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Molecular characterization of wheat germplasm for stripe rust resistance genes (*Yr5*, *Yr10*, *Yr15* & *Yr18*) and identification of candidate lines for stripe rust breeding in Kashmir

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici (Pst)*, is an important disease of wheat in Jammu and Kashmir state of India. The leading cultivars and breeding lines of wheat in the state were evaluated for stripe rust resistance at the experimental fields of the Faculty of Agriculture, Wadura. The study on molecular characterization and identification of candidate lines for stripe rust resistance genes *Yr5*, *Yr10*, *Yr15* and *Yr18* depicted that gene *Yr5* was found in 14 entries, *Yr10* in 29 entries, *Yr15* in 25 entries and *Yr18* in 9 entries including reference lines, while the susceptible control did not amplify any of the resistance genes used in the study. Single gene based resistance was detected in 9 entries, two gene based resistance was detected in 20 cultivars, three gene based resistance was detected in 8 cultivars and all four gene based resistance was detected in 1 cultivar (HPW-42). Such validation information is valuable and can efficiently be used in devising future breeding strategies in building a long lasting defense against stripe rust fungus.

Keywords: Molecular breeding, molecular markers, wheat germplasm, yellow rust resistance

Introduction

The diseases of wheat, particularly the fungal diseases, are important yield constraints in almost all wheat-growing environments[1]. Among the three rust diseases, stripe or yellow rust of wheat caused by *Puccinia striiformis* is a devastating foliar disease and is considered of immense importance in almost all the wheat growing parts of the world[2,3]. Year after year, the susceptible wheat cultivars suffer from stripe rust (*P. striiformis* Westend f. sp. *tritici*) disease, which increases innoculum build up and poses major threat to wheat cultivation in India. Though remarkable progress has been made in breeding for stripe rust resistant varieties in India but the subsequent evolution of pathogenic races at much greater pace has challenged the breeding programmes[4]. And stripe rust continues to pose a threat to wheat cultivation worldwide[5]. The timely application of fungicides against this obligate parasite can provide some control but their use adds to the production costs. Moreover, the use of fungicides is considered unfriendly to the environment. Thus breeding for resistance is the most effective and efficient control strategy, as it does not add up to the input cost of farmers and is environmentally safe[6]. The identification and knowledge of the stripe rust resistance genes in commonly used parental germplasm and released cultivars is very important for utilizing them to control the rust in full potential. The long term and economical strategy could thus be resistance breeding through deployment of effective rust resistance genes over space and time. Under such circumstances, it has become imperative to characterize the set of germplasm lines for identification of stripe rust resistance genes, *viz.*, *Yr5*, *Yr10*, *Yr15* and *Yr18*.

Various programmes aimed at developing resistant cultivars require diverse, well-characterized and effective resistance genes. Till now, nearly 61 major genes conferring resistance to stripe rust (*Yr1* to *Yr61*) have been identified and designated[7]. The genes expressing at adult plant stage have special significance because the cultivars having such genes

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have shown resistance that has remained effective for longer durations[8,9]. So far, many genes or gene complexes conferring linked or unlinked durable stripe rust resistance have been identified and designated, *viz.*, *Yr18-Lr34*[10-12], *Yr29-Lr46*[13-14] and *Yr36-GpcB1*[15]. In South Africa and Mexico *Yr18* can be detected in wheat (*Triticum aestivum* L.) seedlings at low temperatures, but resistance is most effective in adult plants in the field[16]. The molecular tagging of gene *Yr5* by Chen *et al*[17] (*STS-7/STS-8*); gene *Yr10* by Wang *et al*[18] (*Xpsp3000*); gene *Yr15* by Murphy *et al*[19] (*Xbarc8*) and genes *Yr18-Lr34* by Suenaga *et al*[12] (*Xgwm295)*, Spielmeyer *et al*[20] (*Xgwm1220*), Bossolini *et al*[21] (*SWM10*) and by Laugdah *et al*[22] (*cssfr1*) has consequently facilitated detection of these genes in segregating populations and germplasm collections worldwide.

The outbreak of stripe rust in temperate areas of Jammu and Kashmir state is a matter of great concern owing to the fact that rust innoculum generated in these areas may act as a reservoir of innoculum for rust initiation in North-West plain zone of the country, the food bowl of the country. Therefore, there is urgent need to curtail this rust in Kashmir and Ladakh regions of Jammu and Kashmir State on priority basis so that the same may not spread to North-West plain zone and thus the threat to the National Food Security Mission (NFSM) be prevented. Thus, it is needed to characterize the wheat germplasm for stripe rust resistant genes, *viz*., *Yr5*, *Yr10*, *Yr15* and *Yr18*, and therefore these genes be validated in the available wheat germplasms. Molecular markers for these resistance genes have been intensively used in wheat breeding programmes both in India and worldwide. Genes *Yr5*, *Yr10, Yr15* and *Yr18* are still effective against the most virulent Indian pathotype 78S84[23] and are used to determine their presence in wheat cultivars and breeding lines in combination with their phenotype data of the polyhouse and field tests. The pathotype 78S84 was initially detected in 2001 and it remained in low frequency. However, favourable climatic conditions helped its build up over a very large area in 2008. The pathotype 78S84 was found virulent on genes *Yr9* and *Yr27* and its avirulence-virulence formula was found to be *Yr1*, *4, 5, 10, 11, 12, 13, 14, 15, 16, 18/2, 3, 6, 7, 8, 9, 27.* With this background information, the present study was undertaken to screen the wheat germplasm lines for identifying stripe rust resistance based on genes *Yr5*, *Yr10*, *Yr15* and *Yr18.*

Materials and Methods

The materials consisted of 39 wheat genotypes/germplasm lines (Table 1) collected from different sources. They were planted in randomized complete block design with two replicates for screening in both field and greenhouse conditions for stripe rust resistance at Regional Research Station, SKUAST-K, Wadura. Analysis of the population structure based on a Bayesian Markov Chain Monte Carlo approach was performed with Structure version 2.1 software (http://pritch.bsd.uchicago.edu/structure.html)[24]. The university released cultivar Shalimar Wheat-1 was used as susceptible control. Disease severity was recorded following modified Cobb scale devised by Peterson *et al*[25] and 0-9 scale as described by McNeal *et al*[26]. The resistant genotypes were validated for effective stripe rust resistance genes using associated markers. DNA of these genotypes was extracted following CTAB method as modified by Saghai-Maroof *et al*[27]. PCR was carried out for each resistance gene using associated markers (Table 2) and amplified products were resolved on agarose gel to look at gene specific amplifications.

Results and Discussion

The proposed investigation was based on the hypothesis that the identification of sources of resistance against race 78S84 and molecular detection of known effective resistance genes *Yr5,Yr10, Yr15* and *Yr18* will give enough indication of stripe rust resistance genes available to wheat breeders for starting future breeding programmes. Saini *et al*[28] screened 20 Indian and 61 exotic wheat cultivars, 8 near-isogenic lines and 2 leaf rust-susceptible wheat cultivars (Agra Local and Morocco) against stripe rust artificially inoculated with the pathogen in experiments conducted in Ludhiana, Punjab and Bajaura, Himachal Pradesh, India. Zhou *et al*[7] also screened a set of wheat germplasm lines for subsequent molecular validation of stripe rust resistance genes *Yr5, Yr10* and *Yr15.* The terminal disease severity (TDS) was recorded at adult plant stage following standard procedures and scales developed by McNeal *et al*[26] and Peterson *et al*[25].

Phylogenetic Analysis

The phylogenetic analysis based on molecular data grouped the genotypes into 3 clusters (Fig. 1), and the same was confirmed by Bayesian clustering model (structural analysis) by identification of 3 sub-populations (Fig. 2) in 39 lines under study. The

Table 1—List of wheat genotypes (cultivars and breeding lines) used in the study

No.	List of wheat germplasm	Pedigree	Source/Origin
1	Shalimar Wheat-1	BSP 93-21(Selection from EIGN 98)	SKUAST-Kashmir
2	SKW-355	-	SKUAST-Kashmir
3	HPW-42	VEERY(SIB)/3/PAVON-76(SIB)/(SIB)CIANO-67//JARAL-66/(SIB)ORIZABA	DWR, Karnal, Haryana
4	DH-114	Doubled haploid Line	DWR, Karnal, Haryana
5	HPW -155	BT 2549/FATH	DWR, Karnal, Haryana
6	FLW-16	UP2338/*Triticm spelta album*	DWR, Flowerdale, Shimla
7	FLW-10	WH542/Moro	DWR, Flowerdale, Shimla
8	FLW-21	UP2338/Centurk//UP2338/*Yr15*	DWR, Flowerdale, Shimla
9	*Yr5-NIL*	*Yr5*/6*AOC	CIMMYT, Mexico
10	*Yr10-NIL*	*Yr10*/6*AOC	CIMMYT, Mexico
11	*Yr15-NIL*	*Yr15*/6*AOC	CIMMYT, Mexico
12	*Yr18-NIL*	*Yr18*/3*AOC	CIMMYT, Mexico
13	BEZOSTAYA	Lutescens-254-53	FAWWON, Turkey
14	SERI	CM-33027-F-15M-500Y-0M-0ZMB	FAWWON, Turkey
15	SULTAN95	AGRI/NACOZARI-F-76 (Turkey - winter wheat)	FAWWON, Turkey
16	KATIA1	HEBROS/BEZ1 (Turkey - facultative wheat)	FAWWON, Turkey
17	KONYA	KANRED/TENMARQ//P-211-6/3/2183/CO-652643/LANCER	FAWWON, Turkey
18	SKUAST-K1	AGRI/NAC//KAUZ/4/55.1744/MEX67.1//NO57/3/ATTILA	FAWWON, Turkey
19	SKUAST-K2	PYN/PARUS/3/VPM/MOS83-11-4-8//PEW/SHARK-1	FAWWON, Turkey
20	SKUAST-K3	PSK/NAC//SABALAN/3/TAM200/KAUZ	FAWWON, Turkey
21	SKUAST-K4	KARL//CTK/VEE/3/F1502W9.01/4/STEPHENS	FAWWON, Turkey
22	SKUAST-K5	LRC/SERI/MEX-DW/BACA//VONA/3/LAGOS-7/4/SHARK/F4105W2.1	FAWWON, Turkey
23	SKUAST-K6	OK82282/SNB//AGRI/NAC/3/SHARK/F4105W2.1/4/SHARK/F4105W2.1	FAWWON, Turkey
24	SKUAST-K7	TAM200/KAUZ//SHARK-1/3/KRISTAL	FAWWON, Turkey
25	SKUAST-K8	JI5418/MARAS//SHARK/F4105W2.1/3/SHARK/F4105W2.1	FAWWON, Turkey
26	SKUAST-K9	PYN//TAM101/AMI/3/KRC66/SERI/4/SARDARI/5/TAM200/KAUZ	FAWWON, Turkey
27	SKUAST-K10	SOM//1D13.1/MLT/3/VORONA/3/TOB*2/7C//BUC	FAWWON, Turkey
28	SKUAST-K11	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/BECUNA-6	FAWWON, Turkey
29	SKUAST-K12	ATTILA*2/PBW65//YAKAR	FAWWON, Turkey
30	SKUAST-K13	KAMBARA1/KALYOZ-17	FAWWON, Turkey
31	SKUAST-K14	DORADE-5/3/ES14/SITTA//AGRI/NAC	FAWWON, Turkey
32	SKUAST-K15	TAM200/KAUZ//BECUNA-6	FAWWON, Turkey
33	SKUAST-K16	PRL/2*PASTOR//GUN91/MNCH	FAWWON, Turkey
34	SKUAST-K17	KAMBARA1/ZANDER-17	FAWWON, Turkey
35	SKUAST-K18	ROSHAN96/MERCAN-2	FAWWON, Turkey
36	SKUAST-K19	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/BAGCI2002	FAWWON, Turkey
37	SKUAST-K20	GANSU-1/MEZGIT-4	FAWWON, Turkey
38	SKUAST-K21	ATTILA/2*PASTOR//YUMAI 29	FAWWON, Turkey
39	SKUAST-K22	PASTOR//HXL7573/2*BAU/3/F12.71/COC//ATTILA	FAWWON, Turkey

Table 2—List of primers used for molecular validation of stripe rust resistance genes

No.	Stripe rust resistance gene	Details of primer sequences		Type of primer	Allele size (bp)	Reference
1	*Yr5*	Sequence (5′-3′)	GTACAATTCACCTAGAGT	*STS*-7	478	Chen *et al* [17]
		Sequence (5′-3′)	GCAAGTTTTCTCCCTATT	*STS*-8		
2	*Yr10*	Forward Sequence(5′-3′)	GCAGACCTGTGTCATTGGTC	*Xpsp3000*	260	Wang *et al*[18]
		Reverse Sequence(5′-3′)	GATATAGTGGCAGCAGGATACG			
3	*Yr15*	Forward Sequence(5′-3′)	GCGGGAATCATGCATAGGAAAACAGAA	*Xbarc8*	190	Murphy *et al*[19]
		Reverse Sequence(5′-3′)	GCGGGGGCGAAACATACACATAAAAACA			
4	*Yr18*	Forward Sequence(5′-3′)	TTGATGAAACCAGTTTTTTTTCTA	*Cssfr1*	517	Laugdah *et al*[22]
		Reverse Sequence(5′-3′)	GCCATTTAACATAATCATGATGGA			

Bayesian clustering model also presented the genotypes under study in the form of structural plot (Fig. 3), wherein indicating their mixture at genome level in line no. 10, 38, 1 and 7 between the individuals of group 2nd (with green colour) and group 3rd (with blue colour). Similarly there is small mixture (may be an introgressed gene) in the lines 15, 29, 10, 38, 1 and 7 from the genomes of group 1

(red coloured) and group 2 (green coloured). The other individuals in all the 3 sub-groups are without admixture. The analysis shows that the marker data of genes *Yr5*, *Yr10*, *Yr15* and *Yr18* could discriminate all the 39 lines into 3 distinct groups at genome level.

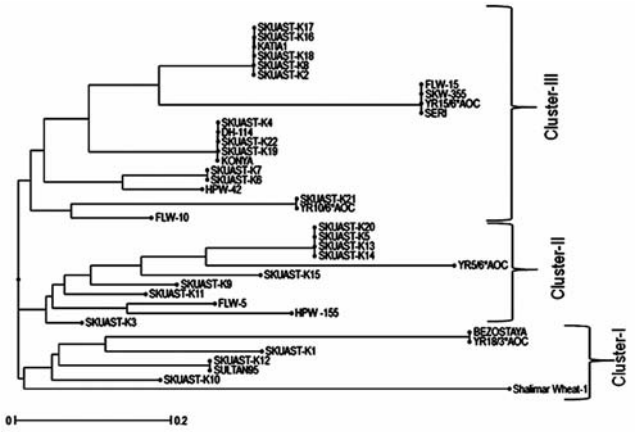

Fig. 1—Phylogenetic analysis based on genotypic data of four stripe rust resistance genes.

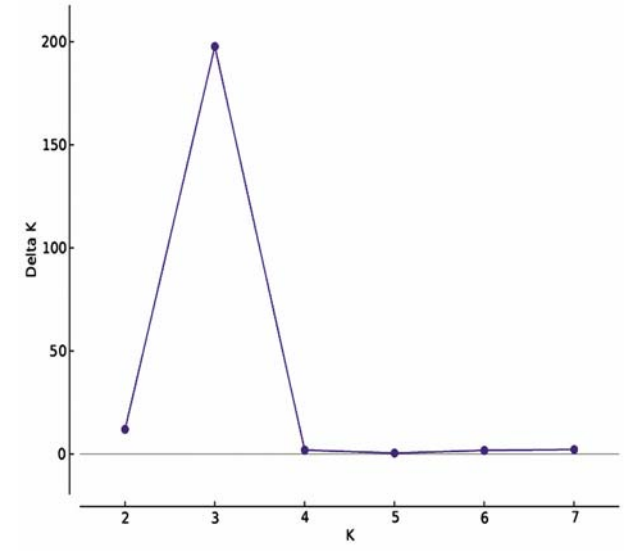

Fig. 2—Bayesian clustering results based on genotypic data of four *Yr* genes.

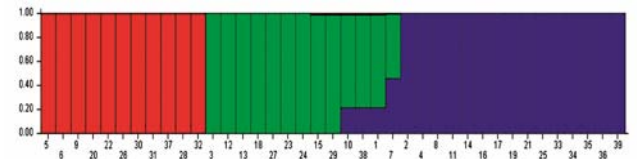

Fig. 3—Structural plots based on Bayesian clustering of genotypic data of four *Yr* genes.

Validation of Stripe Rust Resistance Genes

The breeding of resistant varieties is the key measure to control yellow rust disease, but the conventional breeding method is of low efficiency. Marker assisted selection (MAS) can significantly improve the breeding efficiency[29]. A fundamental prerequisite for MAS application in conventional breeding is the availability of tightly linked DNA markers. This can dramatically increase the speed at which resistant varieties are developed and it can thus be an effective tool for plant breeding[30]. Markers can be used to characterize parental material better, thereby improving the efficiency and effectiveness of parental selection for crossing and to track genes in segregating progenies through the selection process[31]. So, the associated markers for stripe rust resistance genes *Yr5*, *Yr10*, *Yr15* and *Yr18* were selected based on their tight linkages.

The effective stripe rust resistance genes *Yr5*, *Yr10*, *Yr15* and *Yr18* were validated on our germplasm during the present study and these genes have been found responsible in imparting resistant phenotypes in many cultivars.

Validation of Gene *Yr5*

The gene *Yr5* was detected in 14 entries (Fig. 4) including the cultivar HPW42 carrying *Yr5* in heterozygous condition. The stripe rust resistance gene *Yr5* was originally derived from *Triticum spelta* var. *album*. Using classic genetic analyses, it was identified to be dominant and named as *Yr5,* which was also confirmed using several crosses tests against North American races of *P. striiformis* f. sp. *tritici*[32]. By investigating two codominant STS primers *YrSTS7/8* and *YrSTS9/10* in 114 BC7F3 lines[17], it has

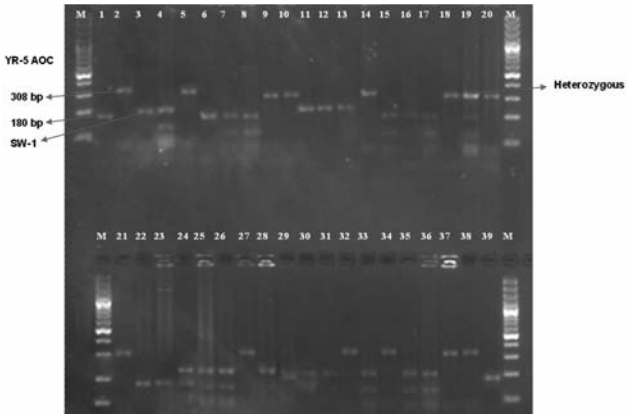

Fig. 4—PCR Amplification of *Yr5* digested products by DPN2-II enzyme, resolved on 3% agarose gel yielding bands 308 bp as resistant and 181 bp as susceptible.

been concluded that these markers are completely linked to *Yr5*. Based on epidemiological studies, *Yr5* was found effective against all rust virulent races in North America[33] and Iran[34]. This gene had shown high levels of resistance to stripe rust in China[32,18]. Also, in surveys of resistance genes in Pakistan[33], *Yr5* and *Yr15* were identified to be effective against all *Pst* races. The fact that *Yr5* was effective in Iran and its surrounding countries makes it a good candidate for wheat breeding programmes. In the present study, *Yr5* was detected in 14 cultivars and the results are in accordance with the findings of Chen *et al*[17] and Zhou *et al*[7].

Validation of Gene *Yr10*

The stripe rust resistance gene *Yr10* was molecularly validated in 29 entries (Fig. 5), of which 3 cultivars, namely, HPW-42, SKUAST-K2 and SKUAST-K6, were heterozygous. The dominant gene *Yr10* was first identified in PI178383 line and located on the short arm of chromosome 1B. This gene is found race specific and has been reported effective against all races in China[35], Iran[34] Pakistan and USA[33]. Some reports suggest that this gene is linked to some morphological traits. Its close linkage with glume brown color (*Rg1*) is used to identify it at maturity of the crop. However, this gene is expressed at the final stage of plant growth and makes it inappropriate for early selection of resistance to yellow rust. Bariana *et al*[36] verified close association between *Yr10* and *Gli-B1* by genetic analysis of the cultivar Moro. Regarding these facts, the *Xpsp3000* marker is suitable for identifying resistant genotypes at different plant growth stages[18]. Singh *et al*[36] designed two primer pairs (*Yr10 F/R* & *Yr10 F1/R1*) based on the *Yr10* sequence and produced markers completely linked to *Yr10*. Further, in the present study, *Yr10* was detected in 17 cultivars and the results are in accordance with the findings of Bariana *et al*[36]. Bariana *et al*[36] reported that varieties with gene *Yr10* amplify a 258-260 bps fragment and those lacking this gene amplify only 240 bps band fragment, which is confirmed in the present investigation.

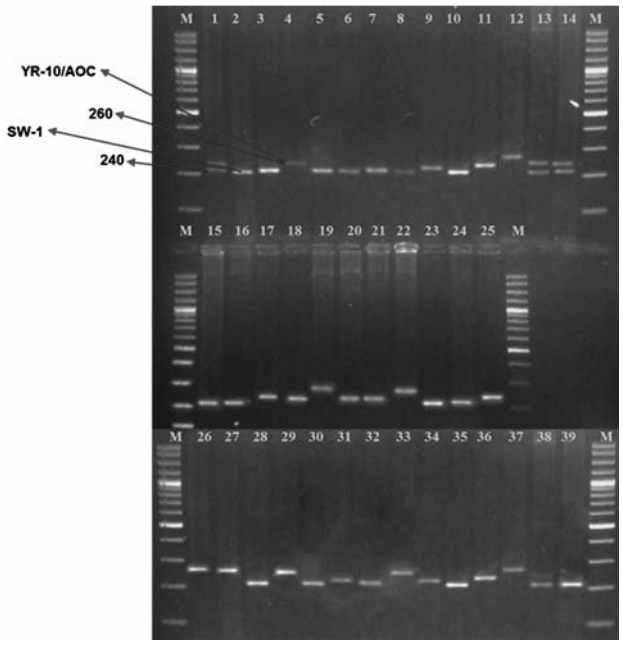

Fig.5—PCR amplification of *Yr10* gene using marker *Xpsp*3000, resolved on 3.0% agarose gel yields two types of fragments, *viz*., 240 bp as susceptible and 260 bp as resistant.

Validation of Gene *Yr15*

The stripe rust resistance gene *Yr15* was detected in 25 entries (Fig. 6), depicting heterozygous condition of this gene in 5 genotypes, *viz.*, HPW-155, FLW-10, SKUAST-K9, SKUAST-K10 and SKUAST-K11, and producing both resistance and susceptibility specific fragments as heterozygotes. The gene *Yr15* derived from *T. dicoccoides* is located on chromosome 1BS. Sun *et al*[37] used RFLP and RAPD markers in an F_2 population of 123 individuals created from the cross between the resistant *Yr15* donor line *T. dicoccoides* G-25 and a susceptible line *T. durum* cultivar D447. One RAPD marker, *OPB131420* was present in all 123 individuals and segregated in ratio of 3:1, indicating normal segregation for a dominant allele. Linkage analysis using marker and trait data found that the distal marker *OPB131420* is 27.1 cM away from the resistance gene. Linkage analysis using the RFLP probes identified *Nor1* as being 11.0 cM proximal from the gene. More recent mapping efforts by Murphy *et al*[19] have identified two SSR markers,

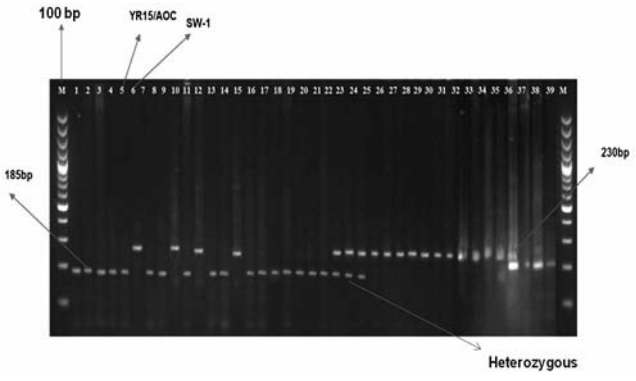

Fig. 6—PCR amplification of *Yr15* gene by using *Xbarc8*, which produces two fragments, *viz*., 185 bp as resistant and 230 bp as susceptible.

Xbarc8 and *Xgwm413*, which appear to be completely linked in their mapping with *Yr15* in a population of 136 BC7F4 lines. This complete linkage may be due to the fact that *Yr15* is an introgression from a wild wheat species *T. dicoccoides* and recombination can be expected to be reduced in the region surrounding *Yr15*[38]. Markers *Xbarc8* and *Xgwm413* are diagnostic for *Yr15*. In the present study, *Xbarc8* was used which showed the presence of *Yr15* gene in 23 entries. So, this gene was present in maximum cultivars showing its effectiveness against the predominant and virulent pathogen 78S84.

Validation of Gene *Yr18*

The nine entries (Fig. 7), viz., HPW-42, Bezostaya, Sultan95, SKUAST-K1, SKUAST-K6, SKUAST-K7, SKUAST-K10 and SKUAST-K12, were found to carry stripe rust resistance gene *Yr18.* The leaf rust resistance gene *Lr34*, first described by Dyck[10], has been shown to enhance leaf rust resistance in combinations with other resistance genes[39]. Another feature of *Lr34* resistance is that it has remained genetically inseparable from the adult plant resistance (APR) gene *Yr18*[40]. Apart from leaf rust resistance, the genes *Lr34/Yr18* also confer moderate resistance to stripe or yellow rust[11]. Co-segregation of the dual APR gene *Lr34*/*Yr18* with other traits, such as, leaf tip (*Ltn1*), powdery mildew [*Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal] and tolerance to Barley yellow dwarf virus (*Bdv1*) have been documented[11,20]. These multi-pathogen resistance traits have made the *Lr34*/*Yr18* locus one of the most valuable gene regions for disease resistance breeding in wheat. Because *Lr34*/*Yr18* resistance is predominantly expressed at the adult plant stage and can also be masked by the effects of other major genes, there is considerable interest in developing effective methods for its easy detection. Development of molecular markers for *Lr34*/*Yr18* has long been a major objective for MAS in wheat. Some of the earlier markers, such as, *Xgwm295* and *Xgwm1220*, identified on chromosome 7DS in quantitative trait loci regions associated with *Lr34*/*Yr18*/*Ltn1*[12], have been limited in breeding applications due to their low diagnostic capability across various wheat germplasm. For detection of this gene complex, more reliable molecular markers *SWM10*[23] and *csLV34*[41] were developed. Five allele-specific markers (*cssfr1-cssfr5*) were developed by Lagudah[22] based on a 3 bp deletion in exon 11 of the *Yr18* gene, which are closely linked to the *Lr34*/*Yr18*/*Ltn1*/*Pm38* locus and have been shown to provide a much wider diagnostic ability for this multi-pathogen resistance trait in diverse wheat cultivars. In the present study, the primer *cssfr1* was used for tracking gene *Yr18* and was validated across nine entries. The validation results are in accordance with the findings of Laugdah *et al*[22].

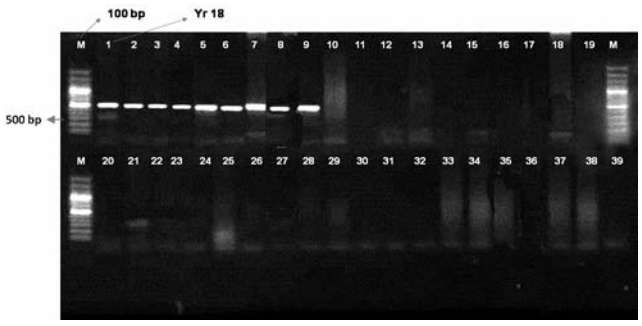

Fig. 7—PCR amplification of *Yr18* using the marker *Cssfr-1*, resolved on 1% agarose gel produces a resistant band of 500 bp.

To conclude, the single gene based resistance was detected in 9 entries, two gene based resistance was detected in 20 cultivars, three gene based resistance was detected in 8 cultivars and all four gene based resistance was detected in 1 cultivar (HPW-42). Such validation information is valuable and could be shared with other concerned centers in the country. As the cultivar HPW42 was the only entry validating all the four resistance genes, the cultivar is a gold mine of effective stripe rust resistance genes and thus can efficiently be used in devising future breeding strategies in building a long lasting defense against this fungus. The present investigation reports diverse sources of resistance against stripe rust across the wheat germplasm in the form of candidate lines for stripe rust breeding in Kashmir.

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