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MARKER ASSISTED DETECTION OF UNDERUTILIZED POTENTIAL *Yr* GENES IN ELITE WHEAT BREEDING LINES

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SUMMARY

In order to assess the resistance levels and presence of less exploited *Yr* genes for genetic improvement of wheat stripe rust resistance in Indian subcontinent, 15 parents (12 lines, 3 testers) along with their 36 F_{1s} were evaluated for adult plant resistance in screening nurseries by infecting with predominant stripe rust races *46 S 119*, *78 S 84* and also screened with SSR markers tightly linked to currently effective resistance genes *Yr5*, *Yr10, Yr15*. Out of 51 wheat genotypes, 38 showed adult plant resistance, 11 classified as intermediate while 2 identified as susceptible based on ACI scores. On molecular screening, PBW 639 amplified marker *Xwnc175* linked to *Yr5* whereas HD 3065, HPW 211 and WH 1100 showed the presence of *Yr10* using *Xpsp3000*. All these results suggested that *Yr5* and *Yr10* are present among elite wheat genotypes which also showed significant field resistance. It will further cater the immediate need of resistance donors with superior genetic background, and could be utilized in wheat stripe rust resistance breeding in South East Asian countries.

Key words: Wheat, yellow rust, SSR, race, resistance, screening

Key findings: In the current scenario, the genes *Yr5* and *Yr10* may play a crucial role in the yellow rust breeding programme as they have shown field resistance and are effective against the prevalent races of yellow rust pathogen. Hence, detection of these genes in can be very useful in wheat improvement programmes.

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INTRODUCTION

Wheat is one of the world's most significant food crops which ranks first among cereals in production and serves as the staple food of about 36% of the entire world population. It is also the prime cereal crop of India ranks second among key cereal crops. The proposed target of 100 million metric tons by 2030 has to be met to feed the ever burgeoning population with an increase in production at the rate greater than 1 million metric tons per annum (Sharma *et al.*, 2011). However, wheat production is constrained by a number of diseases. Yellow (stripe) rust is one of the most predominant diseases of wheat in both North West Plain Zone (NWPZ) and Northern Hills Zone (NHZ), caused by *Puccinia striiformis* f.sp. *tritici* is one of the major foliar diseases of wheat worldwide, which severely damages wheat production every year, causing yield losses from 10 to 70% (Chen, 2005). As a result of this major wheat growing area show productivity level of around 1.75 t/ha, which is rather low as compared to the national average of 3.118 t/ha (Gupta and Kant, 2012). These zones are selectively prone to yellow rust as the pathogen been reported to be prevalent at

higher altitudes and cool and temperate regions where wheat is grown (Boyd, 2005). Being airborne, local races can migrate to other areas and quickly become regionally and often globally predominant. However, virulence for certain genes or gene combinations may still be absent regionally which provides scope for their subsequent utilization in the development of resistant cultivars which is the most competent, cost-effective and ecological friendly safeguard measures against the destructive pathogen in the context of food security (Chakravarty, 2011).

Identification and deployment of race-specific resistance genes ensure effective protection against the disease (Shah *et al*., 2010) by screening the genotypes with linked gene specific molecular marker. It is more effective and rapid method to postulate the status of a gene in resistant genotypes which can be used authentically for gene pyramiding against serious diseases such as rusts quickly (Kesawat and Das, 2009). SSR or microsatellites are useful tools for molecular genetic analysis (Miah *et al*. 2013), as they are abundant and display high levels of polymorphisms in many plant species, including hexaploid wheat and are more informative than any other marker system. High-density wheat SSR genetic maps have been constructed (Wu *et al.,* 2015a) which make tagging yellow rust resistance genes in wheat cultivars possible. SSR markers have been identified for *Yr5***,** *Yr10* and *Yr15* (Murphy *et al.,* 2009). Finding new germplasm with new resistance genes and pyramiding different resistance genes to breed multiline cultivars may increase the durability of resistance (Wen *et al.* 2008). Therefore, this study investigates the extent of genetic variability for yellow rust resistance through field and molecular screening in the available genotypes and to select parents as donors of *Yr* genes in further resistance breeding programme.

MATERIALS AND METHODS

Plant material and experimental site

The experimental material for this investigation comprised 36 F_{1s} along with 15 parents (12 lines and 3 testers) (Table 1). All 51 genotypes were planted in stripe rust screening nursery in 3 replications consisting of 2 rows (1 m long) of each entry with 10 seeds per row with inter and intra row spacing of 23 cm. and 10 cm., in a randomized block design (RBD) at plant pathology block of the Norman E. Borlaug Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India, situated at latitude 28.9700°N, longitude 79.4100°E, during November, 2012-13.

Table 1. List of wheat genotypes and their pedigree used in the study.

No.	Line	Pedigree
1.	DBW 71	PRINIA/UP2425
2.	DBW 74	RWP2008-26/WBLLL*2/BRAMBLING
3.	HD 3059	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES
4.	HD 3065	PBW65/2*PASTOR
5.	HPW 211	MO88/MILAN
6.	KO 307	K 8321/UP 2003
7.	PBW 639	HW2019/PBW49
8.	PBW 644	PBW175/HD2643
9.	PBW 658	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI
10.	Raj 4237	PBW226/RAJ1972
11	UP 2596	CPAN 3004 M
12.	WH 1100	PBW65/2*PASTOR
	Tester	
13.	DPW 621-50	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HU ITES
14	FLW 21	UP2338/CENTURK//UP2338/YR15
15.	PBW 550	WH 594/RAJ 3858/W 485

Field evaluation for stripe rust resistance

Each entry of the nursery was bordered with susceptible to stripe rust spreader 'Agra local'. Artificial epiphytotic conditions were created in the block by inoculating the nursery material, from tillering to the stage of appearance of disease, in late afternoons with a uniform spray of spore suspension containing a mixture of urediospores of different stripe rust (*P. striiformis*) pathotype prevalent in Indian NWPZ since 2006 onwards (*46 S 119, 78 S 84*) (DWR, Flowerdale) through an automizer. The inoculum used in the this study has the virulence against most of *Yr* genes present in Indian cultivars and avirulence for *Yr5, Yr10, Yr15, Yr (CD), Yr(Mega), Yr(Hobbit), Yr(SP)* and *Yr (China-84*).

Source Inoculum was obtained from the Regional Station, Directorate of Wheat Research, Flowerdale, Shimla, India. Spore inoculum was produced by dissolving urediniospores at a rate of 1 g/litre with an approximate concentration of 10,000 spores/ml in the suspension. The higher concentration of spores was used in order to create maximum artificial disease pressure under field conditions and spraying with plain water in the late afternoon on each second day for a fortnight was done in order to make conditions conducive for spore multiplication and disease development. After successful disease development, rust severity (percentage of leaf area with symptoms) was determined by phenotypic observation and recorded from 0 to 100% of rust infection on 5 selected plants within each genotype according to the modified Cobb scale (Peterson *et al.*, 1948) which relies on visual observations for rust severity and it is common to use the following intervals: Trace, 5, 10, 20, 40, 60, 100% infection. The term trace (T) was used below 5% severity. Three consecutive reading for disease incidence on all selected plants was recorded after 7 days interval gap. Observations on field response (response value) of individual plants within each population to the type of stripe rust infection were recorded according to Loegering scale (Khan *et al.*, 2011) (Table 2).

Table 2. Disease reaction and their associated response value as adopted by Loegering (1959).

Disease Reaction	Observation	Response value
No disease	O	0.0
Resistant	R	0.2
Moderately Resistant	MR	0.4
Moderately Resistant to Moderately Susceptible	MR-MS	0.6
Moderately Susceptible	MS	0.8
Susceptible	S	1.0

Where, O: No visible infection; Tr: trace severity of resistant type infection; R: resistant (necrotic areas without or with minute uredia); MR: moderately resistant (small uredia present surrounded by necrotic areas); MS: moderately susceptible (medium uredia with no necrosis but possibly some distinct chlorosis); S: susceptible (large uredia and little or no chlorosis present). Severity and field response readings are usually combined. For example, Tr: Trace severity with a resistant field response; 5MR: 5% severity with a moderately resistant field response; 60S: 60% severity with a susceptible field response.

Severity and reaction were recorded together with severity first. The coefficient of infection (CI) for the rust was calculated in the manner used in CIMMYT and IRN (USDA) (Irfaq *et al.*, 2009).

C.I. = Severity of infection × Response value

The average coefficient of infection (ACI) was derived from the sum of CI values of each entry divided by the number of replications as per Loegering scale. Categorization of genotypes based on ACI values was done in following manner: ACI value 0.1-10 = Resistant; 10.1-30 = Intermediate; 30.1 and above = Susceptible.

Plant genomic DNA extraction, use of molecular markers and genotyping

The genomic DNA from each genotype was isolated from young leaves of 20 days old seedlings grown in the field. DNA was extracted from genotypes using CTAB (Cetyl trimethyl ammonium bromide) method and quantified using UV- Vis spectrophotometer at

260 nm. Amplification was done under proper PCR conditions using *Yr* linked SSR primers *Xwmc175* and *Xgwm501* (*Yr5*), *Xpsp3000* (*Yr10*), *Xgwm413* and *Xgwm11* (*Yr15*). Amplifications were performed in a 25 µl reaction mixture containing 2.5 µl Taq buffer (1X) containing [10mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5mM $MgCl_2$], 0.8 mM of dNTPs, 0.04 µM of each forward and reverse primers, 100 ng genomic DNA and 3 units/µl Taq DNA polymerase. Amplified products thus obtained were separated on 2.5% agarose gel for SSR marker using horizontal gel electrophoresis assembly.

The experimental material was screened for presence or absence of *Yr* gene using SSR markers which are linked with *Yr5*, *Yr10* and *Yr15* (Somers *et al.*, 2004). Details of genes and associated primers have been enlisted in Table 3. The amplified products were scored separately for each primer. The PCR products for marker analysis were scored qualitatively in each lane for presence or absence. Only clear and apparently unambiguous bands were scored for each of the primers separately. Further the molecular analysis was correlated with field screening and accordingly conclusions were drawn for each primer-genotype combination.

Table 3. Detail of *Yr* genes and associated primers used in this study.

No.	Gene	Chromosomal position	Source genotypes	Type of resistance conferred	Linked marker	Primer name	Dir.	Primer sequence (5'- 3')	Possible product size	References
1	*Yr5*	2BL	*Triticum aestivum* subsp. *spelta* 'Album'	RS, AS	*Xwmc175*	*WMC 175F*	F	GATAAAATCATTATTGGGTGTCCTTT	251, 257, 253, 277	Somers *et al.* (2004), Mcintosh *et al.* (2008), McGrann *et al.* (2014)
						WMC 175R	R	TTCAAATAATCTTTCATCAGTCAAATG		
					Xgwm501	*WMS 50 F*	F	ACTTACATGAATTATCTTTCTTGGTCC	176	
						WMS 501R	R	CGTATTCAAATAATCTTTCATCAGTCA		
2	*Yr10*	1BS	'Moro'	RS, AS	*Xpsp 3000*	*PSP 3000F*	F	TGTTTTGGAGAAGAGTGATTC	240, 260	Bariana, *et al.* (2002)
						PSP 3000R	R	TGTGCATGCAAATTCTTACT		
3	*Yr15*	1BS	*Triticum turgidum* var. *dicoccoides* G-25	RS, AS	*Xgwm413*	*WMS F413*	F	TTTTTGGCTTATTAGACTGACTT	91, 95, 88, 90	Somers *et al.* (2004), (Mcintosh *et al.*, 2008) (Cheng *et al.*,2014)
						WMS R413	R	TTGCCATAAAATACAAAATCC		
					Xgwm11	*WMS 11F*	F	AAAAGGAACCTCAAGTGACA	213, 202	
						WMS 11R	R	GAAAATGAGGGAGTGAGATG		

Table 4. Reaction of different genotypes to yellow (stripe) rust.

	Testers	DPW 621-50	FLW 21	PBW 550	
Lines	ACI values	8.33 (R)	25 (I)	31.67 (S)	
DBW 71		10 (R)	5 (R)	9 (R)	33.33 (S)
DBW 74		18.67 (I)	2.07 (R)	9.67 (R)	11 (I)
HD 3059		1 (R)	1 (R)	5.33 (R)	32.67 (S)
HD 3065‡		8.67 (R)	6.67 (R)‡	6 (R)‡	8.67 (R)‡
HPW 211‡		10 (R)	5 (R)‡	8 (R)‡	9.33 (R)‡
KO 307		20.67 (I)	1 (R)	23.33 (I)	26.67 (I)
PBW 639†		5.33 (R)	1 (R)†	4.33 (R)†	10 (R)†
PBW 644		9.33 (R)	1 (R)	1 (R)	11.67 (I)
PBW 658		1 (R)	4.67 (R)	1 (R)	8.33 (R)
Raj 4237		11.67 (I)	7.33 (R)	8.67 (R)	12.67 (I)
UP 2596		9 (R)	1 (R)	5 (R)	15 (I)
WH 1100‡		9.67 (R)	1 (R)‡	7 (R)‡	8 (R)‡

L X T CROSSES

† Presence of *Yr5* and ‡ *Yr10* associated allele in parents and crosses. Categorization of genotypes based on ACI values (in parenthesis); 0.1-10 = Resistant (R); 10.1-30 = Intermediate (I); 30.1 and above = Susceptible (S).

RESULTS

The resistance performance of wheat lines to Indian NWPZ predominant stripe rust races

Data about the disease reaction showed a range of infection type within wheat varieties. Out of 15 parents tested, 10 parents (9 lines viz. DBW 71, HD 3059, HD 3065, HPW 211, PBW 639, PBW 644, PBW 658, UP 2596, WH 1100 and one tester DPW 621-50) showed resistant (R) reaction except lines DBW 74, KO 307, Raj 4237 and tester FLW 21 which were categorized as intermediate (I). However one line, PBW 550 showed susceptible (S) reaction to yellow (stripe) rust. Among the 36 crosses, 28 crosses exhibited resistant reaction to yellow rust, 6 were intermediate whereas 2 were found susceptible (Table 4).

Molecular screening of stripe rust resistance in wheat lines

PCR amplification of *Xwmc175* (linked with *Yr5)* and *Xpsp3000* (linked with *Yr10)* showed polymorphic bands on agarose gel. No amplified bands were observed with primers linked with *Yr15* gene.

Molecular screening of genotypes for *Yr5*

Marker *Xwmc175* was found to be polymorphic and exhibited amplified product of 277 bp in positive control Avocet/*Yr5*. Among 15 parents, only line PBW 639 showed a band of 277 bp using *Xwmc175* primer (Figure 1) and no positive testers were found. The F_{1s} of this positive line with all 3 negative testers were also screened to check if the gene was inherited in the next generation. All the F_{1s} viz. PBW 639 x DPW 621-50, PBW 639 x FLW 21 and PBW 639 x PBW 550 showed 277 base pair band associated with the presence of the gene which was earlier detected in parent PBW 639 and band inherited from tester thus showing codominant nature of marker and served as true hybridity test of F_{1s}. (Figure 2). The perusal of the Table 4 revealed that PBW 639 showed resistant response under field conditions. All the 3 crosses involving the line PBW 639 also found to be resistant. Upon correlating with the field and lab results it can be suggested that resistance for yellow rust in PBW 639 may be due to *Yr5* gene.

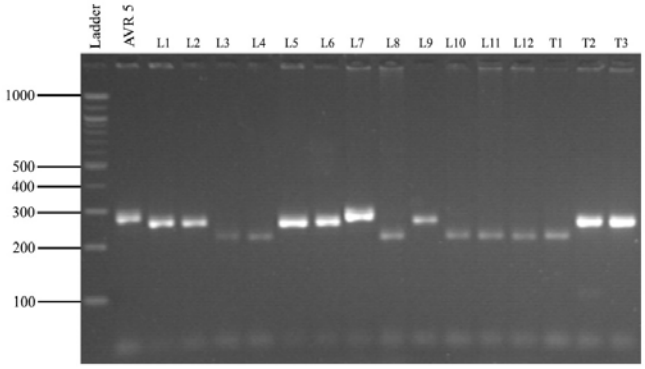

Figure 1. Amplification profile of *Xwmc 175* marker for fifteen parental wheat genotypes. First lane AVR5 (Avocet/*Yr5*) shows amplified band associated with *Yr5*. L1 to L12 lanes represent lines and T1 to T3 lanes represent testers. PBW 639 (L7) shows presence of *Yr5*.

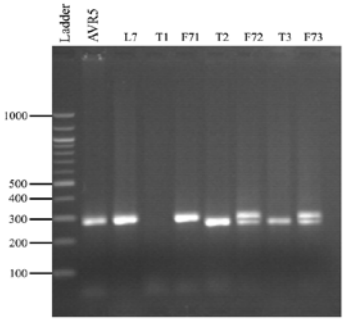

Figure 2. Band pattern of *Xwmc 175* marker for PBW 639, testers and their F_{1s}.

Yr5, a yellow rust race-specific R-gene effective at both seedling and adult plant growth stages, was described first in 1966 by Macer in *Triticum spelta album* (Macer, 1966). This gene confers resistance to most of the races known. *Yr5* is located on chromosome arm 2BL, 21 cM away from the centromere. The line Avocet/*Yr5* (*Yr5*/6*AVS) was included in the study as it is established donor of *Yr5*. This donor line was developed at the Plant Breeding Institute, Sydney, Australia by backcrossing the *Yr5* gene donor, *Triticum spelta album*, with the recurrent susceptible spring wheat genotype Avocet. Based on epidemiological studies, *Yr5* is effective against all rust virulent races in North America and Iran. This gene is known to show high levels of resistance to stripe rust in China and Turkey. Also, in surveys of resistance genes in the Caucasian region and middle Asia and Pakistan, *Yr5* and *Yr15* were identified to be effective against all *Pst* races. The fact that *Yr5* is effective in Iran and its surrounding countries makes it a good candidate for wheat breeding programmes. *Yr5* has not been extensively used in wheat breeding programmes and consequently is still potentially effective against *Puccinia striiformis* on many continents (Wellings *et al.*, 2012).

Molecular screening of genotypes for *Yr10*

The *Xpsp3000* primer pair for *Yr10* was found to be polymorphic and produced a fragment of 260 bps in the positive control Avocet/*Yr10*. The result indicated that a band of 260 was amplified in 4 parents viz. HD 3065, HPW 211 and WH 1100 and absent in all 3 testers (Figure 3). Upon screening crosses of these 4 lines with negative testers using the same primer resulted in same 260 bps band in all the F1 crosses showing codominance of marker and true hybrid combination (Figure 4). These findings were similar to those reported by Bariana *et al.* (2002) who indicated varieties with *Yr10* amplify a 258-260 bps fragment and those lacking this gene amplify 240 bp band. Field data of disease reactions in these parents and their F_{1s} indicate resistant reaction (ACI < 10%) which indicate presence of *Yr10.*

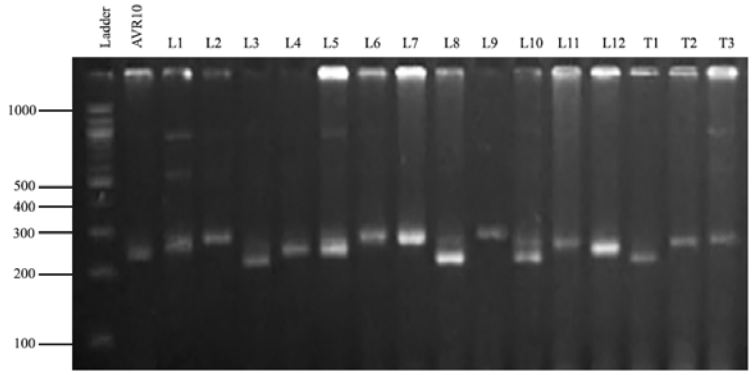

Figure 3. Amplification profile of *Xpsp 3000* marker among fifteen parents of bread wheat. First lane AVR10 (Avocet/*Yr10*) shows amplified band associated with *Yr10*. HD 3065 (L4), HPW 211 (L5) and WH 1100 (L12) shows presence of *Yr10.*

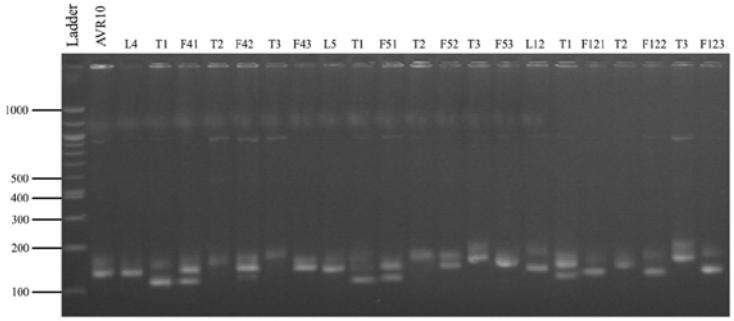

Figure 4. Band pattern of *Xpsp 3000* marker for HD 3065, HPW 211, WH 1100, testers and their F_{1s}.

Dominant gene *Yr10* is a seedling resistance gene for yellow rust have been identified in the wheat cultivar Moro in PI178383 line (donor of the brown glumed *Yr10* source) (Chen and Line, 1992) and has been assigned to chromosome 1B in telomeric region of short arm closely linked to Gliadin gene (*Gli-B1)* (Payne *et al.,* 1986). Its linkage with genes responsible for morphologic traits e.g. glum brown colour (*Rg1*) can be used to identify it at the mature stage but expression at the final stage of plant growth makes it inappropriate for early selection of resistance to yellow rust. A Close association between *Xpsp3000* marker and *Gli-B1* has also been reported. *Gli-B1* is one of the wheat storage protein genes express in endosperm and improves plant resistance to abiotic stresses. A close association between *Yr10* and *Gli-B1* by genetic analysis of the cultivar Moro has been found. The unique genetic associations of *Yr10vav* and *Yr10* with specific alleles of *Gli-B1* and *Xpsp3000* will be useful in marker-assisted selection and gene pyramiding. Certain Australian wheat, however, possessed the *Yr10* linked *Xpsp3000* allele but not *Yr10*, indicating a necessity to conduct disease response tests and/or *Gli-B1* assays to confirm the presence of *Yr10* (Bariana *et al.,* 2002). *Yr10* has been reported to be effective against all races in China, Iran, Pakistan, USA and India (Chatrath *et al.*, 2007).

Out of 10 parental lines showing field resistance, 5 lines DBW 71, HD 3059, PBW 644, PBW 658 and UP 2596 showed a resistant response to yellow rust races but molecular studies indicates these genotypes do not carry *Yr5*, *Yr10* and *Yr15*. For genotypes, having positive reaction in the field for rust resistance but not showing likely presence of *Yr5* or *Yr10*, it can be generalized that they must be having other effective *Yr* genes which could not be detected with the linked markers used in the study or lack the corresponding band which could be as a result of recombination (Robert *et al.,* 2000). However, close genetic distances have been reported to exist between reported marker gene combinations. Though the chance of recombination is very low but could not be neglected. Yet, another effective gene could be responsible for resistance in these genotypes.

Marker validation depends on effective marker/trait linkage. Various approaches for marker validation in genetic association are suggested by Konig (2010) which emphasized strength and consistency of results as major condition for association. To validate the presence of *Yr5* and *Yr10*, an independent F_2 population can be developed from cross between positive parents (PBW639, HD3065, HPW211 and WH1100) and a highly susceptible line which can be tested for phenotypic segregation in field and genotypic segregation on gel analysis by proposed markers for respective genes. Similar validation works has been conducted by Cao *et al.* (2012) to validate presence of *Yrq1* using Pinchun16 (highly susceptible) and RIL290 (carry *Yrq1*) as parents. Bernardo *et al.* (2013) assessed usefulness of markers associated with Ug99 effective genes in MAS using 10 donor lines for resistance. Validation of SSR markers linked with *YrC591* using wheat line C591 (carrying *YrC591*) as parent was done by Xu *et al.* (2014) and found them effective for MAS.

DISCUSSION

As a result of the continued evolution of rust pathogen against known resistant sources, several yellow rust resistance genes have been recognised in wheat since 1966 (*Yr1*) till now (*Yr53*) which sum up to a total of 70 catalogued genes. Deployment of many such resistance genes results in a subsequent appearance of virulence in the pathogen population which called for effective exploitation and utilization *Yr*, such as pyramiding different resistant genes. It would be realized only after identifying genetic stocks containing unutilized potential resistant gene. In the current scenario the genes *Yr5* and *Yr10* are of crucial importance in yellow rust breeding programme as they are effective against the prevalent races of yellow rust pathogen in NWPZ.

All the wheat genotypes found positive for *Yr5* and *Yr10* through molecular marker assisted screening showed significant lower ACI values**.** MAS in F_{1s} of cross combination involving one positive parent for *Yr5* or *Yr10*, give the expected results. Therefore these markers should be useful in early generation MAS in yellow rust breeding programme since alleles associated with presence of *Yr5* and *Yr10*, when amplified always confers field resistance to wheat genotypes. Hence detection of these genes in

PBW639, HD3065, HPW211 and WH1100 can be very useful in wheat improvement programmes which have the good genetic background for the agronomic traits. As the seedling and race specific genes *Yr5* and *Yr10* are effective against *Pts* pathotypes, their combination is expected to extend the useful life of resistance. Thus, they can be crossed to generate genetically diverse populations in which effective selection for high resistance and yield can be accomplished. However, it would be also a great advantage to transfer *Yr5* and *Yr10* to promising lines.

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