8, J24; 9, PBN51; 10, DWR16; 11, DWR162; 12, HD1925; 13, HD2189; 14, HD2501; 15, K9644; 16, NI747-9; 17, PBN142; 18, Vinata; 19, Malviya 318; 20, HI385; 21, HI1077; 22, HI784; 23, HW2004; 24, Chinese Spring.

Table 1. *Sr*2 gene analysis of Indian wheat varieties for SSR marker GWM533 and CAPS marker CsSr2 with known status of this gene based on morphological data.

Zone	Wheat line	[a]*Sr*2 resistance	GWM533	CsSr2	BspHI SNP
	C 306	NA	-	Null	
	Choti lerma	NA	+	+	+
	CPAN 1676	-	+	+	
	CPAN 3004	+	+	Null	
	DBW 16	+	+	+	
	DBW 17	-	-	Null	
	DL 153-2	-	+	+	
	DL 784-3	-	+	+	
	HD 1941	NA	Null	Null	
NWPZ	HD 1981	NA	+	Null	
	HD 2009	NA	-	Null	
	HD 2177	NA	+	+	+
	HD 2204	NA	+	Null	
	HD 2270	NA	+	+	
	HD 2285	+	+	+	
	HD 2687	+	+	+	+
	IWP 72	-	+	+	
	K 68	NA	+	+	
	Kalyan sona	-	-	Null	

Table 1. Continued.

Zone	Wheat line	[a]*Sr2* resistance	GWM533	CsSr2	BspHI SNP
	Kharchiya 65	-	+	Null	
	KRL 14	-	-	Null	
	KRL 19	-	+	+	
	KSML 3	NA	+	Null	
	Lalbahadur	NA	+	+	
	MLKS 11	NA	Null	+	
	Motiya	NA	-	+	
	NW 1076	NA	-	+	
	PBW 12	NA	-	+	
	PBW 54	NA	-	Null	
	PBW 65	-	-	Null	
	PBW 120	-	-	+	
	PBW 138	-	-	+	
	PBW 154	NA	-	+	
	PBW 175	+	+	+	
	PBW 222	-	-	+	
	PBW 226	-	Null	Null	
	PBW 299	-	Null	+	
	PBW 343	+	+	+	
	PBW 373	+	+	+	
	PBW 396	+	+	+	
	PBW 502	+	+	Null	
	PBW 509	-	+	+	
NWPZ	PBW 550	-	+	+	
	PV 18	NA	+	Null	
	Raj. 1482	NA	Null	Null	
	Raj. 1972	-	-	+	+
	Raj. 2184	+	+	+	
	Raj. 3077	NA	+	+	
	Raj. 3765	+	+	+	
	Sonak	NA	Null	Null	
	UP 301	NA	-	Null	
	UP 368	NA	Null	+	
	UP 2003	NA	Null	+	
	UP 2113	NA	-	+	
	UP 2121	NA	-	+	
	UP 2338	+	+	+	+
	UP 2382	-	-	Null	
	UP 2425	+	-	+	
	WG 357	NA	-	Null	
	WG 377	NA	+	+	
	WH 157	NA	-	+	
	WH 283	-	+	+	
	WH 291	-	+	+	
	WH 416	NA	+	+	
	WH 533	+	+	+	
	WH 542	-	+	+	+
	WH 711	+	+	+	+
	WL 410	-	+	+	
	WL 711	+	+	+	

Table 1. Continued.

Zone	Wheat line	[a]*Sr2* resistance	GWM533	CsSr2	BspHI SNP
	WL 1562	NA	+	+	
	WL 2265	NA	+	+	
NWPZ	HD 2329	-	+	+	
	HPW 251	+	+	+	
	NIAW 917	+	+	+	
	AKW 1071	NA	+	+	
	DL 788-2	+	+	+	
	DL 803-3	-	+	Null	
	Durgapura 65	NA	-	+	+
	GW 10	NA	-	+	
	GW 18	NA	+	Null	
	GW 40	NA	+	+	
	GW 89	NA	+	+	+
	GW 173	+	+	+	
	GW 190	+	+	+	+
	GW 273	+	+	+	
	GW 496	NA	+	+	
	GW 503	NA	+	+	
	HD 2236	NA	+	+	
	HD 2278	+	+	+	+
CZ	HD 2327	NA	-	+	
	HI 385	NA	-	+	
	HI 617	NA	+	Null	
	HI 784	NA	+	Null	
	HI 1077	+	-	+	
	HI 1418	NA	+	+	
	HI 1454	+	+	+	
	HW 2004	-	-	+	
	HYB 65	-	+	Null	
	HYB 633	NA	Null	+	+
	J 24	NA	-	Null	
	J 405	-	+	Null	
	Lerma Rajo	NA	+	+	
	Lok 1	+	+	+	
	MP 4010	+	+	+	+
	Narbada 4	NA	+	+	
	Narbada 112	NA	+	Null	
	Ajanta	NA	+	Null	
	AKAW 3722	NA	+	+	
	DWR 39	NA	+	+	
	DWR 16	NA	+	+	
PZ	DWR 162	+	+	Null	
	DWR 195	+	+	+	
	HD 1925	NA	+	+	
	HD 2189	+	+	Null	
	HD 2501	+	+	+	
	HD 2781	+	+	+	+
	HD 2833	-	-	+	

Table 1. Continued.

Zone	Wheat line	[a]*Sr*2 resistance	GWM533	CsSr2	BspHI SNP
	HUW 510	+	+	+	
	HW 657	NA	-	+	
	K 9644	+	+	+	
	NI 747-19	NA	+	Null	
	NI 917	NA	Null	Null	
	NI 5439	-	+	Null	
	NI 5643	NA	+	Null	
PZ	NIAW 34	-	-	+	
	NIAW 301	NA	-	Null	
	PBN 51	NA	-	+	
	PBN 142	NA	+	+	
	PBW 533	-	-	+	
	Raj. 4037	+	-	Null	
	SarbatiSonara	NA	-	+	
	Vinata	NA	+	Null	
	HD 2135	+	+	+	
	Malviya 318	+	+	+	
SHZ	HW 741	+	-	+	+
	[b]Positive control	+	+	+	+
	[c]Negative control	-	-	+	-

NWPZ, North western plain zone; CZ, Central zone; PZ, Peninsular zone; SHZ, Southern hill zone; +, presence of the gene, -, absence of the gene; NA, not available; a, *Sr*2 gene scoring on the basis of morphological marker (Bhardwaj et al., 2011); b, Positive control: Hope; c, negative control: Chinese Spring.

morphological marker information for *Sr*2 gene was available for 71 genotypes only and out of that, 38 varieties confirmed the presence of this gene. Among these 38 genotypes, presence of *Sr*2 was confirmed in 35 genotypes at molecular level except for three varieties (HI 1077, Raj. 4037 and UP 2425) with CsSr2 and GWM533 in the present study.

DISCUSSION

*Sr*2 gene is effective in the adult plant stage against all known pathotypes of stem rust including recently described Ug99 race and its variants in wheat (Singh et al., 2011). This adult plant resistance (APR) gene shows recessive inheritance and is closely associated with *Lr*27, one of the two complementary genes involved in conferring resistance to leaf rust and with pseudo-black chaff (PBC) which involves melanin pigmentation of the glumes and stem, particularly below the uppermost node (McIntosh et al., 1995). PBC is used as phenotypic marker to monitor the presence of *Sr*2 gene during wheat breeding program. Recently, various gene specific and closely linked molecular markers for stem rust resistance genes including *Sr*2 were reported (Spielmyer et al., 2003; Mago et al., 2011).

In the present study, 92 varieties amplified diagnostics bands of 120 bp for GWM533 and restricted fragments of 172, 112 and 53 bp for CsSr2 for *Sr*2 gene in a set of 135 bread wheat varieties of highly productive north-west plains and stem rust prone central-southern regions of India). Bhardwaj et al. (2011) reported gene matching of Indian wheat varieties for the presence of the *Sr*2 gene under All India Coordinated Wheat and Barley Improvement Project (AICW & BIP). This information was used in the present study to compare molecular markers based characterization of Indian wheat varieties. Of the 135 selected varieties, *Sr*2 gene matching data was available for 71 varieties and this gene was reported in 38 varieties released for north-west and central-southern regions of India. Among these 38 genotypes, presence of *Sr*2 was confirmed in 35 genotypes with molecular markers CsSr2 and GWM533. Three genotypes viz. HI 1077, Raj. 4037 and UP 2425 that were scored negative for *Sr*2 gene during molecular markers assisted screening in the present study were reportedly confirmed for this gene with morphological marker. It is possible that some lines presumed to carry *Sr*2, were wrongly classified and lack the gene due to external factors influencing host pathogen interaction during gene

matching study (Bhardwaj et al., 2011; Mago et al., 2011). Moreover, during host pathogen interaction study, scores of only 4 and 5 were found to be apparent observation for precise confirmation of *Sr*2 during seedling chlorosis test (Brown, 1997).

In this study, GWM533 was able to predict *Sr*2 gene accuracy in 89.4%, whereas CsSr2 was found compatible only in 24% lines with presumed *Sr*2 status in Indian wheat varieties. However, when these markers were used in combination, then the accuracy in 92% lines was observed for this gene at molecular level. Therefore, it was observed that these two molecular markers in combination would provide more accurate information at molecular level. Therefore, it is concluded that determining the presence of *Sr*2 gene with a combination of these two markers may necessarily deliver a comprehensive assessment for this gene at molecular level and it is suggested to enhance the accuracy of this important stem rust resistance gene pyramiding during stem rust breeding programme.

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