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ARTICLE

Phenotypic and Molecular Assessment of Wheat Genotypes Tolerant to Leaf Blight, Rust and Blast Diseases

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ABSTRACT

Globally among biotic stresses, diseases like blight, rust and blast constitute prime constraints for reducing wheat productivity especially in Bangladesh. For sustainable productivity, the development of disease-resistant lines and high yielding varieties is vital and necessary. This study was conducted using 122 advanced breeding lines of wheat including 21 varieties developed by Bangladesh Wheat and Maize Research Institute (BAMRI) with aims to identify genotypes having high yield potential and resistance to leaf blight, leaf rust and blast diseases. These genotypes were evaluated for resistance against leaf blight and leaf rust at Dinajpur and wheat blast at Jashore under field condition. Out of 122 genotypes tested, 20 lines were selected as resistant to leaf blight based on the area under the diseases progress curve (AUDPC) under both irrigated timely sown (ITS) and irrigated late sown (ILS) conditions. Forty-two genotypes were found completely free from leaf rust infection, 59 genotypes were identified as resistant, and 13 genotypes were identified as moderately resistant to leaf rust. Eighteen genotypes were immune against wheat blast, 42 genotypes were categorized as resistant, and 26 genotypes were identified as moderately resistant to wheat blast. Molecular data revealed that the 16 genotypes showed a positive 2NS segment among the 18 immune genotypes selected against wheat blast under field conditions. The genotypes BAW 1322, BAW 1295, and BAW 1203 can be used as earlier maturing genotypes and the genotypes BAW 1372, BAW 1373, BAW 1297 and BAW 1364 can be used for lodging tolerant due to short plant height. The genotypes WMRI Gom 1, BAW 1349 and BAW 1350 can be selected for bold grain and the genotypes WMRI Gom 1, BAW 1297, BAW 1377 can be used as high yielder for optimum seeding condition but genotypes BAW 1377 and BAW 1366 can be used for late sown condition. The selected resistant genotypes against specific diseases can be used in the further breeding program to develop wheat varieties having higher disease resistance and yield potential.

KEYWORDS

Wheat blast; leaf blight; leaf rust; 2NS marker; grain yield

1 Introduction

Wheat (*Triticum aestivum* L.) is the staple food of over half of the human population and ranks second only after rice in terms of production and acreage [1,2]. In South Asia, its consumption is multiplying due to the rapidly increasing population [3–5]. Wheat has attained the status of a profitable and secure crop for farmers in Bangladesh during the last four decades [6,7]. A variety of factors including high yielding varieties which are resistant to biotic and abiotic stresses, modernized production technology packages and effectively disseminated research outputs to the farming community are the attributes that have assisted in boosting wheat productivity (3.49 t ha^{-1}) [8,9]. However, a sharp decline in wheat area and production has been recorded in the country recently due to its competition with other cash crops as well as the incidence of different diseases [7].

Diseases play an important role in wheat production all over the world including Bangladesh. There are over 200 diseases of the wheat crop but in Bangladesh, among them, three diseases including leaf rust (*Puccinia triticina*), leaf blight and seedling blight (*Bipolaris sorokiniana*) are considered economically drastic through reducing wheat growth and yield [10,11]. Moreover, rust caused by *Puccinia triticina* Eriks is also posing a major threat to the sustainable production of wheat worldwide [12]. Leaf rust incidence usually occurs in mid-February, while its severity attains peak at the end of March under Bangladesh's agro-climatic conditions. These diseases get optimal environment (hot and humid) and ultimately the severity of this disease has reached an alarming level having the potential to inflict yield losses by 40%–100% [13–15]. The existing wheat varieties in Bangladesh have no absolute resistant gene against these diseases; while other abiotic stresses (i.e., heat, drought, waterlogging, weed infestation and poor plant nutrition) tend to increase the severity of these diseases [16,17]. Generally, yield losses of wheat depend on the susceptibility level of genotypes, agro-environmental conditions and crop growth stage [18]. These diseases have the potential to impart drastic effects including complete crop failure especially in case of susceptible varieties under hot climate. There exist a considerable research gap and limited information regarding susceptibility and resistance/tolerance reaction of the cultivated wheat varieties and germplasm against different diseases under agro-ecological conditions of Bangladesh.

In February 2016, the wheat blast (WB) caused by *Magnaporthe oryzae Triticum* [19] has emerged a great threat for the sustainability of wheat production in Bangladesh [20]. The initial year, the WB was spotted in eight districts and affected 15,000 hectares (about 16%) wheat area in Bangladesh, with upto 100% yield losses in some [21]. The disease was first reported in 1985 in Paraná, Brazil and has since spread throughout many of the important wheat-producing areas of Brazil and to the neighboring countries of Bolivia and Paraguay. Blast is now considered a major threat to wheat production in South America.

Scientists observed that CIMMYT line Milan appeared to contain high levels of resistance against WB under field condition [22]. Although, other cultivars with this resistance source are now being widely deployed, but the genetic basis of the resistance in Milan has not yet been established [22]. Therefore, there is an urgent need for the documentation of new sources of resistance to WB. It is well-documented that WB resistance genes have come from *Aegilops ventricosa* (Zhuk.) Chennav on wheat. This translocation carries a 25 to 38 cM distal segment of chromosome arm 2NS from *Aegilops ventricosa* to the distal region of chromosome arm 2AS in wheat. The *A. ventricosa* 2NS/2AS translocation carries resistance genes *Rkn3* against root-knot nematodes (*Meloidogyne* spp.), *Cre5* against the French pathotype *Ha12* of the cereal cyst nematode (*Heterodera avenae* Wollenweber), and *Lr37, Sr38*, and *Yr17* against some races of wheat leaf, stem and stripe rust [23,24].

To meet the threat of WB, Bangladesh Wheat and Maize Research Institute (BWMRI), with the technical support of the International Maize and Wheat Improvement Center (CIMMYT), Mexico, has developed and released a new wheat 'BARI Gom 33' [25]. Before releasing the variety, several multilocation trials, and laboratory screening were done both in 2016 and 2017 at Jashore (natural condition)-Bangladesh, Bolivia (natural condition), and in the Maryland USA in Laboratory condition and found WB resistant. The wheat variety also provides 5%–8% more yield and 40–45 ppm Zn-enriched than exiting varieties than exiting wheat varieties Bangladesh [7,25]. Besides these, the variety is moderately resistant against leaf blight and leaf rust diseases [7,25].

The researchers of BWMRI-Bangladesh are trying to find out the resistance sources for the development of wheat varieties for the sustainability of wheat production in the modern era to find out the resistant wheat genotypes against blight, rust and WB diseases through phenotyping and molecular assessment, and to identify high yielding promising genotypes based on yield and yield attributing traits.

2 Materials and Methods

2.1 Plant Materials and Locations of Evaluation

One hundred twenty-two high yielding advanced lines of wheat including 21 varieties developed from 1968 to 2018 by Bangladesh Wheat and Maize Research Institute (BAMRI) were evaluated against Bipolaris leaf blight (BpLB) and leaf rust under field condition at Dinajpur, Bangladesh (Tab. 1). The same genotypes were evaluated against wheat blast at Regional Agricultural Research Station (RARS), Jashore, one of the wheat blast hotspots area of Bangladesh.

2.2 Climatic Conditions

Weather information such as daily maximum (Max.), minimum (Min.) and mean temperatures, as well as rainfall, were recorded in each location (Fig. 1) from November 2018 to March 2019. For recording the weather data during crop stages, a HOBO U12 Family of Data Loggers was set in both locations.

2.3 Seed Sowing and Agronomic Management

The genotypes were planted in 5 m long 8 row-plots with 20 cm spacing between rows and 60 cm between entries. The materials were planted following an alpha-lattice design with two replications. The trial was set under irrigated timely sown (ITS) on 21 November 2018 and irrigated late sown (ILS) conditions on 25 December 2018 at Dinajpur. In the case of Jashore, the genotypes were sown on 23 November 2018 for ITS and 26 December 2018 for ILS condition. Seed rate for all genotypes was120 kg ha^{-1}. For minimizing seedling infection and also ensuring better germination, seeds of all wheat genotypes were treated with a fungicide namely 'Provax-200 WP' (containing carboxin and thiram). For controlling soil-borne insects, Furadan 5G at 10 kg ha^{-1} (Carbofuran) was incorporated with soil at final land preparation. For proper growth and development, BWMRI recommended fertilizers, namely N, P, K, S and B, respectively were applied at the rate of 100, 27, 40, 20, and 1 kg ha^{-1} during final land preparation. In the case of nitrogen fertilizer, 2/3 was applied as basal dose during final land preparation with other fertilizers. The remaining 1/3 N fertilizer was applied as top dress immediately after the first irrigation (20 days after sowing (DAS)). Three irrigations were applied during the whole cropping season. The first irrigation was applied at 20 DAS, while the second and third irrigation were applied at 55 and 75 DAS, respectively. Weeds were controlled by a post-emergence herbicide namely 'Affinity 50.75 WP' (Carfentrazone + Isoproturon (ready-mix formulation) at 1.25 kg ha^{-1}, which was applied at 10 days after first irrigation.

Table 1: List of the tested BWMRI wheat varieties/genotypes and their pedigrees

Serial No.	Variety/ genotype	Pedigree	Serial No.	Variety/ genotype	Pedigree
1	Khery	Landraces	62	BAW1341	GOURAB/HUW234 + LR34/PRINIA// KRONSTAD F2004
2	Sonora	YT 54/N10B//2*Y 54 II 8469–2Y-6C-6Y-4C-2Y-1C-0 MEX	63	BAW1342	BIJOY/SHA3/SERI// SHA4/LIRA/3/CHIR1 BD11DI252S-099DI-050DI-050DI-030DI-1DI
3	Kylansona	PJ/GB 55, II 8156	64	BAW1343	BIJOY/EMB16/CBRD// CBRD
4	Sonalik	1154–388/AN/3/YT54/ N1OB/LR64 II 18427–4R-1 M	65	BAW1344	BIJOY/BAW996// BIJOY
5	Kanchan	UP301/C3061187–1–1P-5P-5JO-0JO	66	BAW1345	BAW1129/BIJOY
6	Sourov	NAC/VEE (NL 560)	67	BAW1346	SOURAV/BAW 1055
7	Gourob	TURACO/CHIL	68	BAW1347	BIJOY/3/HUW234 + LR34/PRINIA// KRONSTAD F2004
8	BARI Gom21	MRNG/BVC//BLO/ PVN/3/PJB-81	69	BAW1348	BIJOY/BARI GOM 28
9	BARI Gom22	KAN/6/COQ/F61.70// CNDR/3/OLN/4/PHO/ 5/MRNG/ALDAN// CNO	70	BAW1349	BARI GOM 26/3/ OASIS/3*ANGRA// 708E
10	BARI Gom23	NL297*2/LR25	71	BAW1350	BARI GOM 26/3/ OASIS/3*ANGRA// 708E
11	BARI Gom24	G. 162/BL 1316//NL 297	72	BAW1351	GOURAB/FANG60// BAW 1037
12	BARI Gom25	ZSH 12/HLB 19// 2*NL297	73	BAW1352	BIJOY/3/OASIS/3/ *ANGRA// 708E
13	BARI Gom26	ICTAL 123/3/RAWAL 87//VEE/HD 2285	74	BAW1353	KANCHAN/3/FANG 60// RL 6043/4*NAC// BIJOY
14	BARI Gom27	WAXWING*2/VIVISTI	75	BAW1354	GOURAB//BIJOY
15	BARI Gom28	CHIL/2*STAR/4/BOW/ CROW//BUC/PVN/3/ 2*VEE#10	76	BAW1355	GOURAB/FANG 60/3/ PF 70354/MU//BOW

(Continued)

Table 1 (continued)

Serial No.	Variety/ genotype	Pedigree	Serial No.	Variety/ genotype	Pedigree
16	BARI Gom29	SOURAV/7/KLAT/ SOREN//PSN/3/BOW/ 4/VEE#5. 10/5/CNO 67/ MFD// MON/3/ SERI/6/ NL297	77	BAW1356	HUW 234 + LR 34/ PRINIA//KRONS-099JE-050JE-030JE-030JE-2JETAD// PRODIP
17	BARI Gom30	BAW 677/Bijoy	78	BAW1357	SHATABDI/ GARUDA//AKBAR/ GOURAB
18	BARI Gom31	KAL/BB/YD/3/ PASTOR	79	BAW1358	PRODIP/BAW 1075
19	BARI Gom32	SHATABDI/GOURAB	80	BAW1359	GOURAB/BIJOY
20	BARI Gom33	KACHU/SOLALA	81	BAW1360	T.DICOCCONCI9309/ AE.SQUARROSA (409)//MUTUS/3/ 2*MUTUS/8/REH/ HARE//2*BCN/3/ CROC_1/….
21	WMRI Gom 1	Shatabdi/ BARI Gom 24	82	BAW1361	PAURAQ*2/3/T. DICOCCONPI94625/ AE.SQUARROSA (372)//SHA4/CHIL/4/ QUAIU#1/SOLALA// QUAIU #2
22	BAW1203	Shatabdi/GOURAB	83	BAW1362	KVZ/PPR47.89C// FRANCOLIN#1/3/ 2*PAURAQ/4/ UP2338*2/KKTS*2// YANAC
23	BAW1208	BARI Gom 26/ BARI Gom 25	84	BAW1363	MELON//FILIN/ MILAN/3/FILIN/5/ CROC_1/AE. SQUARROSA …
24	BAW1243	CY 8801/BAW966// BAW 1074	85	BAW1364	Plant 1
25	BAW1154	CY 8801/ BARI Gom 25	86	BAW1365	Plant 4
26	BAW1272	BARI Gom 24/ SW 89. 5422// BAW 1051	87	BAW1366	SOURAV/4/PFAU/ SERI.1B//AMAD/3/ WAXWING
27	BAW1280	BAJ #1*2/TECUE #	88	BAW1367	SOURAV/4/PFAU/ SERI.1B//AMAD/3/ WAXWING

(Continued)

Table 1 (continued)

Serial No.	Variety/ genotype	Pedigree	Serial No.	Variety/ genotype	Pedigree
28	BAW1286	SW89–5124*2/FASAN	89	BAW1368	SOURAV/4/PFAU/ SERI.1B//AMAD/3/ WAXWING
29	BAW1147	OASIS/3*ANGRA//708	90	BAW1369	SOURAV/4/PFAU/ SERI.1B//AMAD/3/ WAXWING
30	BAW1254	Borloag 100	91	BAW1370	SOURAV/4/PFAU/ SERI.1B//AMAD/3/ WAXWING
31	BAW1290	BARI Gom 21/ BL 3503	92	BAW1371	SOURAV/4/PFAU/ SERI.1B//AMAD/3/ WAXWING
32	BAW1293	BARI Gom 24// BAW 968/BARI Gom 21	93	BAW1372	SOURAV/4/PFAU/ SERI.1B//AMAD/3/ WAXWING
33	BAW1295	BAW 923/BAW 824// SWARNA BD09DI1920S-099DI-050DI-030DI-30DI-6DI-0DI	94	BAW1373	SOURAV/4/PFAU/ SERI.1B//AMAD/3/ WAXWING
34	BAW1296	BAW 968/BARI Gom 21//BARI Gom 25	95	BAW1374	#
35	BAW1297	BAW 968/BARI Gom 21//BARI Gom 25	96	BAW1375	SOURAV/3/HUW234 + LR34/PRINIA// KRONSTAD F2004
36	BAW1299	BAW 968/BARI Gom 21//BARI Gom 25	97	BAW1376	SOURAV/3/HUW234 + LR34/PRINIA// KRONSTAD F2004
37	BAW1303	BARI Gom 24/BL3809	98	BAW1377	SOURAV/3/HUW234 + LR34/PRINIA// KRONSTAD F2004
38	BAW1304	BARI Gom 24/BL3809	99	BAW1378	SOURAV/3/HUW234 + LR34/PRINIA// KRONSTAD F2004
39	BAW1316	SHATABDI/ BL 3503	100	BAW1379	KANCHAN// GOURAB/PAVON 76
40	BAW1317	PRODIP// BAW 968/ SHATABDI	101	BAW1380	SOURAV// GARUDA*2/BAW 748
41	BAW1318	BAW 968/SHATABDI// BAW1059	102	BAW1381	PRODIP//RL 6043/ 4*NAC

(Continued)

Table 1 (continued)

Serial No.	Variety/ genotype	Pedigree	Serial No.	Variety/ genotype	Pedigree
42	BAW1321	PRODIP/BAW972	103	BAW1382	PRODIP//RL 6043/ 4*NAC
43	BAW1322	BL3063/BARI Gom 27	104	BAW1383	PRODIP//RL 6043/ 4*NAC
44	BAW1323	BAW65//BAW 968/ SHATABDI	105	BAW1384	PRODIP/BAW 1151
45	BAW1324	BL3503/3/OASIS/ 3*ANGRA//708E	106	BAW1385	BIJOY/BAW 1075
46	BAW1325	BL3503/3/OASIS/ 3*ANGRA//708E	107	BAW1386	PRODIP/BAW 968/ SHATABDI
47	BAW1326	BL3503/3/OASIS/ 3*ANGRA//708E	108	BAW1387	GOURAB/PRODIP
48	BAW1327	SUFI/PRODIP// BAW972	109	BAW1388	Plant 1
49	BAW1328	BL3503/6/CS/TH.SC// 3*PVN/3/MIRLO/BUC/ 4/MILAN/5/TILHI	110	BAW1389	Plant 4
50	BAW1329	BIJOY/ GARUDA	111	BAW1390	HUIRIVIS #1*2/ MURGA/3/ TACUPETO F2001/ BRAMBLING*2// KACHU
51	BAW1330	BIJOY//GOURAB/ FANG 60	112	BAW1391	VALI/MAYIL
52	BAW1331	BIJOY/ GARUDA	113	BAW1392	68.111/RGB-U// WARD/3/FGO/4/RABI/ 5/AE.SQUARROSA,,
53	BAW1332	PRODIP/ FRANCOLIN #1	114	BAW1393	FRET2/KUKUNA// FRET2/3/YANAC/4/ FRET2/KIRITATI/5/ 2*BOKOTA
54	BAW1333	PRODIP/ FRANCOLIN #1	115	BAW1394	LIVINGSTON/6/ 2*MTRWA92.161/ PRINIA/5/SERI*3//
55	BAW1334	PRODIP/ BAW 1059	116	BAW1395	Rosadun/2008Y3–6871
56	BAW1335	PRODIP/ BAW 1059	117	BAW1396	2006xioaheimai3/ chuanyu// chuanyu64927
57	BAW1336	BAW 1027/BAW 1059/ 3/ OASIS/3*ANGRA// 708E	118	BAW1397	BORL14*2//KFA/ 2*KACHU

(Continued)

Table 1 (continued)

Serial No.	Variety/ genotype	Pedigree	Serial No.	Variety/ genotype	Pedigree
58	BAW1337	BIJOY/ GARUDA	119	BAW1398	KACHU/KINDE// NELOKI/3/BORL14
59	BAW1338	BIJOY//GOURAB/ FANG 60	120	BAW1399	BAJ#1/8/NG8201/ KAUZ/4/SHA7//RPL/ VEE#6/3/..
60	BAW1339	SOURAV/3/ HUW234 + LR34/PRINIA// KRONSTAD F2004	121	BAW1400	BAVIS#1/5/W15.92/4/ PASTOR//HXL7573/ 2*BAU/3/WBLL1
61	BAW1340	SOURAV/3/ HUW234 + LR34/PRINIA// KRONSTAD F2004	122	BAW1401	#

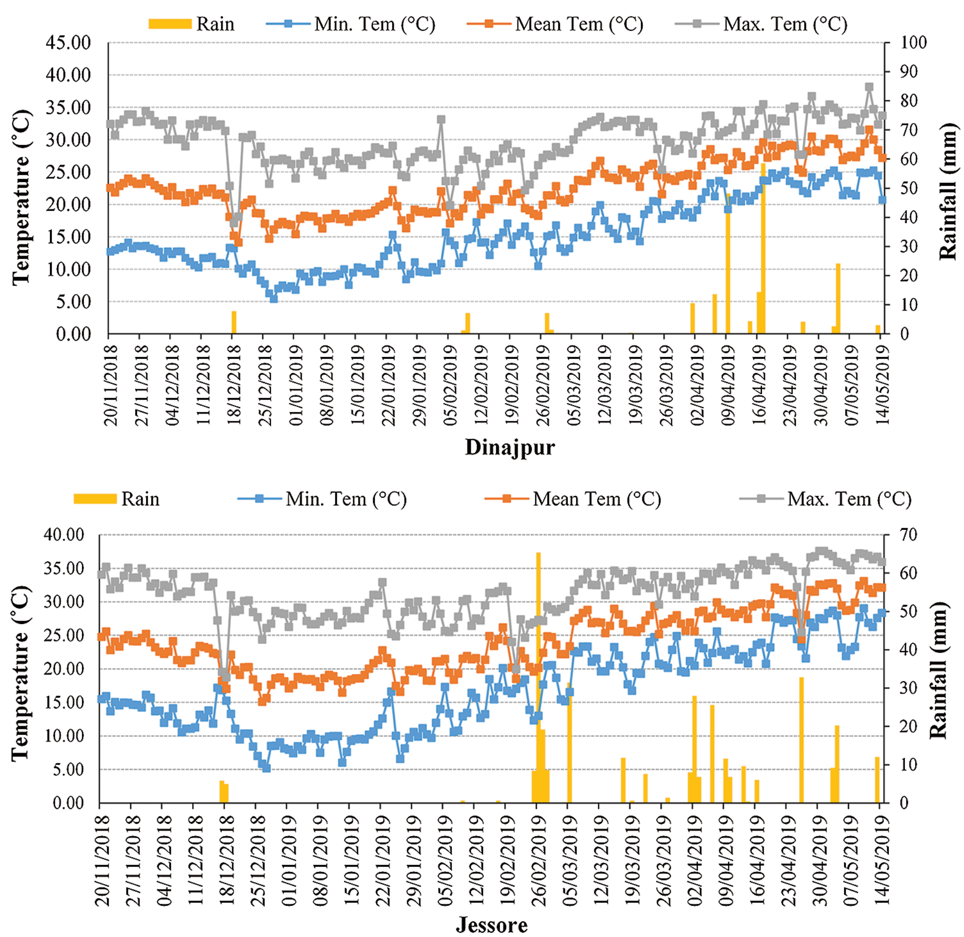

Figure 1: Average daily maximum (Max.), minimum (Min.) and mean temperatures as well as rainfall data recorded in both locations during the wheat season

2.4 Inoculation Procedures

A mixture of susceptible varieties was planted surrounding the experimental plots spreader rows at Dinajpur. The susceptible mixture serves as a substrate for multiplication as well as the distribution of BpLB and rust inoculum. The inoculation of spreader rows was done by spraying the aqueous suspension of uredospores of *Puccinina triticina* for disease development of leaf rust at the booting stage of the crop at Dinajpur. The highly blast susceptible variety 'BARI Gom 26' was sown surrounded by the experiment at Jashore for wheat blast spreader. At Jashore, the spreader rows were inoculated with *Magnaporthe oryzae* pathotype *Triticum* (MoT) spores (20000 spores per mL) for blast symptom development starting from three weeks after sowing and continued until the primary infection was observed. The inoculum of MoT was multiplied at plant pathology laboratory of Regional Agricultural Research Station, Jashore.

2.5 Assessment Procedures of Diseases

The severity of leaf blight was estimated thrice on the double-digit scale (00–99) [26], which started from water ripe to dough stages of Zadoks growth scale [27,28].

2.5.1 Assessment of Leaf Blight

The data about leaf blight were converted to diseased leaf area (DLA) and then the area under the disease progress curve (AUDPC) was calculated as suggested by [17].

$$\% \text{ DLA} = D_1/9 \times D_2/9 \times 100$$

where, D_1, indicates relative disease height; D_2, indicates disease severity.

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+1} + Y_i) \times 0.5]\ [T_{i+1} - T_i]$$

where, Yi, the severity of disease at ith observation, T_i, Time (days) of the ith observation; n, the total number of observations (at least 3 observations).

2.5.2 Assessment of Leaf Rust

The assessment of leaf rust was executed at the dough stage with the help of a modified Cobb scale [29].

2.5.3 Assessment of Wheat Blast

Wheat blast severity was recorded as per the following equation:

Disease severity or index (%) = (spike incidence (%)/100) × (diseased area on spike (%)/100) × 100

2.5.4 Molecular Screening of Wheat Blast

Genomic DNA Extraction

To amplify 2NS translocation region, genomic DNA was extracted from 10 days old seedlings using the modified CTAB method [15]. Briefly, about 200 mg fresh wheat leaves were cut into small pieces (~2 mm) and collected in 2 mL centrifuge tube which contained two sterile steel balls, frozen in liquid nitrogen, crushed the leaves to powder by the high-speed vortex. The cap of the centrifuge tube was carefully opened, added 1 ml (0.8%) of warmed (65°C) CTAB buffer, added 0.4 ml of chloroform and mixed thoroughly, removed the steel balls from the tube by magnet suction; heated in a water bath at 60°C for 15 min. and then incubated at −20°C for 15 min. The sample was centrifuged at 12000 rpm for 10 min at 4°C temperature; the clear supernatant was transferred into a new 1.5 mL centrifuge tube. An equal volume of isopropyl alcohol (isopropanol) was added and mixed gently by inverting (2–3 times). The sample was incubated on ice for 10 min for precipitating the DNA and centrifuged at 12000 rpm for 15 min at 4°C. A very small gel-like pellet was visible on the side and bottom of the tube. The pellet was washed twice with 0.4 ml of 75% chilled ethanol. The final pellet was air-dried and then dissolved in 100 to 500 µl of 1×TE (100 mM Tris-HCl, 10 mM EDTA, pH = 8.0).

PCR Amplification and gel Electrophoresis

Two PCR primers (VENTRUIP 5'-AGGGGCTACTGACCA AGGCT-3'), LN2 (5'-TGCAGCTACA GCAGTATGTACACAAAA-3') and Yr17F (5'-TTATTACCTTGATGAGAAATACAGF-3') Yr17-R (5'-CTGAAATTGGGACTAGCGAAATTA-3') were used for screening wheat blast resistance genes in 2NS segment of wheat germplasms. PCR was performed in a volume of 10 µL using a Verity Thermal Cycler (Applied Biosystems, USA). The reaction mixture contained 40 to 100 ng of genomic DNA, 2× PCR master mix, 10 µM each Primer and ddH_2O up to 10 µL. The amplification program of VENTRUIP-F/LN2-R was as follows: 94°C for 3 min (enzyme activation); 30 cycles of 94°C for 45 sec (melting), 65°C (depending on the specific primers) for 30 sec (annealing) and 72°C for 60 sec (extension); and a final extension at 72°C for 7 min. The amplification program of Yr17-F/Yr17-R was as follows: 94°C for 3 min (enzyme activation); 26 cycles of 94°C for 45 sec (melting), 57°C (depending on the specific primers) for 45 sec (annealing) and 72°C for 45 sec (extension); and a final extension at 72°C for 8 min. PCR products (10 µl each) were run on 1.5% agarose gel and stained with ethidium bromide.

2.6 Agronomic Data Recording

Data on days to heading, days to maturity, plant height, spike m^{-2}, spikelet $spike^{-1}$, grain $spike^{-1}$, 1000-grain weight and grain yield were recorded in the location of Dinajpur. At maturity, the middle five-meter long six rows were harvested to estimate yield.

2.7 Statistical Analysis

Data collected during this study were statistically analyzed using R software [30]. Duncan's new multiple range test (DMRT) at a 5% probability level was used to test differences among mean values [31].

3 Results

3.1 Leaf Blight

Out of 122 genotypes tested, 20 lines were selected as resistant based on AUDPC under both in ITS and ILS conditions. The AUDPC of the selected lines ranged from 39 to 106 in ITS and 43 to 114 in ILS condition (Tab. 2). The lowest AUDPC was found from the genotype of BAW 1339 and BAW 1342 int ITS and BAW 1329 in ILS condition.

3.2 Leaf Rust

The severity of leaf rust varied among different advanced genotypes and varieties tested under field condition (Tab. 3). The varieties/advanced lines showed 0% to 40% severity with different types of disease reaction, while 80% severity with susceptible reaction was displayed in spreader rows. Based on rust severity, genotypes were categorized in highly resistant (0% severity), resistant (1%–10% severity), moderately resistant (11–30% severity), moderately susceptible (31%–50% severity), susceptible (51%–75% severity) and highly susceptible (76%–100% severity) groups. Out of 122 genotypes, 42 genotypes including 9 varieties were completely free from leaf rust infection, 59 genotypes showed resistance, 13 genotypes showed moderate resistance and 8 genotypes showed moderately susceptible reactions against leaf rust diseases.

3.3 Wheat Blast

Out of 122 genotypes, 18 genotypes including BARI Gom 33 were immune against wheat blast under field condition, 42 genotypes including BARI Gom 30 and BARI Gom 32 were categorized as resistant (0.2%–10% disease index), 26 genotypes were categorized as moderately resistant (11%–30% disease index), 12 genotypes were identified as moderately susceptible (31%–50% disease index), 9 genotypes were classified as susceptible (51%–75% disease index), 15 genotypes were classified as highly susceptible (76%–100% disease index) to blast disease (Tab. 4).

Table 2: Performance of resistant wheat lines selected against *Bipolaris* leaf blight

SL No.	Genotype	AUDPC	
		ITS	ILS
1.	BARI Gom-21(SHATABDI)	91	98
2.	WMRI Gom 1	59	79
3.	BAW 1203	74	111
4.	BAW1304	66	54
5.	BAW1318	94	86
6.	BAW1321	47	98
7.	BAW1323	77	109
8.	BAW1325	104	86
9.	BAW1326	100	98
10.	BAW1329	88	43
11.	BAW1334	47	109
12.	BAW1335	75	114
13.	BAW1336	102	49
14.	BAW1339	39	100
15.	BAW1340	43	105
16.	BAW1341	106	105
17.	BAW1342	39	64
18.	BAW1344	70	88
19.	BAW1356	77	102
20.	BAW1360	77	109
21.	BAW1365	49	57

3.3.1 Molecular Screening of Wheat Genotypes for Blast Resistance Using 2NS Markers

Development and dissemination of resistant/tolerant wheat varieties would be the most effective way to control the fearsome wheat blast disease. It has been proved that 2NS translocation from *Aegilops ventricosa* expresses resistance to wheat blast in the most background. Therefore all of the selected genotypes were screened to identity wheat blast-resistant genotypes using the 2NS marker. Out of 122 genotypes, 16 genotypes including one variety (BARI Gom 33, BAW 1254, BAW 1280, BAW 1328, BAW 1338, BAW 1358, BAW 1360, BAW 1362, BAW 1363, BAW 1374, BAW 1390, BAW 1391, BAW 1394, BAW 1397, BAW 1399 and BAW 1401) showed positive 2NS segment (Figs. 2, 3). Importantly, genotypes with 2NS translocation showed immune disease reaction under field condition.

3.4 Yield Parameters

The results revealed that significant variation was present among wheat varieties for grain yield-related parameters (Tab. 5).

Table 3: The response of wheat genotypes to leaf rust under field condition and their classification based on disease severity

Lesion area (%)	Variety/genotype	Disease reaction
0	Gourab, Shatabdi, Sufi, Bijoy, BARI Gom 27, BARI Gom 29, BARI Gom 30, BARI Gom 31, BARI Gom 33, BAW 1194, BAW 1203, BAW1243, BAW1280, BAW1286, BAW1304, BAW1316, BAW1324, BAW1338, BAW1339, BAW1340, BAW1341, BAW1343, BAW1347, BAW1358, BAW1360, BAW1361, BAW1362, BAW1363, BAW1365, BAW1366, BAW1373, BAW1375, BAW1376, BAW1378, BAW1390, BAW1391, BAW1394, BAW1397, BAW1398, BAW1399, BAW1400, BAW1401 = 42	Highly resistant
1–10	Kheri, Kanchan, Sourav, BARI Gom 25, BARI Gom 32, BAW1321, BAW1317, BAW1318, BAW1352, BAW1357, BAW1369, BAW1370, BAW1377, BAW1385, BAW1386, BAW1389, BAW1396, BAW1328, BAW1336, BAW1367, BAW1295, BAW1332, BAW1333, BAW1335, BAW1348, BAW1353, BAW1293, BAW1355, BAW1356, BAW1290, BAW1296, BAW1297, BAW1323, BAW1326, BAW1329, BAW1337, BAW1346, BAW1359, BAW1208, BAW1344, BAW1154, BAW1272, BAW1325, BAW1327, BAW1330, BAW1349, BAW1350, BAW1351, BAW1354, BAW1364, BAW1393, BAW1254, BAW1299, BAW1368, BAW1371, BAW1372, BAW1374, BAW1387, BAW1303 = 59	Resistant
11–30	Sonora 64, Kalyansona, BARI Gom 28, BAW1342, BAW1392, BAW1322, BAW1331, BAW1345, BAW1380, BAW1388, BAW1334, BAW1379, BAW1395 = 13	Moderately resistant
31–50	Sonalika, Prodip, BARI Gom 26, BAW1147, BAW1381, BAW1384, BAW1382, BAW1383 = 08	Moderately susceptible
51–75	-	Susceptible
76–100	-	Highly susceptible

The results revealed significant differences among the wheat genotypes regarding plant height, heading days, maturity days and other yield attributes (Tab. 5). Ample variability among wheat genotypes for yield attributes offers an excellent opportunity to exploit their genetic potential to breed new cultivars having a higher potential for grain yield. The coefficient of variation was low for days to heading and days to maturity (1.2%), plant height (2.4%), and Spikelet spike-1 (4.7%). Thirteen genotypes had higher thousand-grain weight than the involved released 3 check varieties Fentalle, Amibara and Werer-2. Genotypes able to maintain high 1000-grain weight under high-temperature stress may possess a high level of heat tolerance.

Table 4: The response of wheat genotypes to blast under field condition and their classification based on disease severity

Disease severity (%)	Variety/genotype	Disease reaction
0	BARI Gom 33, BAW1280, BAW1254, BAW1328, BAW1337, BAW1338, BAW1353, BAW1358, BAW1360, BAW1362, BAW1363, BAW1374, BAW1390, BAW1391, BAW1394, BAW1397, BAW1399, BAW1401 = 18	Highly resistant
0.2–10	BARI Gom 32, BAW1154, BAW1272, BAW1286, BAW1290, BAW1293, BAW1296, BAW1297, BAW1304, BAW1316, BAW1322, BAW1323, BAW1324, BAW1325, BAW1326, BAW1329, BAW1331, BAW1332, BAW1333, BAW1336, BAW1339, BAW1340, BAW1343, BAW1344, BAW1346, BAW1347, BAW1348, BAW1350, BAW1351, BAW1352, BAW1354, BAW1355, BAW1356, BAW1357, BAW1359, BAW1361, BAW1364, BAW1365, BAW1373, BAW1393, BAW1398 = 42	Resistant
11–30	Kheri, Sonora 64, Sonalika, Sourav, Gourab, Shatabdi, Bijoy, BARI Gom 25, BARI Gom 27, BARI Gom 29, BARI Gom 21, BAW1147, BAW1208, BAW1295, BAW1299, BAW1303, BAW1318, BAW 1317, BAW1327, BAW1330, BAW1334, BAW1335, BAW1341, BAW1349, BAW1378, BAW1395 = 26	Moderately resistant
31–50	Kanchan, Prodip, BARI Gom 28, BAW 1203, BAW1243, BAW1345, BAW1379, BAW1380, BAW1385, BAW1387, BAW1392, BAW1396 = 12	Moderately susceptible
51–75	Kalyansona, WMRI Gom 1, BAW1321, BAW1342, BAW1368, BAW1376, BAW1377, BAW1386, BAW1400 = 9	Susceptible
76–100	Sufi, BARI Gom 26, BAW1366, BAW1367, BAW1369, BAW1370, BAW1371, BAW1372, BAW1375, BAW1381, BAW1382, BAW1383, BAW1384, BAW1388, BAW1389 = 15	Highly susceptible

The effect of seeding times revealed that all traits were significantly influenced by seeding time (Supplementary Tab. 2). Days to heading and days to maturity were earlier at late sowing condition. Plant height, number of spikes m^{-2}, grain $spike^{-1}$ was significantly higher at ITS condition. The TGW was higher at ITS condition compared to ILS conditions. The higher yield was achieved under ITS (5102 kg/ha) than ILS (3414 kg/ha) condition.

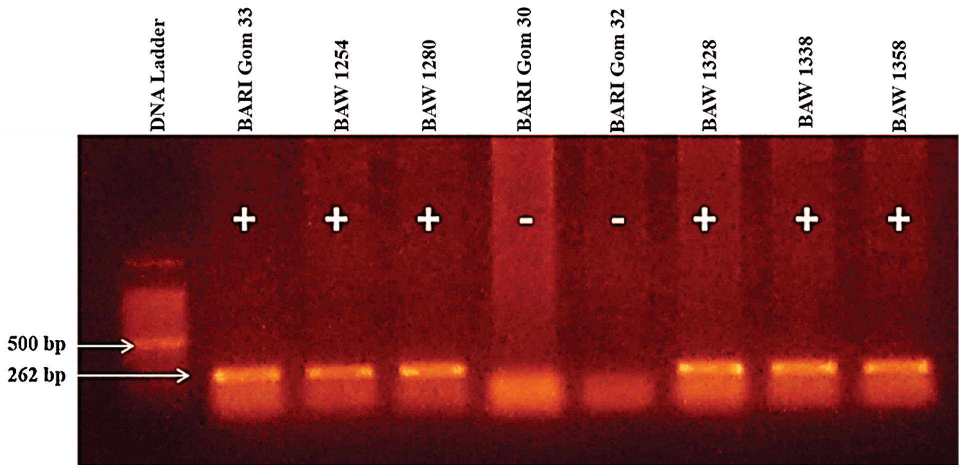

Figure 2: PCR amplification with 2NS specific primers VENTRUIP-F/LN2-R

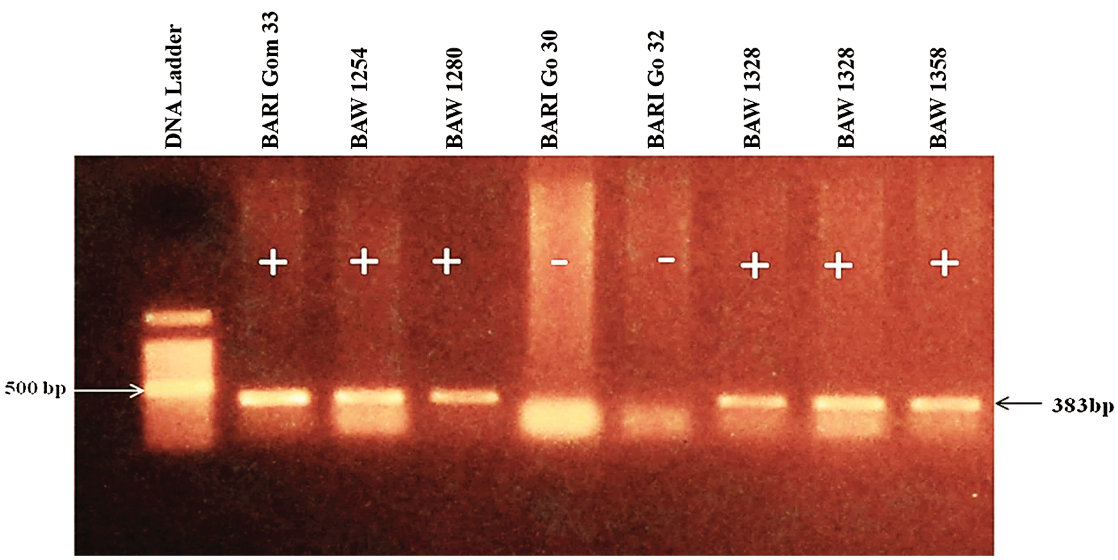

Figure 3: PCR amplification with 2NS specific primers Yr17-F/Yr17-R

The minimum time to heading and maturity was found in WMRI Gom 1, BAW 1203 and BAW 1295. On the other hand, the highest days to heading and maturity were found in BAW 1386 and most of the genotypes headed and matured earlier than the long duration variety Shatabdi (Supplementary Table 3). The lowest plan height was found in BAW 1372 (72 cm) and BAW 1373 (72 cm). There are fifty genotypes sowed lower plant height (<100 cm). The cultivars with short stature are generally tolerant to lodging and result in a significant increase in crop yield. The highest spike m^{-2} was recorded in Khery (623) but the yield was low. The highest spikelet $spike^{-1}$ was found in BAW 1358 and grain $spike^{-1}$ in BAW 1396. Out of 122 genotypes, the highest 1000-grain weight was recorded in BAW 1293 followed by BAW 1380. The lowest 1000-grain weight was showed in Kalyansona. The highest yield was achieved in BAW 1377 (5668 kg/ha), followed by BAW 1397 (5412 kg/ha) and WMRI Gom 1 (5276 kg/ha).

A significant effect was found for seeding time, genotypes and their interactions on yield and yield contributing characters (Supplementary Tab. 4). The lowest heading days found in BAW 1322 (56 days), BARI Gom 32 (56 days) and BAW 1295 (56 days) both under ITS and ILS condition. The lowest maturity days was found in WMRI Gom 1 (96 days) and BAW 1203 (96 days) in ITS condition and BAW 1332 (93 days), BAW 1280 (93 days) in ILS condition. The short stature plant was observed in BAW 1372 (74 cm), BAW 1373 (77 cm), BAW 1297 (88 cm) and BAW 1364 (88 cm) both in ITS and ILS condition. The highest 1000-grain weight was found in BAW 1357 (66.1 g) followed by BAW 1293 (65 g) and BAW 1380 (65 g) in ITS condition but variety WMRI Gom 1 showed the highest 1000-grain weight (62.6 g) followed by BAW 1349 (56.8 g) and BAW 1350 (56.4 g) in ILS condition. The highest yield was achieved in WMRI Gom 1 (6261 kg ha^{-1}) followed by BAW 1297 (6260 kg ha^{-1}), BAW 1377 (6234 kg ha^{-1}) under ITS condition. In ILS condition, the highest yield was found in BAW 1377 (5102 kg ha^{-1}) followed by BAW 1366 (4948 kg ha^{-1}). The genotype BAW 1322, BAW 1295, BAW 1203 can be used as earlier maturing genotypes for the further breeding program. The genotype BAW 1372, BAW 1373, BAW 1297 and BAW 1364 can be used for tolerance to lodging due to short plant height. The genotype BAW WMRI Gom 1, BAW 1349 and BAW 1350 can be used for bold grain. The genotype WMRI Gom 1, BAW 1297, BAW 1377 can be used as a high yielder for optimum seeding condition but genotype BAW 1377 and BAW 1366 for the late sown condition. The genotype BAW 1385, BAW 1366, BAW 1359 and BAW 1394 can be used as terminal heat tolerant.

Table 5: Mean square from the analysis of variance of 122 wheat genotypes evaluated for eight yield and yield attributing traits at BWMRI, Dinajpur, Bangladesh in 2018–2019

Trait	df	HD	MD	PH	SPM	SPS	GPS	TGW	YIELD
G	121	54.15**	26.25**	231.93**	13947.10**	7.77**	142.31**	151.26**	1475360**
R	1	0.82	0.30	1.39	3055.00	0.90	8.39	0.53	201815
ST	1	1779.97**	19591.10**	7585.61**	61201.40**	14.81**	964.34**	8465.28**	350316000**
G*ST	121	15.69**	10.45**	20.53**	3963.31**	2.68**	43.95**	60.26**	516286**
Residual	243	0.62	1.63	6.09	964.23	0.68	10.33	21.84	131883
LSD (0.05)		1.10	1.78	3.44	43.25	1.15	4.48	6.51	505.76
CV (%)		1.2	1.2	2.4	8.6	4.7	7	9.6	8.5

Note: Genotype-Genotype; R-Replication; ST-Sowing Time; df-degree of freedom; HD-heading days; MD-Maturity days; PH-Plant height (cm); SPM-Spike m^{-2}; SPS-Spikelet $spike^{-1}$; GPS-Grain $spike^{-1}$; TGW-Thousand-grain wt. (g), Yield (kg ha^{-1})

4 Discussion

Reduction of wheat yield due to different diseases including spot blotch, rust and blast pose a serious threat to sustainable wheat production worldwide. A couple of research experiments were conducted both under field and laboratory conditions following standard procedures to identify the best genotypes resistant to the above-mentioned diseases including high yield potential by assessing a large number of promising genotypes including varieties. Calculation of the area under the disease-progress curve (AUDPC) as a measure of quantitative disease resistance entails repeated disease assessments [32]. Trails related to diseases assessment require work and time to a large extent. There are some limitations (weather, space) on how frequently assessments can be made. The use of the calculated AUDPC has increased in recent years and can certainly be recommended when, because of either host phenology or growth, monotonically increasing disease progress is unlikely [32]. The field and molecular study tested 122 wheat genotypes for their tolerance against *B. sorokiniana* at the Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur location on the field showed different reactions and 20 lines were selected based on AUDPC under both in ITS and ILS condition, indicating that the genetic variability/variations for the response to leaf blight among the entries. Earlier, it was concluded that wheat cultivars especially cv. Kanchan having higher potential against fungal diseases have been developed through the targeted breeding program and new commercial cultivars have performed better in terms of grain yield [33,34]. On similar lines, a study was executed to identify and screen out spot blotch resistant wheat genotypes from the pool of 1387 genotypes and subsequent performance evaluation was conformed through the field and molecular analyses [35]. Similar to our results, wheat genotypes were sorted out into low and moderate resistance potential against spot blotch disease [36]. It was also reported a set of recombinant inbred lines were screened for spot blotch disease under the natural condition at three hot spot locations in India and few resistant lines were isolated [37]. Recently, it was concluded that only 20 genotypes out of 100 were found to be highly resistant to the disease, whereas others were resistant (28 genotypes), moderately resistant (22 genotypes), moderately susceptible (15 genotypes) and susceptible (15 genotypes) [38].

In Bangladesh, leaf rust which is caused by *Puccinia triticina* has become one of the most pertinent diseases of wheat [39]. The disease becomes very serious if susceptible varieties are sown later than the optimum time and wheat crop experiences terminal heat stress which is congenial for rust development under Bangladesh condition. Genetic resistance is the most economic and effective means of reducing yield losses caused by leaf rust disease [40]. Breeding for disease resistance genotypes is a continuous process and plant breeders need to add new effective genes to their breeding materials. The present investigation deals with new sources of resistance that can be incorporated into wheat to escape yield losses wreaked by the leaf rust disease. In this study, out of 122 genotypes, 42 genotypes including 9 varieties showed completely free from leaf rust infection, 59 genotypes showed resistance, 13 genotypes showed moderate resistance and 8 genotypes showed moderately susceptible reactions. It was concluded that resistant wheat lines might contain any resistant set of *Lr* genes. These new sources of leaf rust resistance can be incorporated into wheat to avoid yield losses and crop failure [41–44].

Previously, wheat blast remained restricted to South America's tropical regions [45], however recently it has spread to South Asian countries especially Bangladesh [46]. The current scenario is that over 15% of wheat area in Bangladesh is under serious threat of this disease. Recently, it is reported that disease-resistant genotypes at both stages (vegetative and reproductive) have been found promising to be used in wheat breeding programs [47,48].

The blast resistance genes come from *Aegilops ventricosa* (Zhuk.) Chennav on wheat [22,49]. In this experiment, conidia of *M. oryzae* pathotype *triticum* inoculated at the reproductive stage, 18 genotypes including BARI Gom 33 were immune against wheat blast under field condition, 42 genotypes including BARI Gom 30 and BARI Gom 32 were categorized as resistant, 26 genotypes were categorized as moderately resistant under field conditions. Among the identified 18 immune genotypes against wheat blast under field test, 16 genotypes including one variety (BARI Gom 33, BAW 1254, BAW 1280, BAW 1328, BAW 1338, BAW 1358, BAW 1360, BAW 1362, BAW 1363, BAW 1374, BAW 1390, BAW 1391, BAW 1394, BAW 1397, BAW 1399 and BAW 1401) showed positive 2NS segment of *Aegilops ventricosa*. This finding indicates a good correlation between the phenotypic and genotypic assessment of the genotypes against blast diseases. Similar research was executed and results revealed that few wheat varieties (BR 18-Terena) showed better performance as compared to others in terms of grain yield and disease tolerance owing to better genetic makeup [50]. Other studies have elaborated considerable differences among wheat genotypes regarding resistance to wheat blast [51–55]. Our findings are in agreement with previously reported results where wheat varieties having lower resistance to the wheat blast were identified and reported in Brazil to develop a genetic pool for developing more resistant varieties [56].

5 Conclusions

Out of 122 genotypes tested, 20 genotypes were found resistant to leaf blight, 42 were found completely free from leaf rust infection, while 59 were identified as resistant, and 13 were identified as moderately resistant to leaf rust both in irrigated timely and late sown conditions. Eighteen genotypes were immune against wheat blast (WB), 42 were characterized as resistant, and 26 were recognized as moderately resistant to WB. Molecular data revealed that the 16 genotypes showed a positive 2NS segment among the 18 immune genotypes selected against wheat blast under field conditions. Besides these, genotypes BAW 1322, BAW 1295, and BAW 1203 were found as earlier maturing genotypes and genotypes BAW 1372, BAW 1373, BAW 1297 and BAW 1364 were recorded as lodging tolerant due to short stature. Three genotypes namely WMRI Gom 1, BAW 1349 and BAW 1350 were categorized as bold grain. The genotypes WMRI Gom 1, BAW 1297, BAW 1377 were found suitable for optimum seeding condition and genotypes BAW 1377 and BAW 1366 were selected for late sown heat stress condition. The selected resistant genotypes against specific diseases and the other selected genotypes for yield and yield attributing traits can be used in a further breeding program to develop disease-resistant high yielding wheat varieties.

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