Molecular Screening of Selected Wheat Genotypes for Fusarium Head Blight Resistance Genes

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Abstract- Wheat, being economically important, is susceptible to various biotic and abiotic factors that can affect its yield. Biotic factors include pests and pathogens, while abiotic factors encompass temperature extremes, light, heat, salinity, and other stressors. One of the biotic stresses is Fusarium head blight (FHB), also known as head scab, a devastating fungal disease that affects small grain cereal crops such as wheat. In this study, we conducted molecular screening of 20 wheat varieties to detect FHB resistance genes. Specifically, we used SSR markers linked to three FHB resistance genes, namely GWM-644 (*FHB-2*), GWM-304 (*FHB-5*), and TaHRC-GSM (*FHB-1*). Among the tested varieties, 18 out of 20 genotypes showed presence of the GWM-644 marker linked to *FHB-2*, while 11 out of 20 genotypes exhibited presence of the GWM-304 marker linked to *FHB-5*. However, TaHRC-GSM linked to *FHB-1* was not detected in any of the tested genotypes. Notably, 7 out of 20 wheat varieties showed presence of both *FHB-2* and *FHB-5* genes, constituting 20% of the total varieties, including Wadan, Israr Shaheed, Khaista-2017, NIFA Lalma, Seher 2006, Pakistan-2013, and Shahkar-13. These findings provide valuable insights for Fusarium head blight control and the potential utilization of these varieties in the field. In conclusion, our study highlights the importance of molecular screening for detecting FHB resistance genes in wheat varieties and provides valuable information for the development of disease-resistant wheat cultivars, contributing to sustainable wheat production.

Index Terms- Wheat; Fusarium head blight; Disease resistance genes; SSR markers; PCR

I. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most essential food grain crop worldwide, accounting for thirty percent of all cereal products and providing approximately twenty percent of food calories for the human race (Reddy, 2004). Wheat is cultivated on 240 million hectares globally (Enghiad *et al*., 2017) and is the main food crop for 40% of the world population. In Pakistan, wheat is the main staple food, with an annual production of 25.45 million tons on an area of 9.25 million hectares. Punjab is the leading wheat-producing province in Pakistan, while Khyber Pakhtunkhwa (KP) produces 1.35 million tons of wheat on 0.78 million hectares (MNSFR 2015-16), with 61 percent grown in barani (rain-fed) areas and 39 percent in irrigated territories. However, wheat production in Pakistan is relatively low compared to other advanced countries due to various factors, including biotic and abiotic stresses, such as pest and pathogen infestations, extreme temperatures, soil hardness, and unbalanced use of fertilizers (Ahmad and Rashid, 2004; Ali *et al*., 2014).

One of the major biotic stresses affecting wheat production is Fusarium head blight (FHB), a devastating fungal disease and a worldwide recognized disease caused by *Fusarium graminearum* (McCartney *et al*., 2016). FHB can cause significant economic losses, with an estimated 21.5% of crop production lost to the disease or insect pests (Savary et al., 2019). The disease was first diagnosed in Nebraska in 1898 and has periodically caused epidemics in North America, with severe outbreaks in 1957, 1982, and 2007 due to changes in climate during the flowering and grain filling phases of wheat (Leonard and Bushnell, 2003; Wegulo *et al*., 2015). FHB can result in sterility of florets, formation of discolored and shrunken grains, and low grain volume weight, leading to reduced grain

yield and quality (Tuite *et al.*, 1990; Panthi *et al.*, 2014). Control strategies for FHB include cultural practices, chemical and biological control, planting resistant cultivars, disease monitoring, and improving harvesting strategies, but these are only moderately effective (Buerstmayr *et al.*, 2009). Pyramiding multiple resistance genes in a single variety is an excellent way to attain durable resistance and, therefore, has been successfully used in breeding for diseases resistance, particularly after the identification of new resistance genes and their associated markers (Ullah *et al*., 2020; Bibi *et al*., 2022; Ullah *et al*., 2023).

Fusarium head blight not only has a significant impact on grain yield, but also poses a threat to human and livestock health due to the production of toxins such as deoxynivalenol (DON) in diseased grains (Wegulo, 2012). Consequently, planting resistant cultivars is considered the most cost-effective, ecologically friendly, safe, and efficient approach for FHB management (Zhang, Yang, *et al*., 2018). As a result, enhancing FHB resistance has become a primary goal in wheat breeding programs. However, currently, the majority of Chinese wheat cultivars are susceptible to FHB, with the exception of the Yangmai, Ningmai, and Emai series of cultivars (Cheng *et al*., 2012). In recent years, FHB has emerged as a significant threat in wheat fields in Pakistan, and there is a need to develop effective strategies to control this disease. Therefore, this study aims to screen selected genotypes of wheat for Fusarium head blight resistance at the molecular level, in order to contribute to the development of FHB-resistant cultivars and improve wheat production in Pakistan.

II. MATERIAL AND METHODS

Plant material

The plant material for this study consisted of 20 varieties of wheat (see Table 1). The seeds of these varieties were obtained from the Gene Bank of the Plant Genetic Resource Institution (PGRI), NARC (National Agriculture Research Centre), located in Islamabad, Pakistan. The lines were planted in the experimental field of the Agriculture Department at Hazara University Mansehra, Pakistan during the 2018-19 wheat growing season. The research was based on a Randomized Complete Block Design (RCBD) with three replications. The rows were kept with a space of 30 cm from each other and all recommended crop management techniques were followed to maintain a healthy crop. Molecular-based screening of the Fusarium head blight resistance genes was performed at the Department of Biotechnology and Genetic Engineering at Hazara University Mansehra.

Table 1 List of 20 wheat varieties to be assessed for Fusarium head blight resistance using molecular markers.

S. No.	Name of Genotype	Status
1	PEERSABAK 5	Crop variety
2	WADAN	Crop variety
3	PAKISTAN 2013	Crop variety
4	KOHAT 2017	Crop variety
5	FAHEEM 2019	Crop variety
6	AARI 2013	Crop variety
7	NIFA LALMA	Crop variety
8	NIFA INSAF	Crop variety
9	PIRSABAK-2015	Crop variety
10	INSAF 127	Crop variety
11	PEERSABAK 2013	Crop variety
12	SHAKAR 19	Crop variety
13	NIFA AMAN 116	Crop variety
14	ZINCOL-2016	Crop variety
15	SEHAR	Crop variety
16	HIS	Crop variety
17	PASINA 2017	Crop variety
18	JOHAR 2017	Crop variety

19	GOLD 2016	Crop variety
20	KHAISTA	Crop variety

Extraction of genomic DNA

The genomic (g) DNA was extracted using CTAB method (Ali *et al*., 2016). Fresh leaves at early seedling stages were collected in eppendorf tubes and put immediately in liquid nitrogen. Crushed with a glass rod and 700µl of heated (60° C) 2x CTAB buffer (50mM Tris-HCl, pH 8.0, 25mM EDTA, 300mM NaCl and 2% CTAB) was added to each sample. The samples were then incubated at 56° C overnight and mixed again with the help of a glass rod. Followed by addition of 700µl Chloroform: Isoamyl alcohol (24: 1) solution and kept at room temperature for 30 minutes. The samples were then centrifuged at 9000 rpm for 20 minutes and a clear supernatant (500µl) was transferred to a newly labeled eppendorf tubes. Then 500µl ice cold isopropanol and 40µl sodium acetate were added to it and incubated at -20° C for at least 1 hour or overnight. The samples were centrifuged at 9000 rpm for 20 minutes to make DNA pellet. The supernatant was discarded, and the pellet was washed with 70% ethanol and dried at room temperature. Then, 40µl TE buffer was added to each sample. For RNA degradation 1µl RNAse was added to each tube and incubated at 37° C for one hour. The quality and quantity of DNA was checked on 1% agarose gel stained with ethidium bromide. The concentration of DNA was adjusted from 20 to 50 ng/µl by using double distilled water and stored at 4°C for further use.

Selection of primers

Molecular characterization of the twenty wheat varieties was performed using three FHB resistance markers: GWM-644 (*FHB-2*), GWM-304 (*FHB-5*), and TaHRC-GSM (*FHB-1*) (see Table 2). Out of these markers, only two were successfully optimized: GWM-644 and GWM-304.

Table 2. Sequence of primers used in this study.

S/No	Marker type	Name of gene	Nucleotides sequence (5' – 3')	Chromosome No
1	SSR	*FHB2*	F- CTGGGTCAAGGCCAAG	6BS
			R- AGGAGTAGCGTGAGGGGC	
2	SSR	*FHB5*	F- AGGAAACAGAAATATCGCGG	5A
			R- AGGACTGTGGGGAATGAATG	
3	SSR	*FHB1*	F- ATTCCTACTAGCCGCCTGGT	3BS
			R- ACTGGGGCAAGCAAATGGT	

Polymerase Chain Reaction

Devos and Gale (1992) protocol was used for polymerase chain reaction. Two microsatellite markers were used during the present study to amplify DNA from twenty wheat genotypes. The details of the primers used in this study are provided in Table 2. Amplification reactions was carried out in 16 ul reaction volumes containing 1µl genomic DNA (20 – 50 ng/µl), 0.5µl each of forward and reverse primers (10 µM / µl), 1.2µl of dNTPs (25 mM each) , 0.4 µl of Taq DNA Polymerase (2 units, Thermo Scientific), 1X Taq Buffer and 1.6µl MgCl2 (2.5 mM). PCR amplification was carried out in DNA Thermal Cycler (Applied Bio System) set at: an initial denaturation of 5 min at 94 °C; 36 cycles of 94 °C for 45 sec, 50 – 62 °C for 45 sec, and 72 °C for 1:30 sec. One additional cycle of 7 min at 72 °C was used for final extension. Amplification products were resolved by electrophoresis on 2% agarose gel run in 1 X TAE buffer. The amplified products were observed under UV light after staining with ethidium bromide (10 ug/ml). The data was scored for the presence or absence of Fusarium head blight resistance genes.

III. RESULTS

Genomic DNA Analysis: The present study investigated 20 different wheat varieties through a molecular analysis. Genomic DNA was extracted from each variety using the CTAB method and assessed for quality using gel electrophoresis on a 1% agarose gel. The results, depicted in Figure 1, indicate high-quality genomic DNA was obtained. The concentration of each

DNA sample was measured with a spectrophotometer and adjusted to a range of 20-50 ng/µL.

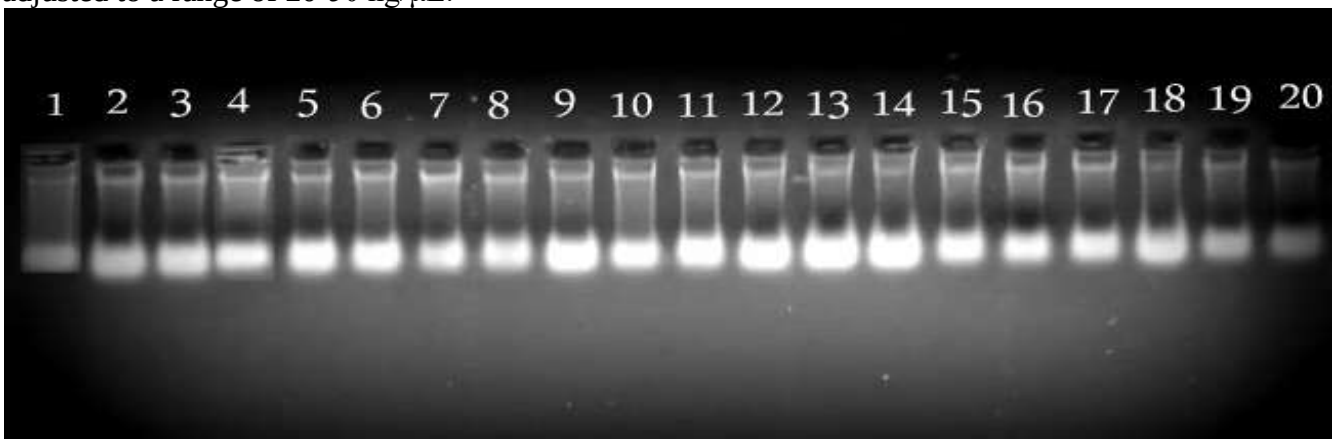


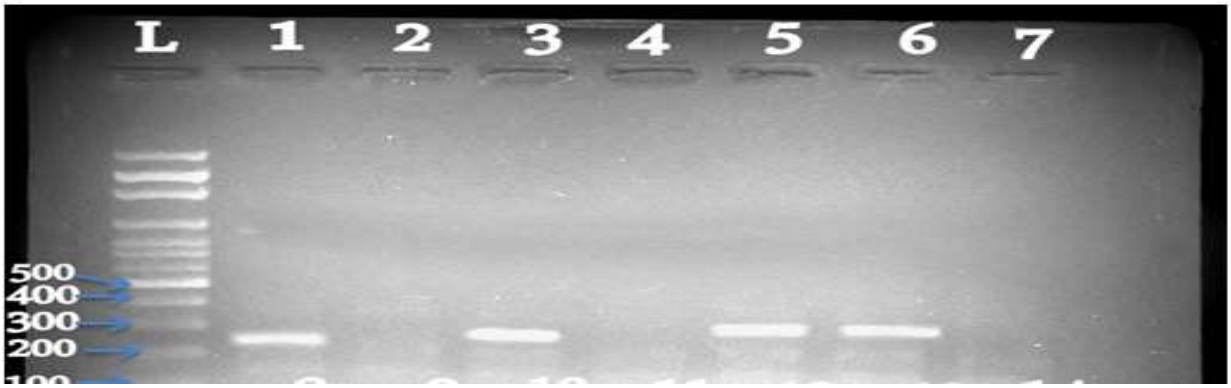
Figure 1 Genomic DNA extracted from 20 selected genotypes of wheat and resolved on 1% agarose gel. (1= Nifa Insaf, 2= Nifa aman, 3=Wadan, 4=Pasina-2017, 5=Israr Shaheed, 6= Pirsabak-2013, 7= Pirsabak-2015, 8= Khaista-2017, 9=NIFA Lalma, 10= Seher-2006, 11=Shahkar-13, 12= Gold-2016, 13=Johar-2017, 14=Faheem 2019, 15=Kohat 2017, 16= AARI, 17=Ihsan 16, 18=Shahkar 13, 19=Johar 2017, 20=Fatehjhang).

PCR analyses for Fusarium head blight resistance genes

In the molecular screening of Fusarium head blight resistance genes in 20 wheat varieties, two SSR markers (GWM-644 for *FHB-2* and GWM-304 for *FHB-5*) were used. The presence or absence of these genes was determined by scoring the bands obtained from PCR amplified products on gel electrophoresis, with a positive sign (+) indicating the presence of genes and a negative sign (-) indicating their absence.

Results showed that 18 out of 20 wheat genotypes had the expected amplicon size of 234 bp for *FHB-2* gene using GWM-644 primer (Figure 2 & 3). For *FHB-5* gene, amplification bands of 206 bp were detected in 11 out of 20 wheat genotypes using GWM-304 primer (Figure 4). Specifically, wheat varieties NIFA Insaf, Wadan, Pasina-2017, Israr Shaheed, Pirsabak-2013, Pirsabak-2015, Khaista-2017, NIFA Lalma, Seher-2006, Pakistan-2013, Shahkar-13, Gold-2016, and Johar-2017 showed the presence of FHB-2 gene in 90% of the varieties, while wheat varieties NIFA Aman, Wadan, Israr Shaheed, Kohat-2017, Khaista-2017, NIFA Lalma, Seher-2006, Pakistan-2013, Ihsan-16, Shahkar-13, and Fatehjang-16 showed the presence of FHB-5 gene, constituting 55% of the total 20 wheat varieties (Table 3).

Notably, 7 out of 20 wheat varieties (Wadan, Israr Shaheed, Khaista-2017, NIFA Lalma, Seher-2006, Pakistan-2013, and Shahkar-13) showed the presence of both FHB-2 and *FHB-5* genes, accounting for 20% of the total wheat varieties (Table 3).



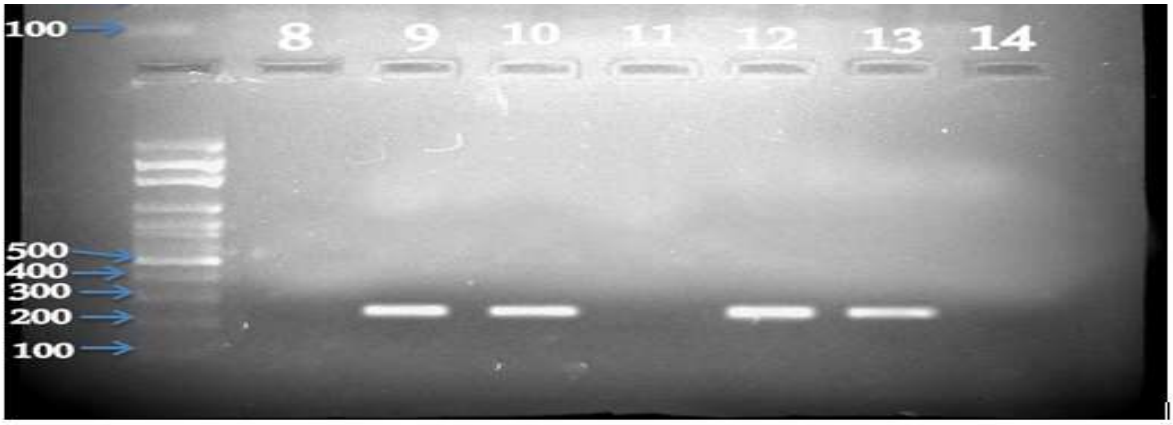


Figure 2 Band pattern of GWM-644 marker (234 bp) depicting PCR amplified products application over 2% agarose gel, for detecting Fusarium head blight gene FHB2 in wheat varieties. [L = Ladder (100 bp); Line 1-14 = Wheat varieties].

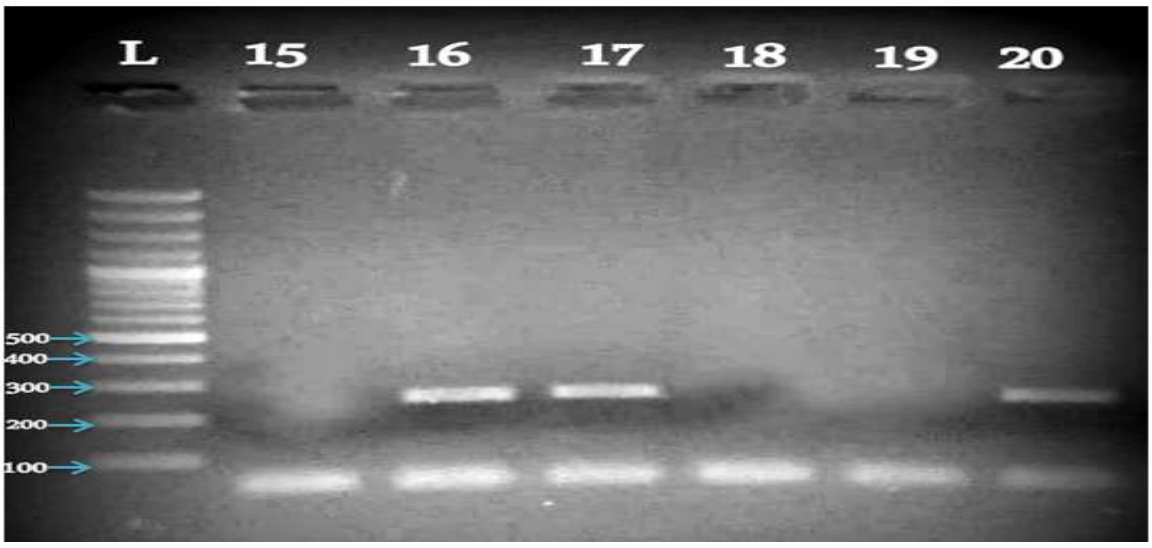


Figure 3 Band pattern of GWM-644 marker (234 bp) depicting PCR amplified products application over 2% agarose gel, for detecting Fusarium head blight gene FHB2 in wheat varieties. [L = Ladder (100 bp); Line 15-20 = Wheat varieties].

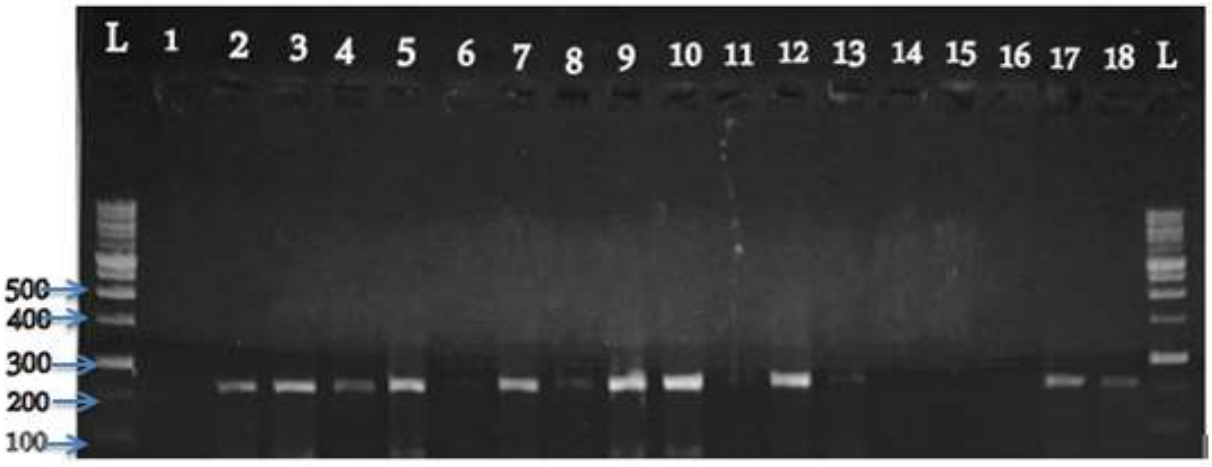


Figure 4 Band pattern of GWM-304 marker (206 bp) depicting PCR amplified products application over 2% agarose gel, for detecting Fussarium head blight resistance gene FHB5 in wheat varieties. [L = Ladder (100 bp); Line 1-18 = Wheat varieties].

Table 3 PCR based-screening of selected wheat germplasm for the presence of Fusarium head blight resistance genes

S.N	Variety	(*FHB-2*)	(*FHB-5*)
1	NIFA AMAN	-	+
2	NIFA INSAF	+	-
3	WADAN	+	+
4	PASINA 2017	+	-
5	ISRAR SHAHEED	+	+
6	KOHAT 2017	-	+
7	PIRSABAK 2013	+	-
8	PIRSABAK-2015	+	-
9	KHAISTA 2017	+	+
10	NIFA LALMA	+	+
11	AARI 11	-	-
12	SEHER 2006	+	+
13	PAKISTAN 2013	+	+
14	FAHEEM 2019	-	-
15	ZINCOL 2016	-	-
16	IHSAN 16	-	+
17	SHAHKAR 13	+	+
18	GOLD 2016	+	-
19	JOHAR 2017	+	-
20	FATEHJHANG 16	-	+

"+" presence of gene; "-" absence of gene

IV. DISCUSSION

Fusarium head blight (FHB) is a devastating fungal and economically important disease that affects bread wheat, durum wheat, and other small grain cereals (Gilbert & Tekauz, 2000). *Fusarium graminearum Schwabe* (teleomorph: Gibberella zeae (Schwein.) Petch) is the most prevalent species causing FHB in wheat (Mesterhazy 1995). FHB, also known as scab disease, has spread to crucial wheat-growing regions worldwide, posing a significant threat to wheat production since the early 20th century (Ghimire *et al*., 2020). In the United States, FHB caused an estimated economic loss of about $7.67 billion in wheat and barley from 1993 to 2001 in nine major epidemic states, including Illinois, Indiana, Kentucky, Michigan, Missouri, Minnesota, Ohio, South Dakota, and North Dakota (Nganjee *et al*., 2004). FHB is also ranked as the second most challenging disease in the US Midwest, China, Canada, south Brazil, Paraguay, Uruguay, and Argentina (Savary *et al*., 2019). FHB not only causes yield and quality losses, but it also has significant implications for food and feed safety, as the FHB pathogens contaminate grain with mycotoxins, such as trichothecenes and other toxic fungal metabolites, posing health risks to humans and animals (Dweba *et al*., 2017).

FHB resistance in wheat is quantitative and involves multiple genes. Three types of FHB resistance, type-1, type-2, and type-3, have been identified, and each plays a crucial role in combating the FHB pathogen. Type-1 provides resistance to initial infection, type-2 provides resistance to spread within the spike, and type-3 provides resistance against toxic

contamination in grain (Mesterhazy *et al*., 2005). The entire wheat chromosome has been mapped, with the exception of 7D, and approximately 100 quantitative trait loci (QTLs) for FHB resistance have been identified in wheat (Liu *et al*., 2009). Major FHB resistance genes are located on different arms of chromosomes, with Fhb1 located on chromosome 3B (QTL-*FHB1*), *FHB2* exhibiting rachis resistance located on 6B (QTL-*FHB2*), and *FHB5* located on chromosome 5A (QTL-*FHB5*). QTL-*FHB2* has been mapped as a Mendelian factor, spanning a 4.2 cM region with two simple sequence repeat (SSR) markers, GWM-133 and GWM-644, using a cross-based recombinant inbred value (RIL) between BW278 (resistant parent) and AC Foremos (susceptible parent), and accounting for 24.1% of the resistance to FHB (Cuthbert et al., 2007). Similarly, QTL-*FHB*2 was also mapped on 6BS using a doubled haploid population, which conferred 21% resistance to *FHB* (Yang *et al*., 2003).

In the present study, molecular characterization of FHB resistance using markers revealed that the primer GWM-644 linked with *FHB-2* was detected in 18 out of 20 wheat genotypes, while the primer GWM-304 linked with *FHB-5* produced amplification bands of 206 bp in 11 out of 20 wheat genotypes. Seven wheat varieties out of 20 showed the presence of both *FHB-2* and *FHB-5* genes, constituting 20% of the total wheat varieties. Zhang *et al.* (2021) conducted a study to introduce QTLs into five Chinese wheat varieties through marker-assisted selection from different ecological zones. They developed a total of 14 resistant wheat lines that showed high significance after artificial inoculation over two years. Their findings suggested that by combining FHB-resistant genes like *FHB1, FHB4*, and *FHB5* and creating a pyramid, these varieties exhibited resistance against Fusarium head blight disease. Importantly, there were no negative effects observed on plant height, number of tillers, and number of kernels per spike, 1000 grain weight, flowering time, and yield per unit area. This indicates that FHB-resistant QTLs are crucial for the production of disease-free wheat varieties using marker-assisted selection techniques. The information from this study could be valuable for Fusarium head blight control and field exploitation of these varieties, as well as for future genetic improvement of wheat to generate further variability for the development of future varieties.

V. CONCLUSION

The present study focused on the molecular characterization of Fusarium head blight resistance in recently released wheat varieties. Among the 20 studied wheat varieties, 7 (Wadan, Israr Shaheed, Khaista-2017, NIFA Lalma, Seher 2006, Pakistan-2013, and Shahkar-13) were found to possess both fusarium head blight resistant genes (*FHB-2* and *FHB-5*). These varieties are therefore recommended for future sowing purposes. The findings of this study will be valuable for breeders in terms of the crossing, selection, and deployment of resistant genes to combat Fusarium head blight disease, ultimately contributing to the improvement of wheat productivity in the country.

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