Molecular Validation of Genes for Rust Resistance and Grain Protein Content (*Gpc-B1*) for Wheat Breeding

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Abstract

Rust is one of the most serious fungal diseases that hamper the world's' wheat productivity, with potential yield losses of 20 to 30% globally. Several rust resistance genes (~80) have been identified and introgressed into wheat to develop resistant cultivars. However, validation of these genes using flanking/linked markers in wheat varieties would be of utmost importance for widely used in future breeding programs. In the present study, wheat cultivars; HD2967, PBW800 and PBW723 were previously introgressed for rust resistance genes. HUW234 and HUW468 were previously enhanced for grain protein content (Gpc-B1).

Therefore, these cultivars were validated for rust resistance genes using linked molecular markers and phenotypic screening in natural hot spots. The genes examined included leaf rust resistance (Lr19, Lr34, Lr37 and Lr76), stripe rust resistance (*Yr10, Yr15, Yr17 and Yr70*), *stem rust resistance* (*Sr25 and Sr38*) *and grain protein content linked genes. Analysis of marker validation showed that HD2967 and PBW723 carried all three types of rust resistance genes whereas PBW800 possessed only stripe rust resistance genes Yr10 and Yr15. These wheat genotypes, with different combinations of rust resistance genes were subsequently used in resistance breeding. The validation of linked markers for rust resistance suggested that these prospective lines could be further utilised in marker assisted breeding program.*

Keywords: Wheat, Rust resistance, Molecular marker, Rust pathogens.

Introduction

Wheat is one of the most crucial cereal crops worldwide and serves as a staple food in many regions. It belongs to the 'genus, *Triticum*' and is cultivated in several forms, primarily *Triticum aestivum* L. (common wheat), *T. turgidum* L. (durum wheat) and *T. spelta* L. (spelt wheat). Wheat accounts for over 21% of the total calories and nearly 55% of the carbohydrates in the human diet[26]. Therefore, its role in global food security is indispensable, feeding approximately 40% of the global population[42]. In terms of production, India ranks second after China, with an average productivity of 3.52 tonnes per hectare[36]. However, numerous biotic and abiotic stresses limit the complete genetic potential of modern day cultivars, especially in north eastern plains of India where wheat output is lower as compared to other regions of India.

Among the biotic stresses, diseases caused by rust (stripe, leaf and stem) have been major concern for breeders, farmers and commercial seed producers for more than a decade[20]. In summary, yellow (stripe) rust is caused by *Puccinia striiformis* f.sp. *tritici*, stem (black) rust by *P. graminis* f.sp. *tritici* and brown (leaf) rust by *P. triticina*[21]. According to Singh et al[32], rust infection results in 10 to 30% yield losses globally. *Puccinia* species require favorable environment to produce inoculums before rust is dispersed by the wind[30]. The stem rust pathogen best thrives in warm and humid (~30ºC) environments. The leaf rust pathogens best grow at low humidity and temperatures 20–24°C while temperature ranging from 12 to 20ºC is best suited for stripe rust growth[31].

Using conventional breeding approach, it generally takes seven to twelve years to develop a wheat cultivar that is resistant to rust whereas with marker-assisted selection (MAS), breeding can significantly shorten this timeframe[9]. Several crops including rice, wheat, maize, tea and peanuts, have greatly benefited from the use of the MAS[7-9]. In MAS, various marker such as SSRs (Simple Sequence Repeats) and KASP (Kompetitive Allele Specific PCR) can be used to identify a resistant plant in the early generations.

Nevertheless, owing to their repeatability, robust amplification, co-dominant inheritance, multi-allelic nature, broad genome coverage and ease of accessibility, SSR markers are still famous among plant breeders for MAS and molecular mapping studies[14]. For example, traits like grain weight and grain protein content of popular varieties have been successfully improved using SSR markers[38]. In other studies, several traits including grain quality and rust resistance have been improved using SSR markers[19,41]. Gene pyramiding, which involves introducing many resistance genes into a single cultivar, is another practical method for improving rust resistance. The development of molecular markers that flank or are linked to resistance genes and their application in breeding may expedite the gene pyramiding process as well as reducing costs at the same time[7].

Gene-specific flanking markers are used for the introgression or pyramiding of resistance genes and have

been validated in parental genotypes to enhance the reliability of target gene[10]. Molecular markers are also effective for the introgression of desirable genes into susceptible cultivars and for the detection of desirable genes targeting specific traits during early seedling stage and are useful in introgression. Validation of previously reported molecular markers in our parental genotypes is also required to measure their usefulness in the target breeding program.

Accordingly, in the present study, we have validated known linked markers for rust resistance and grain protein content gene in the parental genotypes for the presence or absence of the genes and its phenotypic expression. The major advantage of this validation is to detect their presence in agronomical superior wheat genotypes, which can be used in future breeding program.

Material and Methods

Plant materials: Seed of improved variety HD2967 were provided by Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India while PBW800 and PBW723 were obtained from Punjab Agricultural University, Ludhiana, Punjab, India, for validation of introgressed genes. The improved wheat lines (HUW234 and HUW468) with enhanced grain protein content (*Gpc-B1*) and high grain weight (*HGW*), popular in the Eastern Gangetic plains were collected from the Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India. These cultivars were validated for their inrogressed gene of rust resistance/grain protein content in the present study (Table 1).

Seeds were grown during the *Rabi* season of 2017-18 at the Agricultural Research Farm, Banaras Hindu University, Varanasi, for gene validation using specific markers. Here a number of improved genotypes (HD2967, HUW234 HUW468, PBW800 and PBW723,) were identified positive using previously linked markers. The same set was subsequently grown in the off-season/summer nursery at PAU Regional Research Station, Keylong, Lahaul Spiti, H.P., for stripe rust screening and at the ICAR-IARI Regional Station, Wellington (T.N.) for leaf and stem rust pathogen screening. The Agra local was used as susceptible check for all three rusts.

Phenotyping for rust infection: Disease evaluation was performed using modified Cobb's scale of 0–100%[24]. This scale was developed to estimate proportion of the area of a stripe or leaf or stem infection by rust pustules. The rust severity percentages were individually calculated for all three rusts[24]. The infection type (IT) was recorded as part of assessment of the host response[27]. The final disease severity score for each individual plant was calculated by multiplying their IT estimation by the corresponding numerical value (Tr-0.1, R-0.2, MR-0.4, M-0.6, MS-0.8 and S-1.0). The scores for each genotype were then averaged to produce the average coefficient of infection (ACI) [27].

DNA extractions and PCR Protocol: A modified CTAB method[28] was employed to extract DNA from 21-day-young leaves of wheat seedlings. The extracted DNA was stored at –20°C for subsequent use. Molecular markers strongly linked to specific genes were selected from previous literature related with leaf rust *(Lr24 and Lr34)*, leaf rust/stripe rust/stem rust (*Lr37/Yr17/Sr38)*, stripe rust *(Yr10 and Yr15*), leaf/stripe rust (*Lr76/Yr70*), leaf/stem rust (*Lr19/Sr25)* and grain protein content (*Gpc-B1*).

The PCR reaction mixture was prepared using 1-2µL of 100ng DNA template, 1.5µL of 10X PCR buffer (MBI Fermentas, Germany), 1µL primers (20 mM) of each forward and reverse primer (Metabion, Germany), 0.2µL of 10 mM dNTPs (MBI Fermentas, Germany), 0.2µL of 25 mM $MgCl_2$ (MBI Fermentas, Germany), 0.2 µL Taq polymerase (3 U/µl) (MBI Fermentas, Germany) and 9.9µL of double-distilled water. PCR reactions were performed in a thermal cycler (Bio-Rad) with the following PCR conditions: initial denaturation at 94°C for 4 minutes followed by 35 cycles of 94°C for 1 minute, ~55-60°C (depending on marker) for 1 minute, extension at 72°C for 2 minutes and a final extension at 72°C carried out for 10 minutes. The PCR conditions varied for diverse primers, mostly with the variation in annealing temperature for each primer (Table 2).

The amplified PCR products were resolved on agarose gel (2.0-2.5%), prepared by adding agarose (6.0-7.5 gm) to 300 ml TAE buffer (1X) in a flask (1000 ml capacity) and boiled carefully till agarose melted completely and finally 9µl (10 mg/ml) ethidium bromide was added to the gel. For each DNA sample, 2 µl of 10X loading dye (MBI, fermentas) was added and the complete mixture was loaded in the agarose gel. Amplified fragments were observed under UV light and gel photographed was taken using gel documentation system (UVP, GelDoc-It®Imager).

Table 1
Description of plant materials

Improved variety	Introgressed gene	Characteristic features
HD2967	*Lr19-Sr25*, *Yr10* and *Lr34*	Resistant to all three rust
PBW723	*Lr37/Yr17/Sr38*, *Lr76/Yr70* and *CRE5*	
PBW800	*Yr10* and *Yr15*	Resistant to stripe rust
HUW234	*Gpc-B1* and *HGW* (HGW=High Grain Weight)	HUW234 and HUW468 have been improved for high grain protein (>3% more) and 20% higher grain weight than original variety[22,38].
HUW468		

Table 2
PCR conditions for primer pairs used to validate molecular markers for different rust-resistant genes

Traits	Gene/QTL	Marker	Position	Marker/ Primer Sequence	Amplicon Size (bp)	Annealing temp./Time	Dominant/Codominant
GPC- B1	*Gpc-B1*	Xucw108	6BS	F-AGCCAGGGATAGAGGAGGAA R-AGCTGTGAGCTGGTGTCCTT	217 (P)	60.5°C, 1 min	Dominant[35]
Leaf /stem rust	*Lr19/Sr25*	Xwmc221	7DL	F-ACGATAATGCAGCGGGGAAT R-GCTGGGATCAAGGGATCAAT	190 bp (P), 230 bp (A)	57°C, 1 min	Co-dominant[12]
	Sr25	STS-GB	7DL	F-CATCCTTGGGGACCTC R-CCAGCTCGCATACATCCA	130 bp (P)	60°C, 1 min	Dominant[18]
Leaf rust	*Lr34*	STS (CsLV34)	7DS	F-GTTGGTTAAGACTGGTGATGG R-TGCTTGCTATTGCTGAATAGT	150 (P), 229 (A)	55°C, 1 min	Co-dominant[17]
Yellow rust	*Yr10*	Psp3000	1BS	F-GCAGACCTGTGTCATTGGTC R-GATATAGTGGCAGCAGGATACG	260 (P), 240 (A)	59°C, 1 min	Co-dominant[39]
Yellow rust	*Yr15*	Xbarc8	1BS	F-GCGGGAATCATGCATAGGAAAACAGAA R-GCGGGGGCGAAACATACACATAAAAACA	185 (P), 230 (A)	64°C, 1 min	Co-dominant[23]
Yellow /leaf rust	*Yr70/Lr76*	Xgwm190	5DS	F-GTGCTTGCTGAGCTATGAGTC R-GTGCCACGTGGTACCTTTG	190 bp (P), 208 bp (A)	59°C, 1 min	Co-dominant[3]
Leaf / stem /yellow rust	*Lr37/Sr38 /Yr17*	Xcmwg682/ VENTRIUP-LN-2	2NS/ 2AS	F-AGGGGCTACTGACCAAGGCT R-TGCAGCTACAGCAGTATGTACACAAAA	262-285 (P)	60.5°C, 1 min	Dominant[13]

*P-Present, A-Absent

Results and Discussion

At an early stage of growth, molecular markers with a tight linked with the resistance genes can aid in the selection of lines carrying one or more such genes[6,33]. A pathogen finds it very difficult to defeat a combination of race-specific and non-race-specific resistance genes. In the present investigation, three Indian wheat cultivars HD2967, PBW800 and PBW723 previously improved for rust resistance, were validated and can be utilised for the gene pyramiding. The cultivars HUW234 and HUW468 were validated for the presence of *Gpc-B1* and *HGW* genes along with rust resistance genes. All five wheat cultivars were validated for the presence or absence of various gene combinations using gene-specific markers (Table 3). Three rust-resistant genes, namely *Lr19/Sr25*, *Yr10 and Lr34*, were confirmed in the HD2967 genotype.

The *Lr19/Sr25* gene linked SSR marker Xwmc221 and GB, produced amplified band sizes of 190 bp and 130 bp respectively. The *Yr10* gene, linked to the SSR marker PSP3000 was amplified with band size 260 bp, The *Lr34* gene linked SSR marker CsLv34 was amplified with band size 150 bp. The wheat cultivar PBW800 was found to carry two yellow rust genes *Yr10* and *Yr15*, where *Yr10* gene was indicated by the SSR marker PSP3000 (260 bp) and the *Yr15* gene was detected by the marker Xbarc8 (185 bp).

The wheat cultivar PBW723 carries several linked genes *Lr37/Yr17/Sr38/CRE5*, as indicated by the marker Xcmwg682/VENTRIUP-LN-2 which amplified 262-285 bp band and the *Lr76/Yr70* gene by Xgwm190 marker, with a band size of 190 bp. Hence, three wheat cultivars (HD2967, PBW800 and PBW723) possessed different rust resistance genes in their background except for the quality trait gene *Gpc-B1*.

However, only the *Gpc-B1* gene, responsible for grain protein content, was present in improved HUW234 and HUW468 genotypes linked to Xucw108 marker and amplified at a band size 217 bp. These validated wheat cultivars for different rust resistant gene and grain protein content can be used for the development of pyramided lines in future wheat improvement programs. Disease severity was assessed at the adult stage in all five wheat varieties for leaf, stem and stripe rusts using the Modified Cobb's method[24] at ICAR-IIWBR, Dalang Maidan, Keylong, Himachal Pradesh. Agra local, well known widely used susceptible cultivar of wheat for all three rust was included in current study as an infector line.

Table 3
The presence or absence of the grain protein content gene (*Gpc-B1*) and all three rust resistance genes based on molecular markers in wheat genotypes.

Genes/QTL	Improved HUW234	Improved HUW468	Improved HD2967	Improved PBW800	Improved PBW723
Gpc-B1	+	+	-	-	-
Lr19/Sr25	-	-	+	-	-
Lr34	-	-	+	-	-
Yr10	-	-	+	+	-
Yr15	-	-	-	+	-
Yr70/Lr76	-	-	-	-	+
Lr37/Sr38/Yr17	-	-	-	-	+

Table 4
Results of phenotypic screening of 5 genotypes and a check cv., Agra local under glass-house conditions against several pathotypes of the leaf and stripe rusts

Wheat rust	Pathotypes	Distribution of wheat growing zone of India	Status	HUW 234	HUW 468	HD 2967	PBW723	PBW800	Agra local
Stripe rust	46S119	NWPZ and	Predominant	S	S	R			S
	78S84	NHZ	Predominant	S	S	R	R	R	S
							R	R	
Leaf rust	77-5	Present in	High virulence	S	S	R	MR	MR	S
	77-8 104-2	uniformly in	Predominant	S	S	R	R	R	S
	12-1	all zones	Predominant	S	S	R	R	R	S
			Predominant	S	S	R	R	R	S
Stem Rust	40A	CZ, PZ and	Predominant	S	S	R	R	S	S
	40-1 117-6	SHZ	Predominant	S	S	R	R	MR	S
			Predominant	S	S	R	R	S	S

*R-Resistant, MR-Moderately Resistant, S-Susceptible, NHZ-Northern Hills Zone, NWPZ-North Western Plain Zone, PZ-Peninsular Zone, CZ-Central Zone, SHZ-Southern Hills Zone.

Phenotypic disease screening revealed that HD2967 and PBW723 were rust-free (0%) while HUW234 and HUW468 showed susceptibility (80%) to leaf and stripe rust. PBW800 exhibited resistance to stripe rust and moderate resistance to leaf rust (15%). Seedling tests with rust pathotypes were conducted at the IIWBR Regional Station, Flowerdale, Shimla. However, in recent years, new virulent races targeting some of these resistance genes have emerged[25]. In the present experiment, the HD2967 was resistant to four leaf rust pathotypes (77-5, 77-8, 104-2 and 12-1), three pathotypes of stem rust (40A, 40-1 and 117-6) and also two stripe rust pathotypes (46S119 and 78S84). Among the leaf rust groups, pathotypes 77-5, 77-8, 104-2 and 12-1 were identified as the most significant emerging races, with pathotype 77-5 being the most predominant[12]. Phenotypic screening across all pathotypes of leaf, stem and stripe rust revealed that genotypes showed different combinations of susceptibility and resistance (Table 4).

Molecular markers for gene validation

Developing rust-resistant cultivars remains the primary method for battling the leaf, stem and stripe rust diseases in wheat; however, the traditional breeding methods are often inefficient and time consuming. To evaluate the potential donor lines, MAS is currently being employed, increasing the effectiveness and efficiency of selection for passing along and tracing genes in later generations through the selection process[40]. In the current study, several rust-resistant genes were validated in improved lines, namely HD2967, PBW800 and PBW723 and for grain protein content in HUW234 and HUW468. Therefore, identified tightly linked markers to the leaf, stripe and stem rust resistance and high grain protein genes were highly recommended for use in future wheat breeding programs.

***Lr19/Sr25* Gene:** The efficacy against pathotypes of leaf rust, *Agropyron elongatum* - derived 7DL translocation harbouring the *Lr19/Sr25* rust gene has also shown effectiveness against stem rust race Ug99 and its variants. Consequently, breeding lines of wheat carrying the *Lr19/Sr25* translocation segment have been developed worldwide. The objective of the present study was to identify wheat genotypes carrying the *Lr19/Sr25* gene linked to the Xwmc221 marker. Additionally, the Gb marker was employed to detect the presence of the *Sr25* gene. The co-dominant Xwmc221 marker yields a 190 bp fragment, indicating the presence of the resistance gene, while a 230 bp band signifies its absence [Figure 1(i)]. Conversely, the GB marker, being dominant in nature, exhibits a single band of 130 bp, confirming the presence of the *Sr25* gene [Figure 1(ii)].

***Lr34* gene:** The development of a gene-specific DNA marker for *Lr34* provides a powerful tool for MAS in wheat breeding, enabling the introduction of slow-rusting resistance to leaf rust[17]. In the present study, presence of the *Lr34* gene, was confirmed using STS marker CsLv34 and validated in the chosen wheat genotypes. Among the five wheat genotypes, only HD2967 exhibited 150 bp fragment, indicating the presence of the *Lr34* gene, while the other genotypes displayed a 229 bp fragment, indicating the absence of the *Lr34* gene (Figure 2). The association of the *Lr34* gene with *Yr18* was referred by Lagudah et al[17]. To reduce future losses from leaf rust epidemics, rapid incorporation of *Lr34*gene into adapted wheat cultivars is crucial[29].

***Yr10* gene:** The presence of the stripe rust resistance gene *Yr10* was confirmed in two donors genotypes, HD2967 and PBW800 using the linked marker Xpsp3000. This dominant gene, located on the short arm of chromosome 1B, was initially discovered in the PI178383 wheat line. Reports from China[34], Iran[1], Pakistan and the United States[8] indicated that *Yr10* is present in race-specific populations and confers resistance against all races of stripe rust. The Xpsp3000 marker, tightly linked with *Yr10*, can also be utilized to detect resistant genotypes at various stages of plant development[39]. According to Bariana et al[4], cultivars possessing the *Yr10* gene amplified a 260–285 bp fragment, whereas genotypes lacking the gene amplified only a 240 bp fragment (Figure 3).

***Yr15* gene:** Among the assessed lines, only PBW800 was found to possess the stripe rust resistance gene *Yr15*. This gene is located on 1BS chromosome derived from *T. dicoccoides*.

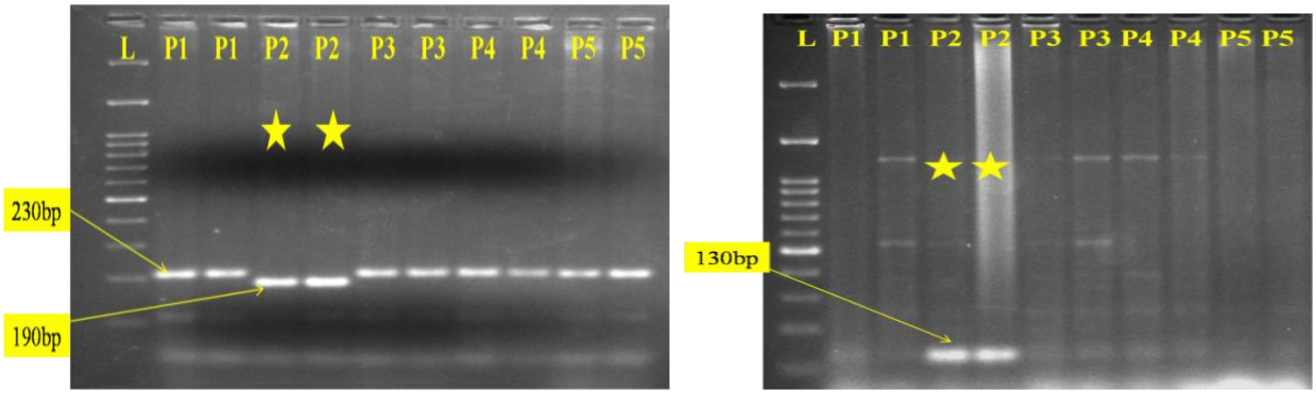

Figure 1: Amplification for (i). *Lr19/Sr25*-linked marker Xwmc221, 190bp(P), 230bp(A) (ii). *Sr25*-linked marker GB, 130bp(P), Ladder-100bp, P1-HUW234, P2-HD2967, P3-PBW800, P4-HUW468 and P5-PBW723

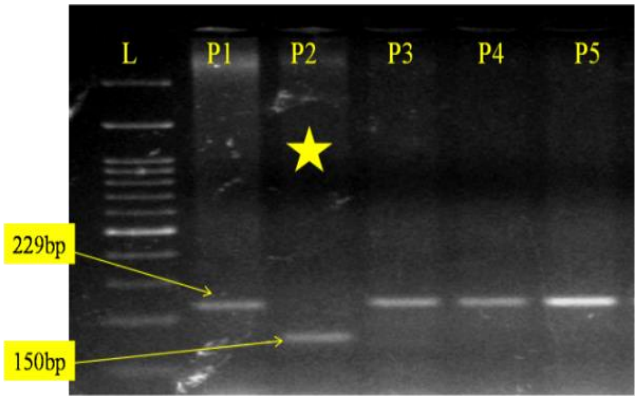

Figure 2: Amplification for *Lr34*-linked marker CsLv34, 150bp(P),229bp(A), Ladder-100bp, P1-PBW800, P2-HD2967, P3-PBW723, P4-HUW234 and P5-HUW468

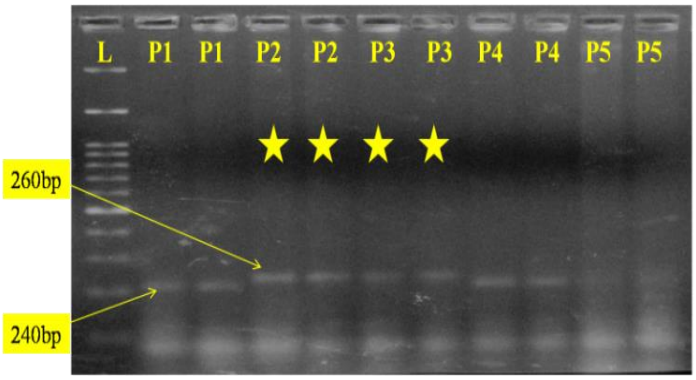

Figure 3: Amplification for *Yr10*-linked marker Psp3000, 260-285bp(P), 240bp(A), Ladder-100bp, P1-PBW723, P2-HD2967, P3-PBW800, P4-HUW234 and P5-HUW468

Recent genetic mapping conducted by Murphy et al[23] revealed that two SSR markers, Xbarc8 and Xgwm413, are tightly linked with *Yr15* within the studied population. The perfect linkage observed between *Yr15* and markers may be attributed to the fact that *Yr15* is an introgression from the wild wheat species *T. dicoccoides* and recombination is limited in the surrounding genomic region[4]. In the current investigation, the Xbarc8 marker, indicative of the presence of the *Yr15* gene at 185 bp and lack at 230 bp, was employed (Figure 4).

***Lr76/Yr70* gene:** The *Lr76* and *Yr70* genes, derived from *Aegilops umbellulata*, are located on chromosome 5D of wheat. The location of the alien introgression has been identified as the 9.47 Mb regions on the short arm of wheat chromosome 5D[3]. Rust resistance genes, both for stem and leaf rust, from *A. umbellulata* were transferred to susceptible wheat cultivar WL711 through induced homoeologous pairing. In the present study, the presence of the *Lr76/Yr70* gene was assessed using the Xgwm190 primer[3]. In this investigation, the amplification of Xgwm190 marker is indicative of the presence of the *Yr70/Lr76* gene at 190 bp and its lack at 208 bp (Figure 5).

***Lr37/Sr38/Yr17* gene:** The *Lr37/Sr38/Yr17* rust resistance gene cluster was successfully transferred to the short arm of bread wheat chromosome 2AS using a segment of *T. ventricosum* (Tausch) Cess. chromosome 2NS[13]. Initially, this cluster gene *Lr37/Sr38/Yr17* was originally introduced into the winter bread wheat line 'VPM1'[4]. Specific primers, Xcmwg682/VENTRIUP-LN-2 and SCAR marker SC-372 were designed to locate this rust resistance gene cluster in commercial wheat cultivars. In wheat lines containing *Lr37/Sr38/Yr17*, PCR products were amplified at 262-285 bp and 300-500 bp respectively while none of the bands were detected in the negative control. PCR amplification using the marker Xcmwg682 resulted in band sizes ranging from 262 to 285 bp, indicating the presence of the *Sr38* gene, while using SCAR marker, SC-372 resulted in band sizes ranging from 300 to 500 bp, indicating the presence of the *Yr17* gene (Figure 6 (i) and (ii)).

***Gpc-B1* gene:** The increase in gpc can be achieved by transferring the *Gpc-B1* gene from *T. turgidum* sp. *dicoccoides* into durum wheat[15]. The genotype Glu269 has been utilised as the donor parent for the introgression of the *Gpc-B1* gene, which confers high GPC in the recipient varieties HUW234 and HUW468[22,37]. Breeding for the *Gpc-B1* gene can be difficult because it is inversely correlated with grain yield, however, some findings suggest that this gene has minimal or no impact on grain yield, protein quality, plant height, or heading date across different genetic backgrounds[11,15].

In the present study, *Gpc-B1* gene was detected in improved HUW234 and HUW468 genotypes, linked to the Xucw108

marker and amplified at a band size 217 bp. Additionally, the *GPC* locus and the stripe rust resistance gene *Yr36* were closely related and it can be tagged using the same *Gpc-B1* markers (https://maswheat.ucdavis.edu/protocols/HGPC) (Figure 7).

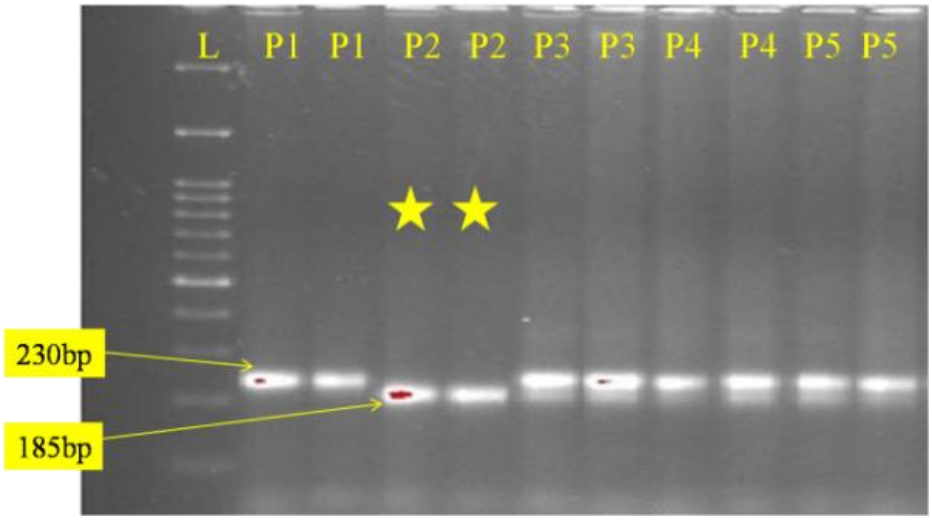

Figure 4: Amplification for *Yr15*-linked marker Xbarc8, 185bp(P),230bp(A), Ladder-100bp, P1-PBW723, P2-PBW800, P3-HD2967, P4-HUW234 and P5-HUW468

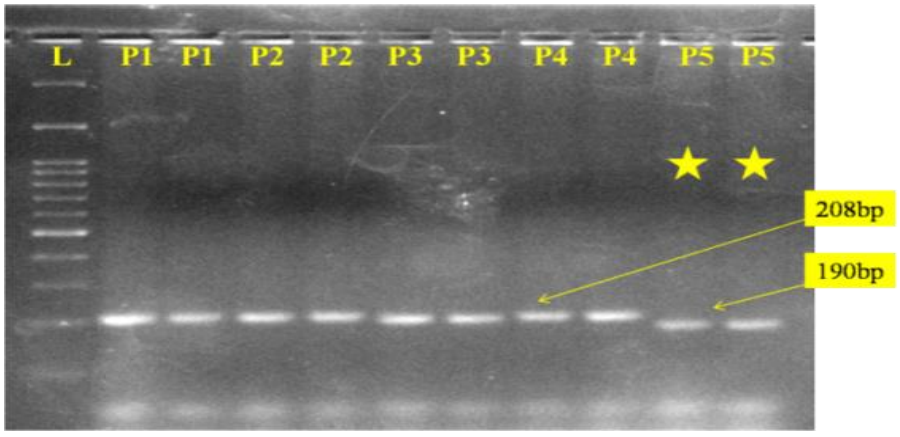

Figure 5: Amplification for *Lr76/Yr70*-linked marker Xgwm190, 190bp(P),208bp(A), Ladder-100bp, P1-HUW234, P2-HD2967, P3-PBW800, P4-HUW468 and P5-PBW723

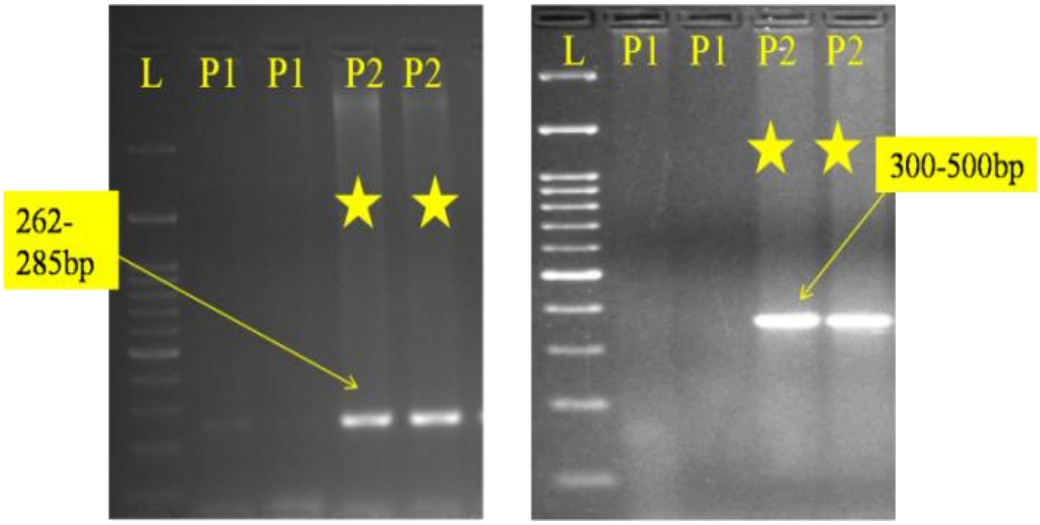

Figure 6: (i) Amplification for *Sr38*-linked marker Xcmwg682/VENTRIUP-LN-2, 262-285bp(P) P1-HUW234/HUW468, P2- PBW723 (ii) Amplification for *Yr17*-linked marker SC-372, 300-500bp(P) P1-HUW234/HUW468, P2-PBW723; Ladder-100bp

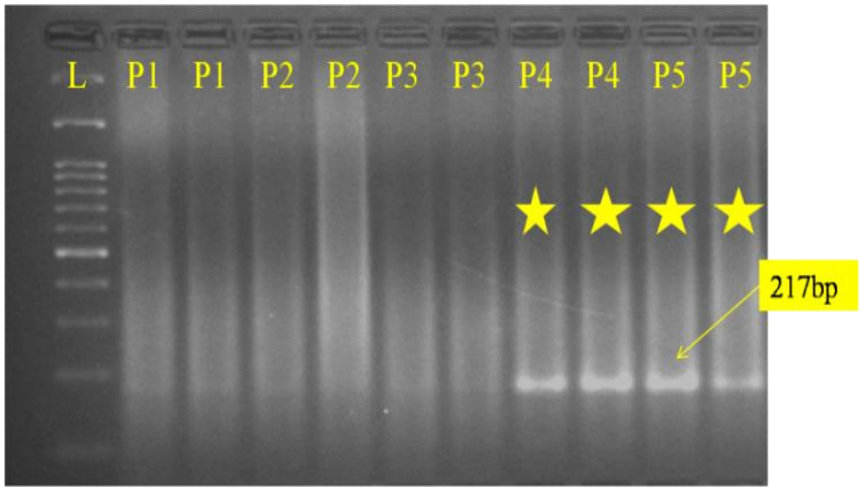

Figure 7: Amplification for *Gpc-B1*-linked marker Xucw108, 217bp(P), Ladder-100bp, P1-PBW800, P2-PBW723, P3-HD2967, P4-HUW234 and P5-HUW468

Conclusion

The identified wheat resistant lines with known genes found in present work can be helpful in marker-assisted breeding programs for multiple genes stacking. The present experiment reveals the presence of *Lr19/Sr25+Lr34+Yr10* in HD2967, *Yr10 + Yr15* in PBW800, *Lr76/Yr70 + Lr37/Yr17/Sr38* in PBW723, *Gpc-B1 + HGW* in both HUW234 and HUW468 respectively. These wheat genotypes contain rust resistance genes for leaf rust (*Lr34*), stripe rust (*Yr10* and *Yr15*) and combination of rust resistance genes like *Lr19/Sr25* (leaf/stem rust), *Lr76/Yr70* (leaf/stripe rust) and *Lr37/Yr17/Sr38* (leaf/stripe/stem rust).

These genotypes can be used as potential donors for leaf, stem and stripe rust resistance in marker-assisted breeding. The knowledge gained in the present investigation about linked molecular markers with rust resistance genes and high grain protein content gene will be highly useful to develop cultivar for rust resistance with enhanced grain protein content.

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