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Short Communication

Validation of molecular markers for stem rust resistance and identification of suitable wheat germplasm targeting Eastern Gangetic plains of India

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Abstract

Five molecular markers were used to identify stem rust resistance genes, namely*, Sr2, Sr22*, *Sr25*/*Lr19, Sr50* and *SrWeb* in eight diverse wheat lines from CIMMYT, Mexico and India that included three popular cultivars of north eastern Gangetic plains of India. One of the CIMMYT line, PMWBIR4 carried all the above mentioned five *Sr* genes, while other lines showed different combinations. The identified wheat genotypes may be useful in stem rust resistance breeding. Validation of linked markers for rust resistance suggested that the markers can be deployed in marker assisted molecular breeding program.

Key word: *Triticum aestivum*, QTL, stem rust, SSR, STS, SCAR, Eastern Gangetic Plains (EGP)

Wheat is a major staple food crop of paramount importance across continents India stands second largest producer of wheat after china (FAOSTAT 2014) with annual production of 95.91mt (Anonymous 2014) of which 80% is produced by Uttar Pradesh, Punjab, and Haryana falling in the Indo-Gangetic region. Since the Green Revolution in India, about 396 wheat varieties have been released for six wheat zones which have played a major role in enhancing productivity. The varieties that dominated during the last two decades are, PBW343 in North Western Plains Zone (NWPZ), HUW 234 in North Eastern Plains Zone (NEPZ) and Lok 1 in Central Zone (CZ) (Joshi et al. 2007). Recently, varieties like HD 2967 and HD 2733 have become popular in NWPZ and NEPZ, respectively. Of several factors affecting wheat production, the major constraints are wheat rust diseases. Among them stem rust is devastating in warmer zones like central and peninsular India (Bhardwaj 2013; Tomar et al. 2014). During the past 3-4 decades, predominant stem rust races *viz*., 21-1, 40A, 40-1, 40-2, 117A, 117-1, 117-2, 117-3, 117-4, 117-5 and 117-6 have been detected and documented, which have rendered important stem rust resistance genes (Nayar et al. 1997, 2001; Bhardwaj et al. 1994; Tomar and Menon 2001; Tomar et al. 2014). Genetic resistance is considered the most reliable way of thwarting wheat rusts because of its efficiency, cost-effectiveness and environment-friendly approach (Todorovska et al. 2009). Molecular markers have proved to be significant for detecting useful genes for specific traits at early seedling stage and introgression of desirable genes into susceptible wheat cultivars. The validation of already reported molecular markers is also required to assess their usefulness in the target breeding program. Therefore, a study was conducted to validate the known markers for stem rust resistance and to detect their presence in some of the agronomically superior wheat genotypes that can be used in ongoing wheat breeding program.

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The material used in the study includes three popular cultivars, HUW234, HUW468 and HUW510 of NEPZ, five advanced lines from CIMMYT *viz*., PMBWIR1, PMBWIR2, PMBWIR3, PMBWIR4 and PMBWIR5. The material was raised in *rabi* 2008-09 at Agricultural Research Farm, BHU, Varanasi. Plants were artificially inoculated with 21A-2 and 40A. Disease severity was recorded at adult plant stage using modified Cobb's scale (Peterson et al., 1948). Seedling test were also conducted at DWR, Regional Station, Flowerdale, Shimla with the pathotypes 40A (62G29), 40A-1 (62G29-1) and 40-2 (58G13-3) during 2009 following the protocol described by McIntosh et al. (1995) while scoring of host response was done according to the scale given by Stakman et al. (1962). The coefficient of infection (CI) was calculated with the help of host response and disease severity (Roelfs et al., 1992).

DNA was extracted from 18 days old seedlings by using modified CTAB method (Saghai-Maroof et al., 1984). The extracted DNA was stored at –20°C for further use. The molecular markers linked to the genes, *Sr2, Sr22, Sr25, Sr36, Sr50* and *SrWeb* used were selected from the published data. The sequence of primers used to amplify DNA markers for Stem rust resistance genes are given in Table 1. The PCR amplifications of DNA markers were performed in a thermal cycler (Biorad). The polymerase chain reaction was performed in 20 µL volume containing 1 µL of 100 ng µL-1 DNA template, 2 µL of 10 x PCR buffer containing 500 mM KCl and Tris-HCl (pH 8.4, MBIFermentas, Germany), 0.5 µL of 10 mMdNTP (MBI Fermentas, Germany), 10 pmol of each primer (Metabion, Germany), 0.8 µL of MgCl2 (MBIFermentas, Germany), 13.5 µL of double distilled water, and 0.2 µL (5 U µL) *Taq*polymerase enzyme (MBI Fermentas, Germany). Five molecular markers linked to each stem rust resistance gene, namely, Xstm559tgag (*Sr2*, 85bp), Xcfa2019 (*Sr22*, 234bp), XSTS*Lr19*-130(*Sr25*/*Lr19*, 130 bp), XIB267 (*Sr50*, 200-300 bp) and Xgwm47 (*SrWeb*, 207 bp) were taken for validation. Molecular data of all the linked SSR and STS markers was scored on the basis of the size of PCR amplicons/ bands in agarose gel. Allelic bands were analysed on the basis of base pair sizes and as present/absent (Table 1).

The results of validation based on the presence/ absence of band in respect of all the five stem rust resistance genes are presented in Table 1. The reactions of eight wheat genotypes against the three pathotypes of stem rust pathogen (40A, 40-1 and 40-2) are given in Table 2. A potentially useful marker stm559tgag was validated by PCR reaction producing a single 85bp fragment in all the genotypes except HUW510 indicating the presence of *Sr2*. This gene is very useful because of its linkage with leaf rust (*Lr27*) and stripe rust resistance (*Yr30*) genes. *Sr2* can be easily identified with black chaff (*Pbc*), a seedling marker linked to the gene (Hayden et al. 2004). The resistance exhibited by *Sr2* makes it a key gene in providing durable resistance in the CIMMYT wheat breeding program (Rajaram et al. 1988) and is recognized as an excellent genetic background for resistance breeding (McIntosh 1988). It is widely used gene and also present in many Indian cultivars and imparting resistance to stem rust.

Table 1. The presence or absence of five stem rust resistance genes based on molecular markers in wheat genotypes

Genotypes/ lines	*Sr*2 (stm559 tgag)	*Sr*22 (cfa 2019)	*Sr*25 (STS Lr19-130)	*Sr*50 (IB267)	*SrWeb* (Xgwm 47)
HUW234	+	+	-	-	-
HUW468	+	+	-	-	-
HUW510	-	+	+	-	-
PMBWIR1	+	+	-	-	-
PMBWIR2	+	+	-	-	-
PMBWIR3	+	+	-	-	-
PMBWIR4	+	+	+	+	+
PMBWIR5	+	+	+	-	-

The gene *Sr*22 is present in all the genotypes as the marker Xcfa2019 was consistently amplified 234bp PCR product. These markers have been validated earlier by Nagaraja et al. (2013) in some Indian genotypes carrying *Sr2* and *Sr22*. For the linked genes *Sr25*/*Lr19*, STS dominant marker STS*Lr19*-130 amplified a single polymorphic band of 130bp in HUW510, PMBWIR4 and PMBWIR5. Bhawar et al. (2011) using the same marker (STS*Lr19*-130) confirmed the presence of *Sr25* in a recombinant HD2687 carrying *Lr19* and *Lr28* genes for leaf rust resistance. Detection of a stem rust virulence against linked gene *Sr25* raised concerns about its usefulness but the virulence has remained confined to a very small area (Tomar et al. 2014). PMBWIR4 is the only genotype carried *Sr50*, validated by IB267 200-300bp

Table 2. Reactions of genotypes to the pathotypes of stem rust pathogen (*Puccinia graminis tritici*)

Genotypes	Pathotypes used and reaction recorded*				Observation
	40A	40-1	40-2	CI	
HUW234	3+	33+	0;	64	*Sr*24, *Sr*25 absent
HUW468	3+	-	;	56	*Sr*24, *Sr*25 absent
HUW510	2	2	-	8	-
PMBWIR 1	3+	3	3	56	*Sr*24, *Sr*25 absent
PMBWIR 2	3+	2	3-	48	*Sr*24, *Sr*25 absent
PMBWIR 3	3+	2p31p2	3	48	*Sr*24, *Sr*25 absent
PMBWIR 4	2	-	0;	2	-
PMBWIR 5	2	2	2	4	-

*62G29(40A) = Most virulent and predominant, avirulent on *Sr*24, *Sr*25; 62G29-1(40-1) = Virulent on *Sr*24, avirulent on *Sr*25; 58G13-3(40-2) = Virulent on *Sr*25, avirulent on *Sr*24; 0;,;,1,2 = Resistant, 2+ = Moderately Resistant, 3 = Moderately susceptible, 33+ & 3+ = Susceptible

amplification(Fig. 1) and *SrWeb*, validated by Xgwm47 amplifying 207bp band (Fig. 2), in addition to *Sr2*, *Sr22* and *Sr25* thus making it a pyramid of five genes displaying multiple disease resistance. The stem rust resistance gene *SrWeb* is located on the chromosome 2BL but has not been exploited at large. Similarly the gene *Sr50*, previously known as *SrR*, is not in much use except being utilized in CIMMYT wheat breeding programme. Stem rust (*Puccinia graminis tritici*) is a

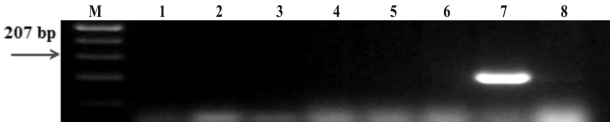

Fig. 1. Validation of stem rust resistance gene *Sr50* by using IB267(200-300bp) M = Ladder 100bp, 1 = HUW 234, 2 = HUW 468, 3 = HUW 510, 4 = PMBWIR1, 5 = PMBWIR2, 6 = PMBWIR3, 7 = PMBWIR4, 8 = PMBWIR5

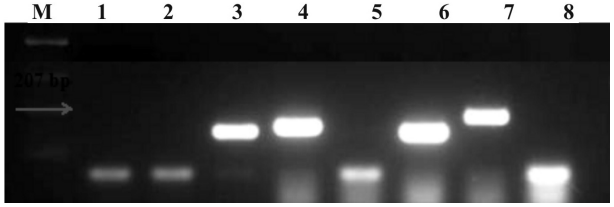

Fig. 2. Picture showing the validation of stem rust resistance gene *SrWeb* with the SSR marker Xwms47/Xgwm47 (207bp). M = Ladder 100bp, 1 = HUW234, 2 = HUW468, 3 = HUW510, 4 = PMBWIR1, 5 = PMBWIR2, 6 = PMBWIR3, 7 = PMBWIR4, 8 = PMBWIR5

major problem worldwide including some zones in central and peninsular India causing yield loss in wheat production if appear in epidemic proportions but has been managed successfully in India through successful deployment of resistance genes in wheat cultivars during the last 2-3 decades (Tomar et al. 2014).

Combination of race specific and non-race specific genes provide great level of resistance because it is more difficult for the pathogen to overcome. The echelon of resistance has been enhanced in Indian wheat cultivars by introgressing multiple rust resistance genes *Sr25* and *Sr26* using MAS (Mallick et al. 2015). Hence, the sources of resistance and validated markers obtained in this study can be used in the ongoing breeding program for stacking of multiple genes. Molecular markers closely linked with gene(s) can facilitate reliable selection of lines carrying one or multiple resistance genes (Bhawar et al. 2011; Sivasamy et al. 2012; Suresh and Malathi 2013). The results of seedling test of the studied genotypes are presented in Table 2. The pattern of seedling reaction in HUW510, PMBWIR4 and PMBWIR5 against 40A and 40-1 indicated that these genotypes do carry the gene *Sr25*, while remaining five genotypes HUW234, HUW468, PMBWIR1, PMBWIR2 and PMBWIR3 do not. Since the gene *Sr24* is ineffective only against 40-1, there is no possibility of this gene being present in these genotypes as these are ineffective against pathotype 40A. Disease scoring in all the genotypes for stem rust resistance in the field was also done following Modified cobb's method (Peterson *et al*., 1948). The coefficient of infection (CI) recorded in all the genotypes (Table 2) revealed low CI only in HUW510, PMBWIR4 and PMBWIR5. The combination of conventional screening methodology along with the use of molecular markers can facilitate breeding and reliable selection of lines carrying multiple resistance genes.

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