UDC 575. 630.11
https://doi.org/10.2298/GENSR2203217G
Original scientific article

ASSESSMENT OF ELITE WHEAT GERMPLASM FOR RESISTANCE TO FUSARIUM HEAD BLIGHT -A THREAT TO WHEAT PRODUCTION IN NORTH-WEST PAKISTAN

Rubina GUL[1], Muhammad FAHIM[2], Sultan Akbar JADOON[3*], Saad Hussain SHAH[1], Ijaz AHMAD[4], Masood AHMAD[5]

[1]Institute of Biotechnology and Genetic Engineering, University of Agriculture Peshawar-Pakistan 25130
[2]Center for Omic Sciences, Islamia College Peshawar-Pakistan
[3]Department of Plant Breeding and Genetics, University of Agriculture Peshawar-Pakistan
[4]Department of Plant Pathology, University of Agriculture Peshawar-Pakistan
[5]Department of Horticulture, University of Agriculture Peshawar-Pakistan

Gul R., M. Fahim, S. A. Jadoon, S. H. Shah, I. Ahmad, M. Ahmad (2022). *Assessment of elite wheat germplasm for resistance to Fusarium head blight -a threat to wheat production in North-west Pakistan*. - Genetika, Vol 54, No.3, 1217-1233.

Fusarium head blight (FHB), caused by *Fusarium graminearum*, affects both quality and quantity of wheat produce. In Pakistan, due to favorable environmental conditions during spring, FHB can cause significant losses to wheat. Recently, we observed FHB in wheat fields, having 34-84% incidence, along river Swat, Northwest Pakistan. Therefore, elite wheat cultivars and candidate lines in Pakistan as well as exotic-near isogenic lines were screened for FHB resistance using molecular markers, specific for *Fhb-1, 2* and *3*. Furthermore, all the germplasm was screened for 2NS Translocation - from *Triticum ventricosum* segment containing cluster of resistance genes for many diseases including FHB. Among Pakistani wheat varieties, Marvi-2000 showed presence of *Fhb-2* and 3 specific bands while wheat cv. Saleem-2000 displayed presence of *Fhb-1* and 2 specific bands. However, among the candidate lines, L-112, L-105, L-106, L-103 and L-129 exhibited *Fhb-1* and 2 specific bands while L-111 alone amplified bands specific to *Fhb-1* and *Fhb-3*. Moreover, 2NS translocation was validated in 2NS near isogenic lines (NILs) obtained from Kansas State University using 2-NS specific marker VENTRIUP and LN2, however, no 2-NS translocation was found in Pakistani varieties as well as candidate lines. In conclusion, none of the Pakistani varieties or candidate lines possessed all

Corresponding author: Sultan Akbar Jadoon, Department of Plant Breeding and Genetics, University of Agriculture Peshawar-Pakistan, sultan@aup.edu.pk

sources of FHB resistance in altogether; however, one aliens NIL (Yaccora-Rojo-2NS) surprisingly not only exhibited 2NS translocation but also *Fhb-1*, 2 and 3 resistant genes.

Key words: disease resistance, food security, Fusarium head blight, mycotoxin, wheat scab

INTRODUCTION

Pakistan has successfully increased its wheat production in past decades due to collaboration with various international organizations. However, the steady fast increasing population, epidemics arising from biotic stresses; the potential emerging threats from climate change and erratic weather, are some of the challenges that demand continuous work for wheat improvement in the country. Among various biotic stresses, Fusarium Head Blight (FHB) caused by *Fusarium graminearum* plays a devastating role in reducing wheat grain quantity and quality worldwide (GILBERT and HABER, 2013). The disease is prevalent in areas with frequent rainfall and humidity particularly during anthesis stage (the most susceptible stage) of wheat (JOHNSON *et al*., 1998; OSBORNE and STEIN, 2007). Besides water availability other factors like air, temperature and light also influence dispersal of inoculums of fungus (DE WOLF, 2003). This disease is widespread in Europe, North and South America and Asia, due to moist weather conditions during wheat growing season (HOLLINS *et al*., 2003). It not only affects wheat yield but also its quality by producing mycotoxins in the grains, rendering it unfit for consumption. Infected FHB kernels accumulate carcinogenic mycotoxins such as tricothecence, deoxynivalenol (DON) and its derivatives and are harmful for humans and animals (PESTKA, 2007). The only option to reduce these toxins contamination is to deploy FHB resistant germplasm in wheat production zones.

One of the best methods to reduce losses caused by FHB is to cultivate resistant varieties (ANDERSON, 2007). However, resistance to FHB is polygenic in nature (BUERSTMAYR *et al*., 2009), making the evaluation and breeding for resistance laborious and non-trivial (HUHN *et al*., 2012; MIEDANER and LONGIN, 2014). Several resistance sources to FHB have been identified in bread wheat in different countries (reviewed in detail by SHAH *et al*., 2017). However, naturally occurring FHB resistant cultivars have sometimes undesirable agronomic characteristics and, therefore, have little commercial value (ANDERSON, 2007; KOSOVA *et al*., 2009).

Few major genes responsible for FHB-resistance have been identified so far. These include *Fhb-1* and *Fhb-2*,, derived from a Chinese variety Sumai-3 (CUTHBERT *et al*., 2006; CUTHBERT *et al*., 2007) and *Fhb-3*, derived from *Leymus racemosus* (QI *et al*., 2008). A fourth source of resistance has been identified in wild relative of wheat i.e., 2NS-Translocation from *A. ventricosum*. 2-NS is a short 25-38cM segment within the Chromosome of *T. ventricosum*. It is translocated to the short arm of bread wheat chromosome 2A. This 2NS segment contains resistance genes for many diseases like leaf rust, stripe rust, stem rust etc. (HELGURE *et al*., 2003).

In the year 2015-16, FHB was spotted in Swat Valley, Northwest Pakistan, putting at risk the food and health security of the local population. A comprehensive literature search of local and international databases (NCBI), revealed no indication of research or reports of

Fusarium Head Blight in Pakistan. Unfortunately, to mitigate the threat of FHB, no systematic data was available on sources of resistances against FHB in wheat cultivars in Pakistan. The presence of Fusarium Head Blight in temperate cum sub-tropical zones of Swat Valley and identification of resistance sources in Pakistani cultivars, candidate lines and wheat alien NILs shall provide a baseline for speed breeding and pyramiding these resistances into promising wheat germplasm, to mitigate health and food security threats in coming years.

MATERIALS AND METHODS

Surveys for FHB of wheat in Northwest Pakistan

In response to a progressive farmer's complaints about a new type of disease affecting wheat heads after milking stage, in district Swat, extensive surveys of adjacent wheat fields were carried out during 2015-16 (Table 1). A total of twenty wheat fields in Barabandai, Kabal and Kanju (Swat) at heading stage were observed and data for characteristic FHB symptoms was recorded. Symptomatic wheat heads were collected randomly from each field surveyed and transported to Plant Pathology Section, at Agriculture Research Institute Mingora, Swat, in Northwest Pakistan. All the collected wheat heads were processed for causal pathogen isolation as described previously (AKBAR *et al*., 2018).

Screening of wheat germplasm for FHB resistance using molecular markers

A total of 30 wheat genotypes including 10 Pakistani varieties commonly grown on farmers field, 10 Pakistani candidate/advanced lines developed at various stations and 10 wheat-alien near Isogenic lines (NILs) from Kansas State University (USA) were screened against FHB resistance genes using molecular markers in present study (Table 1). Seeds of all these genotypes were grown in insect proofed screen house at IBGE, the University of Agriculture Peshawar.

Table 1. List of wheat genotypes studied at the University of Agriculture, Peshawar-Pakistan

S.No.	Germplasm	Source	Description
1	Nowshera-96	Cereal Crops Research Institute (CCRI), Pirsabak Nowshera-Pakistan (BUCK/FLK//MYNA/VUL CM91575-34Y-0M-0Y-1M-0Y)	Approved variety for irrigated areas
2	Marvi-2000	Nuclear Institute of Agriculture (NIA) Tandojam-Sindh (CMH-77 A917/KPV-1600//RL-6010/6*SKA)	Approved variety for irrigated areas
3	Saleem-2000	CCRI, Pirsabak Nowshera- Pakistan (CHAM-6//KITE/PGO ICW 93-0032-7F-0K-0F)	Approved variety for irrigated areas
4	Pirsabaq-2013	CCRI), Pirsabak Nowshera- Pakistan (CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI CMSS97M04005T-040Y-020Y-030M-020Y-040M-28Y-3M-0Y)	Approved variety for irrigated and Rainfed areas

5	Khyber-87	CCRI), Pirsabak Nowshera- Pakistan (KVZ/TRM//PTM/ANA-CM 43930 CM 43903-H-4Y-1M-1Y-3M-2Y-0B }	Approved variety for irrigated and Rainfed areas
6	Bakhtawar-92	Nuclear Institute for Food and Agriculture (NIFA)- Peshawar (JUP/BJYG//URES CM 67458-4Y-1M-3Y-1M-5Y-0B)	Approved variety for irrigated areas
7	Janbaz	Deptt. Of Plant Breeding and Genetics, The University of Agriculture Peshawar- Pakistan (GEN*2//BUC/FILK/3/BUCHIN)	Approved variety for irrigated areas
8	Fakhr-e-Sarhad	Nuclear Institute for Food and Agriculture (NIFA)- Peshawar (PFAU"S"/SERI/BOW "S" CM85295-010-TOPY-2M-0Y-0M-3Y-0M)	Approved variety for irrigated areas
9	Barsat	Nuclear Institute for Food and Agriculture (NIFA)- Peshawar (FRET2 CGSS96Y00146T-099B-099Y-099B-16Y-0B-0SY)	Approved variety for irrigated areas
10	Shahkar-2013	Cereal Crops Research Institute (CCRI), Pirsabak Nowshera-Pakistan (CMH84.3379/CMH78.578// MILAN CMSS93Y006285-7Y-010Y-010M-010Y-10M0Y-3KBY-0KBY)	Approved variety for irrigated areas
11	L-111	Cereal Crops Research Institute (CCRI), Pirsabak, Nowshera-Pakistan	Advanced wheat line
12	L-112	CCRI Pirsabak, Nowshera- Pakistan (YAV79//DACK/RABI/3/SNIPE/4/ AE. SQUARROSA CMSA04Y00649S-028CRE1Y-010M-3SY-03CRE1Y-010M-03Y-0B)	Advanced wheat line approved as ‘Wadan-17’ for sowing in Rain-fed areas
13	L-113	CCRI Pirsabak, Nowshera- Pakistan	Advanced wheat line
14	L-105	CCRI Pirsabak, Nowshera- Pakistan	Advanced wheat line
15	L-106	CCRI Pirsabak, Nowshera- Pakistan (MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4 CMSA02M00279S-040ZTM-040ZTY-040ZTM-040SY-1ZTM-02Y-0B)	Advanced wheat line now approved as ‘Pasina-17’ a short season variety for late sown areas
16	L-103	CCRI Pirsabak, Nowshera- Pakistan	Advanced wheat line
17	L-115	CCRI Pirsabak, Nowshera- Pakistan	Advanced wheat line
18	L-110	CCRI Pirsabak, Nowshera- Pakistan (KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES/7/ CAL/NH//H567.71/3/SERI/4/CAL/ NH//H567.71 /5/2*KAUZ/6/PASTOR CMSS05B00581S-099Y-099M-099Y-	Advanced wheat line approved as ‘Khaista-17’ for sowing in irrigated areas

		099ZTM-2WGY-0B)	
19	L-116	CCRI Pirsabak, Nowshera- Pakistan	Advanced wheat line
20	L-129	CCRI Pirsabak, Nowshera- Pakistan	Advanced wheat line
21	Kern-2NS	Kansas State University (USA)	NIL
22	Kern	Kansas State University (USA)	NIL
23	Anza-2NS	Kansas State University (USA)	NIL
24	Anza	Kansas State University (USA)	NIL
25	Express-2NS	Kansas State University (USA)	NIL
26	Express	Kansas State University (USA)	NIL
27	Yaccora-Rojo-2NS	Kansas State University (USA)	NIL
28	Yaccora-Rojo	Kansas State University (USA)	NIL
29	UC-1037-2NS	Kansas State University (USA)	NIL
30	UC-1037	Kansas State University (USA)	NIL

Molecular marker analysis of wheat germplasm for FHB resistance

Fresh leaves were collected from each genotype for DNA extraction using CTAB protocol (MURRAY and THOMPSON, 1980). For identification and confirmation of FHB resistance in germplasm, a total set of three FHB resistance markers i.e. *Fhb-1F* (ATCATGTCGATCTCCTTGACG) and *Fhb-1R* (TGCCATGCACATTAGCAGAT) primer which amplify 140bp region in germplasm having *Fhb-1* resistance, *Fhb-2F* (TGGTAAAGTCCCTTGMTGAAA) and *Fhb-2R* (GCACCGTTTGTTGACCATCAT) amplify 2100bp region in genotypes having *Fhb-2* resistance and the third primer *Fhb-3F* (CACACGCTCCACCATGAC) and *Fhb-3R* (GTTGAGTTGATGCGGGAGG) give positive bands at 220bp in selected germplasm having *Fhb-3* resistance, were used (PROCUNIER *et* al., 2001). Likewise for identification of 2-NS segment in all selected genotypes 2-NS specific primers VENTRIUP (AGGGGCTACTGACCAAGGCT) and LN-2 (TGCAGCTACAGCAGTATGTACACAAAA) were used (DE FROIDMONT, 1998; HELGUERA *et al*., 2003).

DNA amplification was performed in PCR tubes with 25µl reaction mixture including 0.2µl of Taq Polymerase enzyme, 2.5µl of dNTPs, 25mM $MgCl_2$, 5µl of PCR buffer, 10µl of PCR water, 1µl of each forward and reverse primer and 2µl of template DNA (100ng/µl). The PCR was carried out for; an initial denaturation at 95ºC for 5 min, followed by 38 cycles of denaturation at 95ºC for 40sec, annealing for 30 sec and extension at 72ºC for 40 sec. PCR reaction was terminated by final extension at 72ºC for 10 min and the obtained PCR product were resolved in 2-3% agarose gel stained with ethidium bromide. The gel was then visualized

using Gel documentation system. For analysis scoring of the gel pictures was done and missing values were marked as "0" and the resultant dendograms were obtained using R package.

RESULTS

Incidence of FHB in Northwest Pakistan

Initial survey indicated FHB was limited to wheat cv. Atta Habib (a recently introduced high yielding variety) in villages Kanju, Kabal and Bara-Bandai area of district Swat. Of the total 20 fields surveyed, cv. Atta Habib was being grown on 9 fields and in these fields FHB incidence ranged from 34-84% (Figure 1). Other cultivars grown in the area were Pirsabaq-05, Pirsabaq-13, Pirsabaq-15 and Seher. Interestingly, all of these cultivars were found free from FHB infection. Based on morpho-cultural and microscopic characters, the isolated fungus was identified as Fusarium spp.

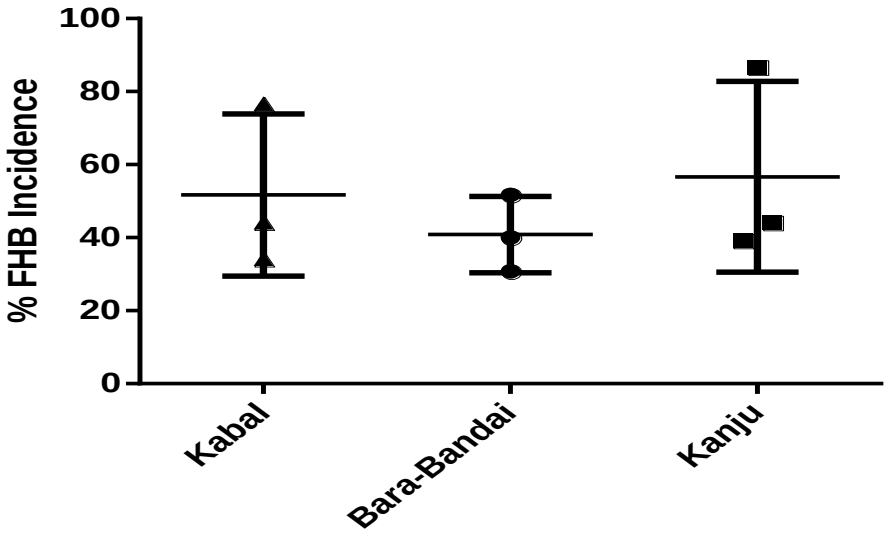

Figure 1. Incidence of Fusarium Head Blight in Northwest Pakistan

Molecular Analysis of Wheat Germplasm for Resistance against FHB

Based on surveys conducted, it can be inferred that Wheat cv. Atta Habib was found highly susceptible to FHB at the reported sites. Molecular markers specific for 2-NS translocation as well as markers for FHB resistances (*Fhb1, Fhb2 and Fhb3*), did not amplify corresponding PCR amplifications. Therefore, studies were then extended to elite Pakistani cultivars, candidate/advanced lines and exotic NILs of 2NS translocation.

Marker based screening of commonly grown Pakistani varieties for FHB resistances

Among the 10 Pakistani wheat varieties only Saleem-2000 was found carrying *Fhb-1* resistance gene by giving a positive band at 140bp while rest of the varieties showed no bands (Fig. 2a). Likewise, when PCR conditions were used for *Fhb-2* marker to amplify 2100bp segment in Pakistani varieties, five out of 10 wheat varieties (Marvi-2000, Saleem-2000, Janbaz, Fakhr-e-Sarhad and Shahkar-2013) reflected positive bands for *Fhb-2* resistance (Fig. 2b). Similarly, another five varieties i.e. Nowshera-96, Marvi-2000, Pirsabak-2013, Khyber-87 and Bakhtawar-92 were found harboring *Fhb-3* resistance genes by displaying a band of approx. 220bp using *Fhb-3* marker. The rest of the varieties showed no amplification with *Fhb-3* marker in PCR (Fig. 2c).

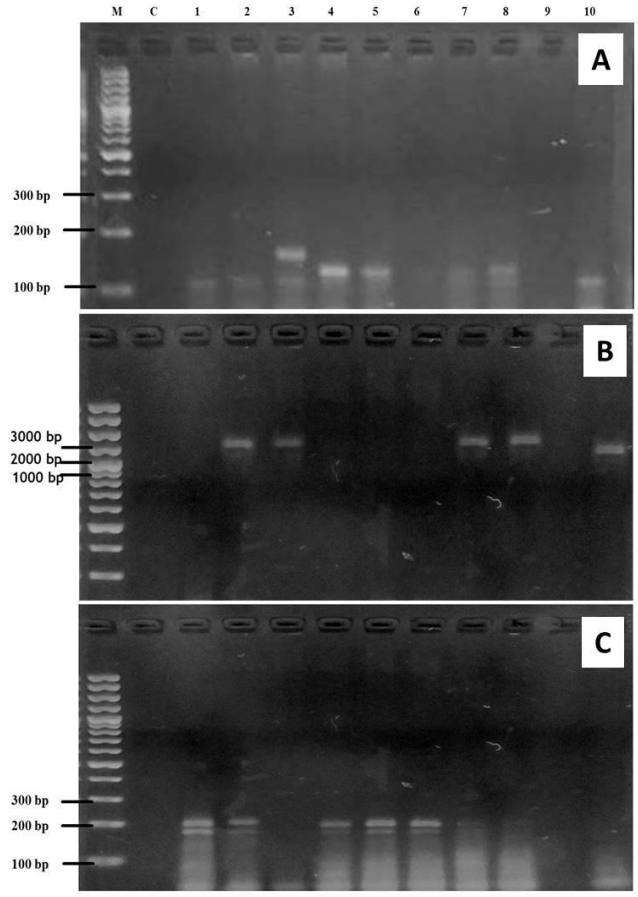

Figure 2. PCR amplification profile of primers (A) Fhb-1, (B) Fhb-2 and (C) Fhb-3 separated on 2, 1 and 2.5% Agarose gel, respectively. M = DNA ladder 100bp, C = negative control and 1 to 10 represents Pakistani varieties.

Screening of candidate wheat lines for FHB resistances

Fhb-1 resistance gene specific band of 140bp was observed in all candidate lines used (111, 112, 113, 105, 106, 103, 115, 110, 129) except line 116 (Fig. 3a). However, *Fhb-2* resistance gene was detected only in lines 102, 105, 106, 103, 116 and 129. In these lines, PCR band of approx. 2100bp in size was amplified showing presence of *Fhb-2* resistance gene (Fig. 3b). Surprisingly, among the ten advanced lines only one line (L-111) showed presence of band at 220bp and thus conferred *Fhb-3* resistant gene (Fig. 3c).

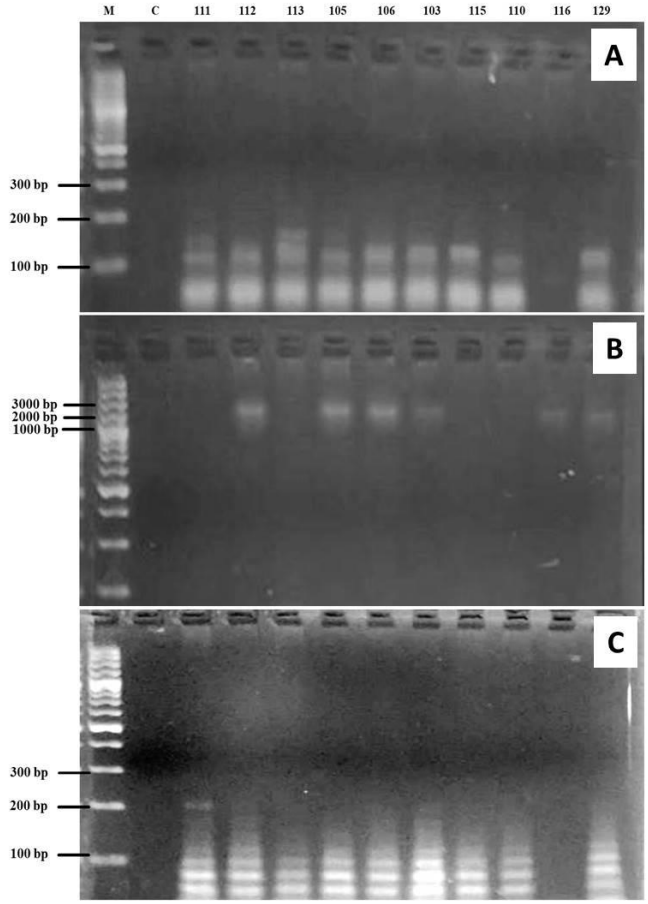

Figure 3. PCR amplification profile of primers (A) Fhb-1, (B) Fhb-2 and (C) Fhb-3 separated on 2, 1 and 2.5% Agarose gel, respectively. M = DNA ladder 100bp, C = negative control and 111 to 129 represents Pakistani advanced/candidate lines.

Screening of wheat-alien Near Isogenic Lines for FHB resistance

Among the exotic NILs, only two lines (Kern-2NS and Yaccora-Rojo-2NS) showed positive banding pattern for *Fhb-1* primer at 140bp position while all other NILs produced no bands indicating absence of *Fhb*-1 resistance gene (Fig. 4a). Similarly 5 of the near isogenic lines (Kern-2NS, Anza-2NS, Express-2NS, Yaccora-Rojo-2NS and UC-1037-2NS) also produced bands at 2100bp indicating presence of *Fhb-2* resistance gene while the other lines having no 2-NS segment did not produce any band at 2100bp (Fig. 4b). Likewise the *Fhb-3* resistance gene was detected in all the NILs except Kern-2NS which gave no band at 220bp as clearly shown in figure 4c.

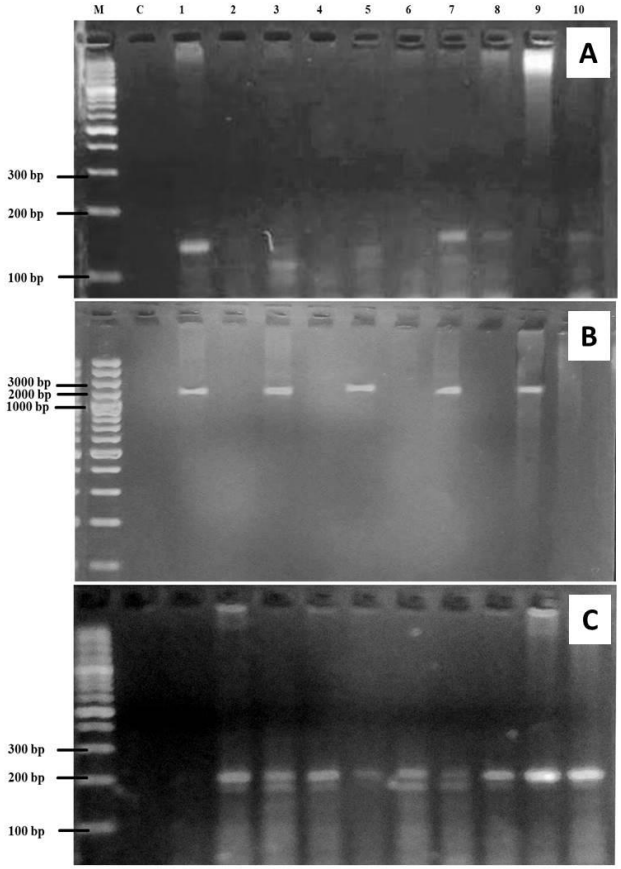

Figure 4. PCR amplification profile of primers (A) Fhb-1, (B) Fhb-2 and (C) Fhb-3 separated on 2, 1 and 2.5% Agarose gel, respectively. M = DNA ladder 100bp, C = negative control and 1 to 10 represents exotic Near Isogenic lines

Screening of Pakistani cultivars, candidate lines and exotic NILs for 2-NS Translocation

Using 2-NS specific marker, none of the Pakistani varieties as well as candidate lines gave positive results for 2-NS translocation. These results indicated that 2-NS segment was absent in all of these wheat genotypes/lines (Fig. 5a and b). However, five of the ten exotic NILs from Kansas State University (USA) exhibited 2-NS Translocation and a specific band of approx. 259bp was amplified in five NILs i.e. Kern-2NS, Anza-2NS, Express-2NS, Yaccora-Rojo-2NS and UC-1037-2NS (Fig. 5c).

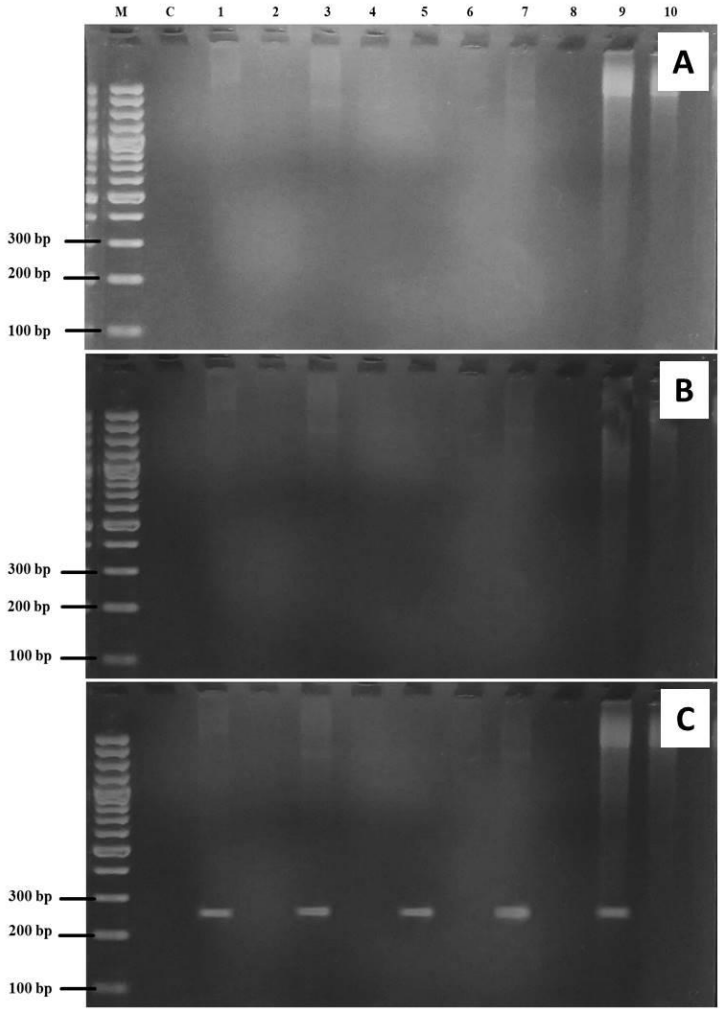

Figure 5. PCR amplification of VENTRIUP/LN2 for (A) ten Pakistani Varieties, (B) ten advanced lines and (C) ten exotic NILs on 2% Agarose gel, M = Marker 100bp, C = negative control.

Cluster Analysis

Cluster analysis was performed for Pakistani varieties, candidate lines as well as exotic lines in order to find out relationship between the respective groups on the basis of four selected primers. Analysis was carried out from binary scoring of the amplifications of the four primers. Dendrogram showed two main clads for Pakistani varieties, each of them further divided into two sub-clads. Pirsabak-2013, Nowshera-96, Khyber-87 and Bakhtawar-92 were found to be residing in one sub-clad showing their close relationship on the basis of four loci (*Fhb-1, Fhb-2, Fhb-3* and 2NS) with same scoring. Similarly in the other clad Fakhr-e-Sarhad, AUP-5008 and Saleem-2000 were occupying single sub-clad with similar scoring. Shahkar-2013 seemed to be more related to the upper clad with Fakhr-e-Sarhad, AUP-5008 and Saleem-2000 while Barsat and Marvi-2000 were more related to the lower clad (Fig. 6a).

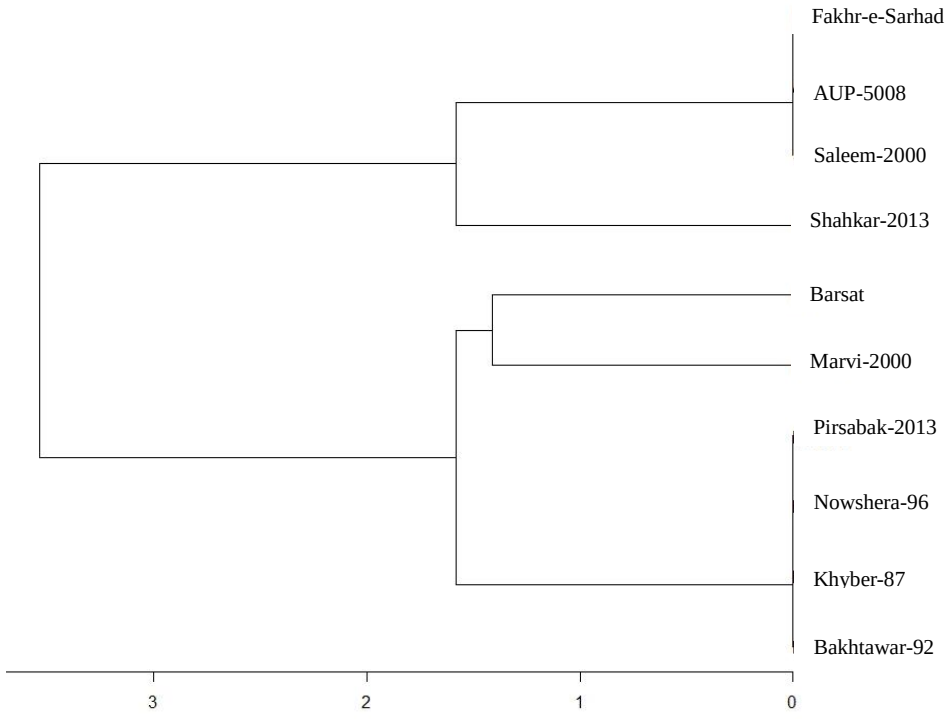

Figure 6(a). Dendrogram constructed for Pakistani varieties from binary scoring of PCR amplification profile of primers; *Fhb-1, Fhb-2, Fhb-3 and 2NS*.

In case of candidate/advanced wheat lines all except L-116 and L-111 appeared in a single clad. In this clad further L-110 was separated from others right after initial clad division while the other lines further formed two sub-clads. Upper sub-clad consisted of L-115 and L-113 while lower sub-clad possessed L-103, L-105, L-106, L-112 and L-129 with the same scoring. It suggests that line L-111 and L-116 are closely related for the four primers used (Fig. 6b).

Dendrogram analysis for alien germplasm also produced two main clads which completely separated 2-NS lines from Non-2NS (Fig. 6c). The clad having 2-NS lines is further divided into two sub clades. One sub-clade has K-2NS and YC-2NS while other has A-2NS, E-2NS and UC-2NS. It suggests that K-2NS and YC-2NS are more closely related to one another as compared to other 2-NS lines on the basis of four studied primers (Fig. 6c).

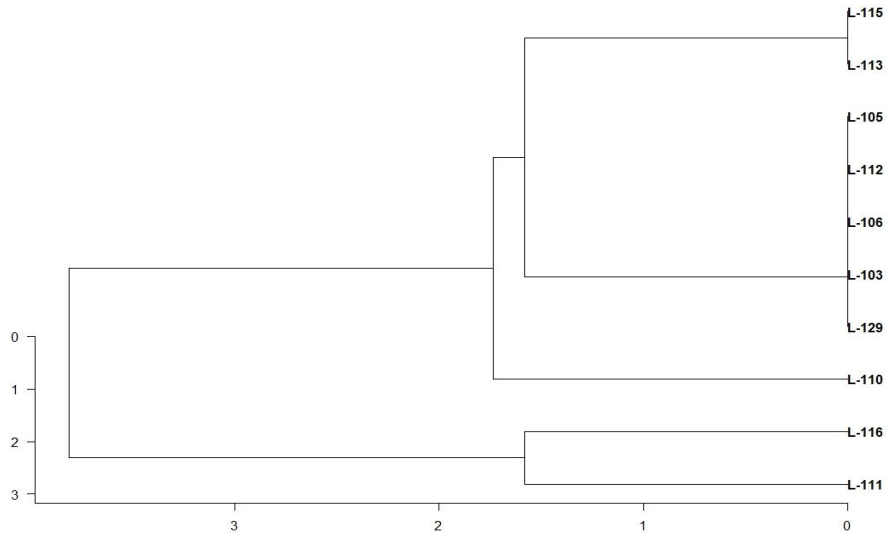

Figure 6(b). Dendrogram constructed for advanced wheat lines from binary scoring of PCR amplification profile of primers; *Fhb-1, Fhb-2, Fhb-3 and 2NS*.

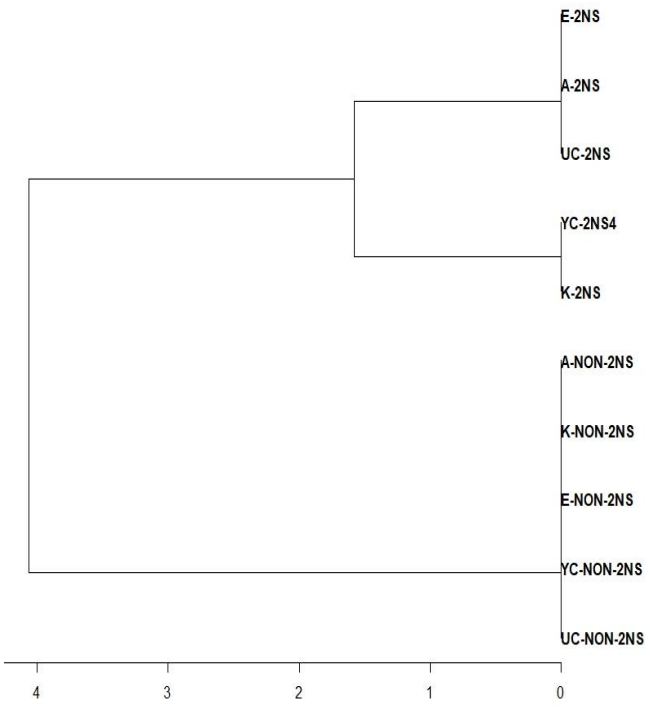

Figure 6(c). Dendrogram constructed for 2NS lines from binary scoring of PCR amplification profile of primers; *Fhb-1, Fhb-2, Fhb-3 and 2NS*.

DISCUSSION

Fusarium head blight (FHB) is caused by *Fusarium graminearum***,** directly affects quantity and quality of the final produce. The quality deterioration is mainly attributed to mycotoxins (e.g., deoxynivalenol, DON, a group 3 carcinogenic toxin) that are serious threat to health security at a concentration of 2000ppb (LI *et al*., 2016) and renders the crop unfit for consumption (DE WOLF, 2003). The disease first time observed in 1989 and caused severe epidemic in USA from 1993-1997 (JOHNSON *et al*., 1998). Although, the FHB is a relative new diseases in in Pakistan, the need for assessment of available elite cultivars prompted us to screen selected wheat germplams for presence of resistance using molecular markers. As FHB is a fungal disease and can easily spread, therefore, it can be of economic importance in Pakistan due to recent shift in climatic change and more importantly, a potential threat for our staple food (MUZAMIL *et al*., 2021; ISLAMIC RELIEF, 2022).

Typical FHB symptoms including premature bleaching of spikelets and reports of *Fusariums graminearum* from other crops in the area (AKBAR *et al*., 2018) indicate that the disease is relatively a new introduction but may become a serious threat in the near future. FHB

was predominantly detected on Wheat cv. Ata-Habib. The lack of resistance, mono-culturing and the use of infected seeds may lead to development of FHB epidemics in near future.

Since, FHB has no known record of endemics or losses in Pakistan, the national breeding program is mainly reliant on CIMMYT germplasm for its wheat improvement against leaf, stem and stripe rust. Therefore, as first line of defense against FHB, the genetic architecture of commonly cultivated wheat varieties, advanced breeding lines and 2NS-carrying near isogenic lines were screened for presence of known FHB resistance using molecular markers. The reported resistances against FHB, including *Fhb-1* gene, *Fhb-2* gene and *Fhb-3* gene (PROCUNIER *et al*., 2001) were found in various combinations in Pakistani wheat. Assessment of wheat cultivars revealed that Saleem-2000 carried *Fhb-1* and *Fhb-2* resistance marker while cv. Marvi-2000, cv. AUP-5008, cv. Fakhr-e-Sarhad and cv. Shahkar-2013 carried *Fhb-2* resistance. Similarly five Pakistani varieties cv. Nowshera-96, cv. Marvi-2000, cv. Pirsabaq-2013, cv. Khyber-87 and cv. Bakhtawar-92 amplified *Fhb-3* resistance marker. On the other hand, cv. Marvi-2000 amplified *Fhb-2* and *Fhb-3* markers but not to *Fhb-1*. Among various reported resistances, *Fhb-1* alone accounts for about 20-25% of total resistance (ANDERSON, 2007). This data suggest that breeding of these varieties should be done so that we can have all the three source of resistance in a single Pakistani variety as naturally resistant wheat varieties against FHB have undesirable agronomic characteristics (ANDERSON, 2007). Recently, it has been reported that *Fhb1*, together with newly identified *Fhb4* and *Fhb5* can reduce the disease severity by 95% (ZHANG *et al*., 2021).

Furthermore, we screened newly developed wheat cultivars from different wheat research stations for FHB resistance markers. The newly developed wheat advanced lines also carried *Fhb-1*, *Fhb-2* and *Fhb-3* resistance markers. We therefore recommend that marker assisted selection (MAS) shall be included in National Breeding Programs for pyrimiding of resistances in elite wheat cultivars. This study also involved a fourth source of uncharacterized resistance to FHB disease i.e. 2-NS translocation. This 2-NS segment contains cluster of genes having resistance genes for many diseases like Lr37, Yr17 and Sr38 etc. (HELGURE *et al*., 2003). At present no work has been reported on 2-NS in Pakistan. Using 2-NS specific primers we screened the Pakistani varieties and candidate lines for 2-NS region but none of them showed positive results. To identify the role of 2-NS region in FHB resistance our study includes 2-NS alien Near Isogenic Lines as exotic germplasm. When these 2-NS lines were screened for FHB resistance the result was outstanding as Yaccora-Rojo-2NS line showed resistance to all the three types i.e. *Fhb-1*, *Fhb-2* and *Fhb-3*. This suggests that 2-NS region is having FHB resistance as well and transfer of this segment through breeding and backcrossing could develop isogenic wheat lines carrying 2-NS that would be having resistance to rust as well as FHB. Moreover, presence of 2NS region in studied exotic 2NS-NILs was confirmed through PCR analysis using VENTRIUP/LN-2 molecular maker. The results clearly displayed presence of band at 259bp in 2-NS positive lines. These results get support from Helguera, et al (2003) who obtained same banding pattern at 259bp for 2-NS positive lines using VENTRIUP/LN-2 molecular marker. Moreover, VALERIE *et al*. (2016) reported that 2-NS segment in bread wheat carry "*Rkn-3*" gene conferring resistance to root knot nematode disease as well.

This study concludes that marker-assisted evaluation of germplasm can be a fast-track tool to mitigate newly identified diseases in developing countries like Pakistan. However, a

concrete effort at national scale is required for pyrimiding various resistance sources into elite germplasm. Furthermore, 2NS-translocation from *A. ventricosum* as potential source of resistance against FHB and newly developing wheat blast disease in south Asia shall be included in national wheat breeding program.

Received, December 10th, 2021
Accepted November 28th, 2022

REFERENCES

AKBAR, A., S., HUSSAIN, K., ULLAH, M., FAHIM, G.S., ALI (2018): Detection, virulence and genetic diversity of *Fusarium* species infecting tomato in Northern Pakistan. PLoS ONE, *13*(9): e0203613.

ANDERSON, J.A. (2007): Marker-assisted selection for Fusarium head blight resistance in wheat. Int. J. Food Microbiol., *119(1-2)*: 51–53.

BUERSTMAYR, H., T., BAN, J.A., ANDERSON (2009): QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed., *128*: 1–26.

CUTHBERT, P.A., D.J., SOMERS, J., THOMAS, S., CLOUTIER, A., BRÛLÉ-BABEL (2006): Fine mapping Fhb1, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). TAG, *112*:1465–1472.

CUTHBERT, P.A., D.J., SOMERS, A., BRÛLÉ-BABEL (2007): Mapping of Fhb2 on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.). TAG, *114:*429–437.

DE FROIDMENT (1998): A co-dominant marker for the 1BL/1RS wheat-rye translocation *via* multiplex PCR. J. Cereal Sci., *27:* 229-232

DE WOLF, E.D., L.V., MADDEN, P.E., LIPPS (2003): Risk assessment models for wheat fusarium head blight epidemics based on within-season weather data. Phytopathology, *93(4)*: 428–435.

GILBERT, J., S., HABER (2013): Overview of some recent research developments in Fusarium head blight of wheat. Can J Plant Pathol., *35*:149–74.

HELGUERA, M., I.A., KHAN, J., KOLMER, D., LIJAVETZKY, L., ZHONG-QI, J., DUBCOVSKY (2003): PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci., *43:* 1839-1847.

HOLLINS, T. W., P., RUCKENBAUER, H., DE JONG (2003): Progress towards wheat varieties with resistance to Fusarium head blight. Food Control., *14:*239–244.

HUHN, M., E., ELIAS, F., GHAVAMI, S., KIANIAN, S., CHAO, S., ZHONG, M., ALAMRI, A., YAHYAOUI, M., MERGOUM (2012): Tetraploid Tunisian wheat germplasm as a new source of Fusarium head blight resistance. Crop Sci., *52:*136–145

ISLAMIC RELIEF (2022): Pakistan Monsoon Floods-2022; Available online at https://reliefweb.int/report/pakistan/pakistan-monsoon-floods-2022-29-august-2022.

JOHNSON, D., G.K., DEMCEY, R.D., FLASKERUD, V., TAYLOR, SATYANARAYANA (1998): Economic impact of Fusarium Head Blight in wheat. Agricultural Economics Report No. 396, Department of Agricultural Economics, North Dakota State University, Fargo, USA.

KOSOVÁ, K., J., CHRPOVÁ, V., ŠÍP (2009): Cereal Resistance to Fusarium Head Blight and Possibilities of its Improvement through Breeding. Czech J. Genet Plant Breed., *45(3):* 87–105.

LI, T., D.D., ZHANG, X.L., ZHOU, G.H., BAI, L., LI, S.L., GU (2016): Fusarium head blight resistance loci in a stratified population of wheat landraces and varieties. Euphytica, *20*: 551-561

MIEDANER, T, C.F.H., LONGIN (2014): Genetic variation for resistance to Fusarium head blight in winter durum material. Crop Pasture Sci., *65*:46–51.

MURRAY, M.G., W.F., THOMPSON (1980): Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., *8(19):* 4321–4326

MUZAMIL, M.R., P., TSCHAKERT, B., BORUFF (2021): An extreme climatic event and systemic vulnerabilities in the face of conflict: insights from the Taliban insurgency in Swat, Pakistan.

OSBORNE, L.E., J.M., STEIN (2007): Epidemiology of Fusarium head blight on small-grain cereals. Int. J. Food Microbiol., *119*:103–108.

PBS (2018): Pakistan Bureau of Statistics, Statistics House, Islamabad-Pakistan.

PESTKA, J.J. (2007): Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim Feed Sci Technol., *137 (3-4)*: 283-298.

PROCUNIER, J.D., J., GILBERT, T.F., TOWNLEY-SMITH, MANITOBA, K., ARMSTRONG, G., FEDAK (2001): Markers for Fusarium Head Blight (Fhb) disease resistance. United States Patent.

QI, L., M., PUMPHREY, B., FRIEBE, P., CHEN, B., GILL (2008): Molecular cytogenetic characterization of alien introgressions with gene Fhb3 for resistance to Fusarium head blight disease of wheat. TAG, *117*:1155–66.

SHAH, L., A., ALI, Y., ZHU, S., WANG, H., SI, C., MA (2017): Wheat resistance to Fusarium head blight and possibilities of its improvement using molecular marker-assisted selection. Czech J. Genet. Plant Breed., *53(2)*: 47-54.

WEINING, S., P., LANGRIDGE (1991): Identification and mapping of polymorphism in cereals based on polymerase chain reaction. TAG, *82*:209–216.

ZHANG, Y., Z., YANG, M.A., HUANG, L., DING, F., DU, Y., JIA, H., LI, G., KONG, Z., RAN, C., GU, Z., MA (2021): Pyramiding of Fusarium Head Blight Resistance Quantitative Trait Loci, *Fhb1, Fhb4*, and *Fhb5*, in Modern Chinese Wheat Cultivars. Front. Plant Sci., 12:694023.

PROCENA ELITNE GERMPLAZME PŠENICE ZA OTPORNOST NA FUZARIOZE KLASA – OPASNOST ZA PROIZVODNJU PŠENICE U SEVERO-ZAPADNOM PAKISTANU

Rubina GUL[1], Muhammad FAHIM[2], Sultan Akbar JADOON[3*], Saad Hussain SHAH[1], Ijaz AHMAD[4], Masood AHMAD[5]

[1]Institut za biotehnologiju i genetički inženejring, Univerzitet za poljoprivredu Peshawar-Pakistan
[2]Centar za Omik nauku, Islamia Coledž Peshawar-Pakistan
[3]Departmant za oplemenjivanje bilja i genetiku, Univerzitet za poljoprivredu Peshawar-Pakistan
[4]Departmant za biljnu patologiju, Univerzitet za poljoprivredu Peshawar-Pakistan
[5]Departmant za hortikulturu, Univerzitet za poljoprivredu Peshawar-Pakistan

Izvod

Fuzarijumska pegavost (FHB), koju izaziva *Fusarium graminearum*, utiče i na kvalitet i na količinu prinosa pšenice. U Pakistanu su povoljni uslovi životne sredine tokom proleća, ali FHB može prouzrokovati značajne gubitke pšenice. Nedavno smo primetili FHB u poljima pšenice, sa 34-84% učestalosti, duž reke Svat, severozapadni Pakistan. Stoga su elitne sorte pšenice i kandidatske linije u Pakistanu, kao i egzotične skoro izogene linije, testirane na otpornost na FHB korišćenjem molekularnih markera, specifičnih za *Fhb-1, 2* i *3*. Štaviše, sva germplazma je pregledana na 2NS translokaciju - iz segmenta *Triticum ventricosum* koji sadrži klaster gena rezistencije za mnoge bolesti uključujući FHB. Među pakistanskim sortama pšenice, Marvi-2000 je pokazao prisustvo *Fhb-2* i *3* specifične trake, dok je pšenica cv. Saleem-2000 je pokazala prisustvo *Fhb-1* i *2* specifičnih opsega. Međutim, među kandidatskim linijama, L-112, L-105, L-106, L-103 i L-129 su pokazivali *Fhb-1* i *2* specifične trake, dok je samo L-111imala trake specifične za *Fhb-1* i *Fhb-3*. Štaviše, 2NS translokacija je potvrđena u 2NS kod izogenih linija (*NIL*) dobijenih sa državnog univerziteta Kanzas koristeći 2-NS specifične markere VENTRIUP i LN2, međutim, nije pronađena 2-NS translokacija u pakistanskim varijantama, kao ni u kandidatskim linijama. U zaključku, nijedna pakistanska sorta ili kandidatska linija nije imala sve izvore otpornosti na FHB u celini; međutim, iznenađujuće jedna izogena linija (Iaccora-Rojo-2NS) ne samo da je pokazla 2NS translokaciju već je sadržala i *Fhb-1, 2* i *3* gene za otpornost.

Primljeno 10.XII.2021.
Odobreno 28. XI. 2022.