Indian Journal of Agricultural Sciences **93** (1): 30–35, January 2023/Article
https://doi.org/10.56093/ijas.v93i1.129567

Characterization of wheat (*Triticum aestivum*) genotypes for multiple fungal resistance using functional markers

BHARAT GARG[1], SHIKHA YASHVEER[1]*, JYOTI TAUNK[2], VIKRAM SINGH[1], NEERU SINGH REDHU[1], JAYANTI TOKAS[3], SAPNA GREWAL[4] and SHALINI MALHOTRA[5]

Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana 125 004, India

Received: 28 October 2022; Accepted: 6 December 2022

ABSTRACT

Wheat (*Triticum aestivum* L.) encounters 15–20% yield loss due to fungal diseases. A study was carried out to analyse the allelic variations in functional genes associated with multiple fungal disease resistance, viz. rusts, smuts and powdery mildew in 58 contrasting wheat genotypes. The experiments were conducted at Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana during 2020–21. A set of 29 simple sequence repeat (SSR) markers was selected for screening, out of which 24 markers showed amplifications (82.7%) and 23 showed polymorphism (95.83%) with a total of 46 alleles. Alleles per locus varied from 1 to 3 with a mean of 1.96 alleles per locus. At a similarity coefficient of 0.66, dendrogram grouped all the genotypes into 2 major clusters. Two and three dimensional plots also confirmed the distribution. Results showed that genotypes PBW 725 and WH 1268 were found to be most diverse at a similarity coefficient of 77%. SSR polymorphism rates were analysed using polymorphism information content, expected heterozygosity, marker index, discriminating power and resolving power values, where first two ranged from 0.03–0.65, and later three ranged from 0.03–1.94, 0.03–0.66 and 0.03–2.00, respectively. Based on these results, 8 proficient markers, viz. Barc232, Swm271, Xbarc124, Xbarc32, Xwmc44, Xgwm296, Gpw5029 and Xwmc557 are suggested for Indian wheat fungal disease resistance profiling. Among these, first two markers (Barc232 and Swm271) were detected in most (57) of the genotypes which are associated with *ut6* and *Lr75* genes, providing resistance to loose smut and leaf rust, respectively. This study can further help in gene pyramiding for producing multiple disease resistant genotypes.

Keywords: Functional markers, Fungal, Rust, Smut, Simple sequence repeats, Wheat

Wheat (*Triticum aestivum* L.) is the world's staple crop, with a production of 774.8 million tonnes (FAO 2021). Wheat production must be increased by 40–60% to fulfill the developing world's expanding requirements (Goutam *et al.* 2015). However, farmers lose huge quantity of their produce annually due to biotic stresses. Wheat infections instigated by fungal pathogens are quite ominous as they cause 15–20% yield loss per annum (Figueroa *et al.* 2018). The most catastrophic consequences on wheat are produced by rust diseases. Wheat stem rust, leaf rust and stripe rust are caused by *Puccinia graminis f.* sp. *tritici*, *Puccinia triticina* and *Puccinia striiformis*, respectively. The rust pathogen exists in two hosts, viz. economic host and alternate host; and wheat is its economic host. Amid the three mentioned rusts, leaf rust is globally present and cause up to 50% loss (Huerta-Espino *et al.* 2011). Fungal control following conventional approaches is simple and efficient, but causes environmental pollutions. Therefore, the best way for genetic control of these diseases is through pyramiding of resistance genes. Screening of genotypes is prerequisite for accomplishing this, which can be achieved through morphological, biochemical and molecular approaches.

Implementation of molecular tools in plant breeding is the most popular strategy among these (Bagge *et al.* 2007). Numerous markers have been assessed and used for marker assisted selection (MAS), among which simple sequence repeats (SSRs) are commonly used. However during MAS, recombination of molecular markers up to some extent can lead to disassociation of target allele and marker, so functional markers (FMs) are used nowadays (Kage *et al.* 2016). Polymerase chain reaction (PCR)-based FMs generated from gene sequences give reliable and high throughput data for identifying allelic compositions (Liu *et al.* 2012). Taking into consideration the importance of

[1]College of Biotechnology, CCS Haryana Agricultural University, Hisar, Haryana; [2]University Centre for Research and Development, Chandigarh University, Mohali, Punjab; [3]College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar, Haryana; [4]Guru Jambheshwar University of Science and Technology, Hisar, Haryana; [5]Pt Jawahar Lal Nehru Government College, Faridabad, Haryana (Maharishi Dayanand University (MDU), Rohtak). *Corresponding author email: shikhayashveer@gmail.com

these markers, present investigation was planned to analyse the allelic variations of fungal disease resistant functional genes in wheat genotypes, which can further help in gene pyramiding for producing multiple disease resistant wheat genotypes.

MATERIALS AND METHODS

An experiment was conducted at the Department of Molecular Biology, Biotechnology and Bioinformatics, Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar, Haryana during 2020–21. Fifty eight genotypes (Table 1) [susceptible/resistant] were screened for multiple fungal resistant alleles.

Molecular markers: Twenty nine SSR markers (Table 2) were chosen for screening genotypes for multiple fungal resistance alleles, based on linkage with specific rust resistance genes. Primer sequences were obtained from GrainGenes (https://wheat.pw.usda.gov/GG3/).

Genomic DNA extraction and PCR amplification: Genomic DNA was extracted from the fresh leaves of 58 wheat genotypes using Cetyltrimethyl ammonium bromide (CTAB) method of Saghai Maroof *et al.* (1984). PCR was executed in Biorad benchtop thermal cycler in a reaction mixture (20 µl) comprising 1X PCR buffer, 1.0 µl of 50 ng DNA, 2.0 µl of 0.37 µM of each primer, 0.5 µl of 0.2 mM dNTP mix, 0.5 µl of 1.5 mM $MgCl_2$, and 0.5 units of Taq DNA polymerase (Thermo Fisher, USA). Reactions were cycled at initial denaturation for 5 min at 94˚C pursued by 36 cycles of 94˚C for 1 min, 45–60˚C (based on primers' Ta) for 45 sec, and 72˚C for 45 sec followed by 7 min last step at 72˚C. Products were differentiated on 2.5% agarose gels and visualized using gel documentation system (Biorad, USA).

Allele scoring and clustering: Genetic similarity was computed by 'SIMQUAL' sub-program of Numerical Taxonomy and Multivariate Analysis System [NTSYS-pc (version 2.02e)]. Unweighted Pair-Group Method with Arithmetic Average (UPGMA) was applied on the distance matrix in SAHN sub-program of NTSYS-pc to create a dendrogram. Two and three dimensional plots were built from principal component analysis (PCA). SSRs polymorphism levels were calculated from polymorphism information content (PIC). Expected heterozygosity and marker index (MI) were calculated as per Powell *et al.* (1996), whereas discriminating power and resolving power (RP) were determined as per Tessier *et al.* (1999), and Prevost and Wilkinson (1999), respectively.

RESULTS AND DISCUSSION

Status of functional markers in wheat genotypes: Out of 29 SSRs screened, only 24 showed amplifications (82.7%) and 23 showed polymorphism (95.83%) with a total of 46

Table 1 Disease profile of the genotypes

Genotype	Origin	Parentage	Disease profile
PBW 725	PAU	PBW621//Glupro/3*PBW 568/3/ PBW 621	R: YR, BR
DBW 71	IIWBR	Prinia/UP 2425	R: YR
WH 1124	CCSHAU	MUNIA/CHTO//AMSEL	R: YR, BR
WH 1105	CCSHAU	MILAN/S 87230//BABAX	R: YR, BR, PM
DPW 621-50	IIWBR+PAU	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	R: YR
DBW 88	IIWBR	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	R: YR, BR
DBW 187	IIWBR	(NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/ KACHU)	R: YR, BR
WH 1025	CCSHAU	C591/PBW231	R: YR, BR
WB 2	IIWBR	T.DICOCCONCI9309/AE.SQUARROSA(409)/3/MILAN/S87230//BAV92/4/2*MILN/S87230 //BAV92	R: YR, BR, PM
DBW 90	IIWBR	HUW468/WH730	R: YR, BR
UP 2565	GBPUA&T	PBW 352/CPAN 4020	R: Rust, PM
WH711	CCSHAU	ALD „S"/HAU//HD2285/3/HFW-17	R: YR
WH 1257	CCSHAU	FRNCLN/3/ND643//2*PRL/2*PASTOR/4/FRANCOLIN#1	R: YR, BR
WH 1258	CCSHAU	CROC_1/AE.SQUARROSA(210)//WBLL1*2/BRAMBLING/3/VILLA JUAREZF2009/5/BAV92//IRENA/KAUZ/3/HUITES*2/4/MURGA	R: YR, BR
WH 1259	CCSHAU	SNB//CMH79A.955/3*CNO79/3/ATTILA/4/CHEN/AEGILOPSSQUARROSA(TAUS)//BCN/3/2*KAUZ/5/KINGBIRD#1	R: YR, BR
WH 1261	CCSHAU	MUNAL#1/FRANCOLIN#1	R: YR, BR
WH 1264	CCSHAU	P12256/P12332//WH1142	R: YR, BR
WH 1263	CCSHAU	P13043/P13038//P13036	R: YR, BR
WH 1265	CCSHAU	P11906/P11925//P11906	R: YR, BR

Contd.

Table 1 (*Concluded*)

Genotype	Origin	Parentage	Disease profile
WH 1266	CCSHAU	MILAN/KAUZ//PRINIA/3/BAV92/4/BAVIS	R: YR, BR
WH 1268	CCSHAU	CHEWINK#1/MUTUS	R: YR, BR
WH 1271	CCSHAU	MILAN/S87230//BAV92*2/3/AKUR	R: YR, BR
WH 1272	CCSHAU	P12968/WH1130//P12892/3/UP2338	R: YR, BR
WH 1274	CCSHAU	BAJ#1/SUP152	R: YR, BR
WH 1276	CCSHAU	P13428/P13471	R: YR, BR
WH 1277	CCSHAU	SOKOLL/WBLL1/4/D67.2/PARANA 66.270//AE.SQ(320)/3/CUNNINGHAM	R: YR, BR
WH 1278	CCSHAU	SHORTENEDSR26TRANSLOCATION//2*WBLL1*2/KKTS/3/BECARD	R: YR, BR
WH 1279	CCSHAU	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/GLADIUS	R: YR, BR
WH 1283	CCSHAU	SHA7//PRL/VEE#6/3/FASAN/4/HAAS8446/2*TRCH/4/WHEAT//2*FASAN/5/CBRD/KAUZ/6/MILAN/AMSEL/7/FRET2*KUKUNA/8/2*WHEAT/ SOKOLL	R: YR, BR
HD 2967	IARI	SOKOLL/WBLL1/4/D67.2/PARANA66.270//AE.SQUARROSA(320)/3/CUNNINGHAM	R: YR
HD 3059	IARI	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	R: YR, BR
HD 3086	IARI	DBW 14/HD 2733//HUW 468	R: YR, BR
WH 1142	CCSHAU	CHEN/Ae.sq(TAUS)//FCT/3/2*WEAVER	R: YR
WH 1252	CCSHAU	SOKOLL/WBLL1/4/D67.2/PARANA66.270//AE.SQUARROSA(320)/3/CUNNINGHAM	R: KB
WH 1080	CCSHAU	PRL/2*PASTOR	R: KB
WH 283	CCSHAU	HD-1981/RAJ-821	R: YR, LR, KB
PBW 723	PAU	PBW343+Lr57/Yr40+Lr37/Yr17	R: YR, BR
PBW 752	PAU	PBW621/4/PBW343//YR10/6*AVOCET/3/3*PBW343/5/PBW621	R: YR
PBW 763	PAU	PBW621/3/YR10/6*AVOCET//4*PBW343/4/2*PBW621/5/PBW621/3/YR15/6AVOCET//4*PBW343/4/2*PBW621	S
PBW 706	PAU	MINO/898.97	S
DBW 17	IIWBR	CMH79A.95/3*CN079//RAJ3777	R: KB
PBW 709	PAU	PBW621/HD2967	S
WH 147	CCSHAU	E4870-C303/S339-PV18	S
PBW 527	PAU	NA	S
WH 1137	CCSHAU	NI623/ATILLA/3*BCN/3/PASTOR	S
WH 1152	CCSHAU	PBW65/2*PASTOR	S
PBW 373	PAU	ND/VG9144//KAL//BB/3//YCO"S"/4/VEE#5 „S"	S
WH 789	CCSHAU	NA	R: YR
PBW 762	PAU	YR5/6*AVOCET//2*PBW550	S
WH 1100	CCSHAU	PBW65/2*PASTOR	S
PBW 158	PAU	NA	S
PBW 677	PAU	PFAU/MILAN/5/CHEN/Ae.Sq.//BCN/3/VEE#7/BOW/4/PASTOR	S
PBW 714	PAU	WG7854/WG7858//NIAW34	S
PBW 695	PAU	PSN/BOW//MILAN/3/2*BERKUT	R: LR, YR, KB
PBW 698	PAU	BW9250*3YR10/6*AVOCET/3/BW9250*3//YR15/6*AVOCET	R: YR
LOK 54	Lokbharti	Raj 3777/WH671	S
WH 1129	CCSHAU	CS/TH.CS//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	S
HUW 540	BHU	NA	S

PAU, Punjab Agricultural University, Ludhiana; IIWBR, Indian Institute of Wheat and Barley Research, Karnal; CCSHAU, Chaudhary Charan Singh Haryana Agricultural University, Hisar; GBPUA&T, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar; IARI, Indian Agricultural research Institute, New Delhi; BHU, Banaras Hindu University, Varanasi; YR, Yellow rust; BR, Brown rust; LR, Leaf rust; PM, Powdery mildew; KB, Karnal bunt; R, resistant; S, Susceptible.

Table 2 Characteristics of functional markers in wheat genotypes

Marker	Linked gene	Trait	Ta (°C)	Allele No.	Obtained fragment size	Expected fragment size	Fragment present	Ch. location
Barc232	*Ut6*	Loose smut	61	2	200-210	206	57	5B
Xbarc124	*Yr69*	Yellow rust	52	3	230-260	249	55	2A
Xbarc32	*Yr59*	Yellow rust	52	2	198-210	165-175	57	5B
Swm271	*Lr75*	Leaf rust	55	2	240-280	--	58	1BS
Xcfd81	*Pm2*	Powdery mildew	51	3	190-280	283	49	4D
Xwmc44	*Lr46*	Leaf rust	55	2	240-260	242	54	1B
Wmc43	*Lr32*	Leaf rust	52	1	320	321	57	3B
Xgwm296	*Lr22a*	Leaf rust	47	2	120-150	121	52	2D, 7D
Barc152	*Sr33*	Stem rust	52	1	240	--	54	1B
Gpw5029	*Ut6*	Loose smut	52	2	210-280	209	48	5A
Wmc44	*Yr29*	Yellow rust	59	3	240-280	242	49	1B
Xwmc557	*Yr59*	Yellow rust	61	3	150-170	298	57	7B
Wmc145	*Yr*	Yellow rust	60	1	210	207	53	6A
Wmc177	*Yr*	Yellow rust	51	3	180-210	184	58	2A
Wmc719	*YrV3*	Yellow rust	61	1	200	219	55	1B
Xcfd233	*Pm43*	Powdery mildew	51	3	240-290	289	54	2D
Barc349	*Yr5*	Yellow rust	52	2	120-140	105	57	2B
Wmc766	*Yr*	Yellow rust	61	3	170-190	179	56	1B
Xgwm118	*Yr*	Yellow rust	61	2	190-220	186	57	1B
Xgwm577	*Stb8*	Zymoseptoria	55	1	150		51	7B
Xwmc41	*Pm43*	Powdery mildew	57	1	160	163	54	2D
Xgwm359	*Yr17*	Yellow rust	55	1	310	212	52	2A
Xwmc175	*Pm43*	Powdery mildew	61	1	250	253	52	2B
Xwgp5175	*Yr59*	Yellow rust	62	1	240	250	58	7B
Barc80	*Lr46*	Leaf rust	52		100-120	—		1B
Xwgp115	*Yr45*	Yellow rust	45		492	—		3DL
Xwgp118	*Yr45*	Yellow rust	47		411	—		3DL
Xgdm35	*Iw*	—	55		250	—		4AL
Xgwm160	*Pm61*	Powdery mildew	59		196	—		4AL

Ta, Annealing temperature; Ch, Chromosome; No., Number.

alleles. Alleles per locus ranged from 1 to 3 with a mean of 1.96 alleles per locus. PCR amplified products ranged from 120–320 bp with an average size of 214.06 bp. Rani *et al.* (2019) also assessed stripe rust resistance in 68 wheat genotypes using 70 markers and identified distribution of 25 *Yr* genes. In the present study, Barc232 functional marker was detected at 206 bp in 57 genotypes. This marker was previously reported by Kassa *et al.* (2014). It is present at 5B chromosome linked to gene *ut6,* which is a loose smut resistance gene. Gpw5029 flanked this gene at a distance of 1.3 cM on the distal side (Singh *et al.* 2017). Li *et al.* (2010) has testified that stripe rust resistance in wheat cultivar Mega was granted by a single dominant gene *YrMe* which is positioned tightly to the chromosome 5BL and flanked by Barc232 and Wmc640 markers. Another functional marker identified in the present study was Swm271, which was amplified at 240 to 270 bp in 57 genotypes. It is present on 1BS linked to the *Lr75* gene. This gene provides partial broad spectrum resistance against leaf rust and is an ideal target for stacking with other disease resistance genes (Singla *et al.* 2017). Bobrowska *et al.* (2022) has checked the diagnostic accuracy of genetic markers for identification of slow rusting locus in wheat. Functional markers identified in the present study can also be validated using similar course of study.

Genetic relationship among wheat genotypes: Similarity coefficient data when subjected to UPGMA tree cluster analysis showed that all the 58 wheat genotypes were clustered into two major groups (Cluster I and II) at a similarity coefficient of 0.66 in the dendrogram (Fig 1). Cluster I had two sub clusters with WH 1268 and WH 1266 in sub-cluster A; and only WH 711 in sub-cluster B. Cluster II also had two sub-clusters, where Lok 54 was present in sub-cluster A and all other genotypes were sorted into sub-cluster B, which was further split into two mini clusters.

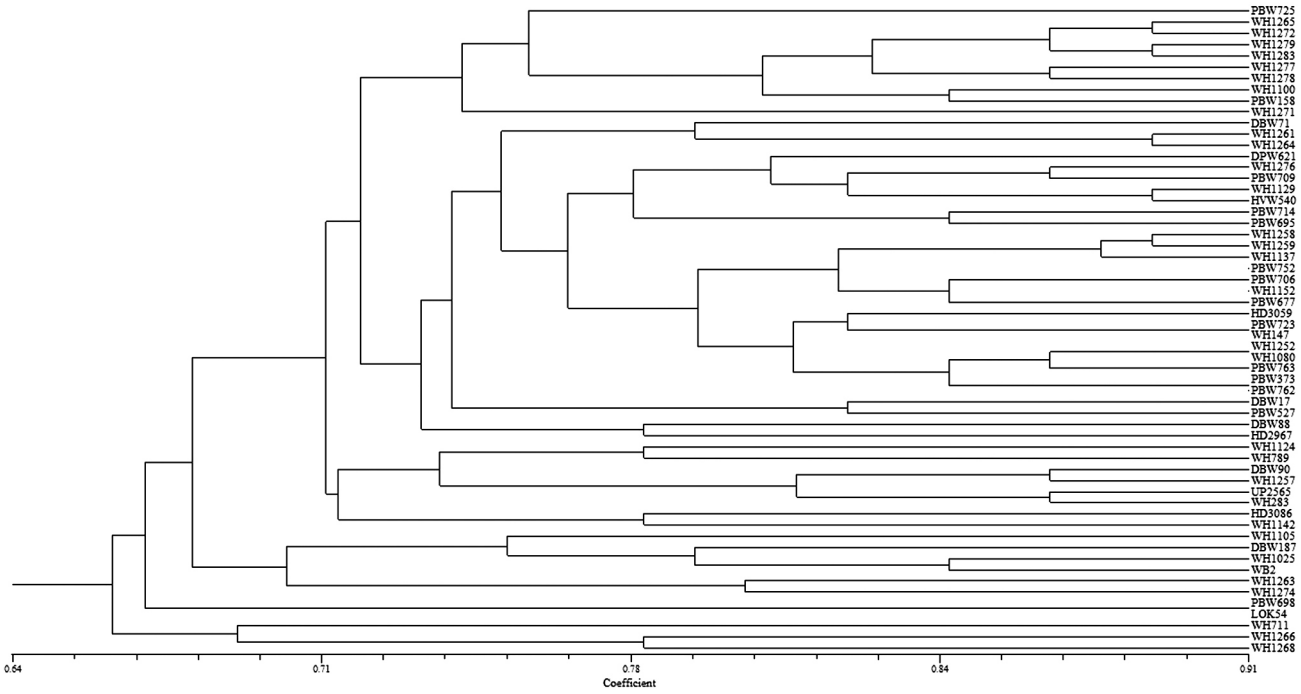

Fig 1 Dendrogram showing clustering pattern of 58 wheat genotypes based on functional markers.

Within the mini-cluster i, micro cluster 'a' had PBW 698 and WH 1274 genotypes; whereas microcluster 'b' had WH 1263, WB 2, WH 1025 and DBW 187 genotypes. Mini-cluster ii also had two micro-clusters, where genotypes WH 789, DBW 90, WH 1257, UP 2565, WH 283, HD 3086, WH 1142 and WH 1105 were present in micro-cluster 'b', whereas rest of the 40 genotypes were present in micro-cluster 'a'. Similar results were revealed by two dimensional and three dimensional PCA scaling of wheat genotypes. Ali *et al.* (2019) also assessed genetic diversity in wheat lines based on PCA based cluster analysis and found that 7 elite lines exhibited linkages of 3 slow rusting genes. PBW 725 and WH 1268 were found to be most diverse at a similarity coefficient of 77%. PBW 725 has high grain yield, is resistant to yellow and brown rusts and has good grain quality. WH 1268 exhibited multiple disease resistance against yellow rust, brown rust and powdery mildew (CCS HAU technical programme manual).

To determine the most informative marker, parameters like PIC, MI, RP and Diversity Index (DI) were evaluated (Table 3). Based on these evaluations, 8 SSR markers, viz. Barc232, Xbarc124, Xbarc32, Swm271, Xwmc44, Xgwm296, Gpw5029 and Xwmc557 were found to be most proficient. Barc232 was found to be correlated with lipoxygenase (LOX) activity by Zheng *et al.* (2022). It is well established that increased LOX activity is involved in hypersensitive response of wheat cells against rust fungi (Nalam *et al.* 2015). Xbarc124, which has been mapped to chromosome arm 2AS, is the marker closest to leaf rust resistance gene *Lr41* (Sun *et al.* 2009), which is located just 1cM from the gene. Stripe rust resistance gene *Yr69* has also been found to be bordered proximally by Xbarc124 (Hou *et al.* 2016). Bobrowska *et al.* (2022) found that Xwmc44

Table 3 Characteristics of functional markers used in the present study

Primer	Expected hetero-zygosity (He)	PIC values	Marker index (MI)	Discrim-inating power (D)	Resolving power (RP)
Barc232	0.34	0.31	0.69	0.35	0.86
Xbarc124	0.52	0.52	1.55	0.53	1.52
Xbarc32	0.42	0.37	0.84	0.43	0.97
Xcfd81	0.50	0.44	1.00	0.51	0.72
Swm271	0.53	0.53	1.60	0.54	1.52
Xwmc44	0.42	0.37	0.84	0.43	0.97
Wmc43	0.03	0.03	0.03	0.03	0.03
Xgwm296	0.45	0.40	0.91	0.46	1.24
Barc152	0.03	0.03	0.03	0.03	0.03
Gpw5029	0.44	0.44	0.88	0.45	1.14
Wmc44	0.54	0.54	1.63	0.55	1.52
Xwmc557	0.57	0.56	1.70	0.58	1.72
Wmc145	0.13	0.13	0.13	0.13	0.14
Wmc177	0.47	0.47	1.41	0.48	1.14
Wmc719	0.07	0.07	0.07	0.07	0.07
Xcfd233	0.56	0.56	1.67	0.57	1.52
Barc349	0.03	0.03	0.07	0.04	0.10
Wmc766	0.65	0.65	1.94	0.66	2.00
Xgwm118	0.13	0.12	0.26	0.13	0.28
Xgwm577	0.10	0.10	0.10	0.10	0.10
Xwmc41	0.07	0.07	0.07	0.07	0.07
Xgwm359	0.10	0.10	0.10	0.10	0.10
Xwmc175	0.03	0.04	0.03	0.03	0.03

PIC, Polymorphism information content.

is the appropriate marker for detection of *Lr46/Yr29* gene's resistance allele. Similarly, *Yr59*, an adult plant resistance gene was positioned on the long arm of chromosome 7B, and was tagged by Xbarc32 (Zhou *et al.* 2014). This marker was also used in marker-assisted backcross selection of *Yr59* to enhance stripe rust resistance in 4 elite wheat cultivars (Zhang *et al.* 2022). Marker Xwmc557 is also linked to *Yr59* gene (Li *et al.* 2022). Like Barc232, Gpw5029 also flanked *ut6* gene (Kassa *et al.* 2014). Expected heterozygosis varied from 0.03 to 0.65 and the PIC value ranged from as low as 0.03 to as high as 0.65 showing the medium level of polymorphism. The MI, discriminating power and RP ranged from 0.03 to 1.9; 40.03 to 0.66; and 0.03 to 2.00, respectively. The size of PCR amplifications varied from 120 bp (Xgwm296, Barc349) to 320 bp (Wmc43).

Present study recommends that diverse wheat variety PBW 725 and WH 1268 can be utilized in future breeding programmes targeted for generating genetic variability in Indian wheat germplasm for fungal disease resistance. Out of 29 markers analysed, 8 proficient markers, viz. Barc232, Xbarc124, Xbarc32, Swm271, Xwmc44, Xgwm296, Gpw5029 and Xwmc557 are suggested for Indian wheat fungal disease resistance profiling.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Indian Council of Agricultural research (ICAR), India for providing National Talent Scholarship to the first author.

REFERENCES

Ali Y, Khan M A, Hussain M, Atiq M and Ahmad J N. 2019. An assessment of the genetic diversity in selected wheat lines using molecular markers and PCA based cluster analysis. *Applied Ecology and Environmental Research* **17**(1): 931–50.

Bagge M, Xia X and Lubberstedt T. 2007. Functional markers in wheat. *Current Opinion in Plant Biology* **10**(2): 211–16.

Bobrowska R, Noweiska A, Spychała J, Tomkowiak A, Nawracała J and Kwiatek M T. 2022. Diagnostic accuracy of genetic markers for identification of the Lr46/Yr29 "slow rusting" locus in wheat (*Triticum aestivum* L.). *Biomolecular Concepts* **13**(1): 1–9.

FAO. 2021. *FAO Publications Series* 2021.

Figueroa M, Hammond-Kosack K E and Solomon P S. 2018. A review of wheat diseases-A field perspective. *Molecular Plant Pathology* **19**(6): 1523–36.

Goutam U, Kukreja S, Yadav R, Salaria N, Thakur K and Goyal A K. 2015. Recent trends and perspectives of molecular markers against fungal diseases in wheat. *Frontiers in Microbiology* **6**: 861.

Hou L, Jia J, Zhang X, Li X, Yang Z, Ma J, Guo H, Zhan H, Qiao L and Chang Z. 2016. Molecular mapping of the stripe rust resistance gene Yr69 on wheat chromosome 2AS. *Plant Disease* **100**(8): 1717–24.

Huerta-Espino J, Singh R P, German S, McCallum B D, Park R F, Chen W Q and Goyeau H. 2011. Global status of wheat leaf rust caused by *Puccinia triticina*. *Euphytica* **179**(1): 143–60.

Kage U, Kumar A, Dhokane D, Karre S and Kushalappa A C. 2016. Functional molecular markers for crop improvement. *Critical Reviews in Biotechnology* **36**(5): 917–30.

Kassa M T, Menzies J G and McCartney C A. 2014. Mapping of the loose smut resistance gene Ut6 in wheat (*Triticum aestivum* L.). *Molecular Breeding* **33**(3): 569–76.

Li Y, Lin R, Hu J, Shi X, Qiu D, Wu P, Goitom G H, Wang S, Zhang H, Yang L and Liu H. 2022. Mapping of wheat stripe rust resistance gene Yr041133 by BSR-Seq analysis. *The Crop Journal* **10**(2): 447–55.

Li Y, Yuan X, Yao Q, He M and Jing J. 2010. Genetic analysis and molecular mapping of stripe rust resistance gene in European wheat cultivar Mega. *Acta Phytopathologica Sinica* **40**(1): 51–56.

Liu Y, He Z, Appels R and Xia X. 2012. Functional markers in wheat: current status and future prospects. *Theoretical and Applied Genetics* **125**(1): 1–10.

Nalam V J, Alam S, Keereetaweep J, Venables B, Burdan D, Lee H, Trick H N, Sarowar S, Makandar R and Shah J. 2015. Facilitation of *Fusarium graminearum* infection by 9-lipoxygenases in *Arabidopsis* and wheat. *Molecular Plant-Microbe Interactions* **28**(10): 1142–52.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S and Rafalski A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* **2**(3): 225–38.

Prevost A and Wilkinson M J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics* **98**(1): 107–12.

Rani R, Singh R and Yadav N R. 2019. Evaluating stripe rust resistance in Indian wheat genotypes and breeding lines using molecular markers. *Comptes Rendus Biologies* **342**(5-6): 154–74.

Saghai-Maroof M A, Soliman K M, Jorgensen R A and Allard R W L. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences* **81**(24): 8014–18.

Singh D P, Sharma A K, Karwasra S S, Jain S K, Pant S K, Sharma I, Bala R, Majumdar V L and Bansal R K. 2017. Resistance in Indian wheat and triticale against loose smut caused by *Ustilago tritici*. *Indian Phytopathology* **70**(1): 131–33.

Singla J, Lüthi L, Wicker T, Bansal U, Krattinger S G and Keller B. 2017. Characterization of Lr75: a partial, broad-spectrum leaf rust resistance gene in wheat. *Theoretical and Applied Genetics* **130**(1): 1–12.

Sun X, Bai G and Carver B F. 2009. Molecular markers for wheat leaf rust resistance gene Lr41. *Molecular Breeding* **23**(2): 311–21.

Tessier C, David J, This P, Boursiquot J M and Charrier A. 1999. Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. *Theoretical and Applied Genetics* **98**(1): 171–77.

Zhang M, Fang T, Zhou X, Chen X, Li X, Feng J, Yang S and Kang Z. 2022.Combination of marker-assisted backcross selection of Yr59 and phenotypic selection to improve stripe rust resistance and agronomic performance in four elite wheat cultivars. *Agronomy* **12**(2): 497.

Zheng W Y, Zhao L, Li Y M, Li J, Zhu Z H and Yao D N. 2022. Association analysis between Lipoxygenase activity and SSR markers in wheat grains. *Cereal Research Communications* **50**(2): 297–303.

Zhou X L, Wang M N, Chen X M, Lu Y, Kang Z S and Jing J X. 2014. Identification of Yr59 conferring high-temperature adult-plant resistance to stripe rust in wheat germplasm PI 178759. *Theoretical and Applied Genetics* **127**(4): 935–45.