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Research Article

Screening rice genotypes for brown spot resistance along with yield attributing characters and its association with morphological traits

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Abstract: Brown spot, caused by *Bipolaris oryzae*, is a devastating disease of rice which can cause yield loss in most rice-growing regions of the world. Breeding for disease resistance is the preferred strategy of managing brown spot. Hence, identification and subsequent development of disease resistance in rice genotypes are crucial. The field resistance of 95 rice genotypes to brown spot was evaluated under water and fertilizer stress during 2017 and 2018. Partial resistance was assessed through reaction type (disease rating) and epidemiological parameters estimates i.e. final brown spot index, area under disease progress curve and apparent infection rate. Disease rating, brown spot index, and area under disease progress curve detected differences in the responses of rice genotypes to disease under field condition, which could be used to study brown spot resistance. Among the genotypes tested, 22 genotypes were resistant to moderately resistant (23.16%) while majority were moderately susceptible to susceptible (76.84%). A significant correlation between leaf angle and area under disease progress curve indicated positive influence of leaf erectness on severity of brown spot disease. Results showed that leaf infection did not significantly affect the number of filled grains per panicle or hundred seed weight, but caused yield decline by decreasing the number of productive tillers. Nevertheless, the infection of rice genotypes from flowering to ripening stages decreased the number of filled grains per panicle and grain weight. The resistant genotypes identified in this study can be exploited for future rice breeding programs to develop promising resistant lines in management of the brown spot disease.

Keywords: field resistance, *Bipolaris oryzae*, grain resistance, leaf angle, *Oryza sativa*, yield parameters

Introduction

Rice feeds nearly half of the world's population and has contributed significantly to global food security. The genetic improvement of this food crop can serve as a major component of sustainable food production. Brown spot (BS) caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker [telomorph: *Cochliobolus miyabeanus* (Ito and Kuribayahi) Drechsler], is the most important fungal disease of rice in irrigated and rain-fed rice environments causing as high as 45% yield reduction in severe

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epidemics (IRRI, 2012). It is a chronic disease of rice which, under favorable conditions, becomes a major threat to yield. This disease affects millions of hectares of rice each year, in pandemic form (Savary *et al.,* 2005; 2011). The disease usually occurs in farms with insufficient inputs like water and fertilizer (Ou, 1985; Barnwal *et al.,* 2013). BS is currently regarded as a serious rice disease worldwide (Barnwal *et al.,* 2013; Mizobuchi *et al.,* 2016). The report of damage caused by BS is increasing under global warming conditions because the optimal temperature for pathogen growth is relatively high (Savary *et al.,* 2011). *B. oryzae* can infect rice in all stages of crop growth. The pathogen causes different diseases in rice such as leaf BS, grain BS, and seedling death. This pathogen causes quantity and quality losses that are associated with the disease incidence on the leaves and grains (Ou, 1985; Lee, 1992). Incidence of *B. oryzae* on grains causes kernel discoloration, which affects the drying, shelling, milling and processing of the rice due to weight loss (Marchetti and Petersen, 1984; Soave *et al.,* 1984). In addition, the quality of grains decreases, which may lead to rejection of deliveries at international market. Because, for rice consumers, whole grains free from defects are preferred and this factor determines the price that growers will receive (Dallagnol *et al.,* 2014). Yield losses due to BS infected grains have been recorded in the range of 16% to 43% (Datnoff *et al.,* 1997). Genetic resistance is considered the most reliable and friendly approach for controlling BS (Sato *et al.,* 2008). Several studies have been conducted to screen cultivars for BS resistance (Mizobuchi *et al.,* 2016; Aryal *et al.,* 2016; Pantha *et al.,* 2017). Several cultivars that have been categorized as resistant did not show complete resistance to BS. Several cultivars, including Tetep, Khazar, IR64, IR50, Usen, Teqing and Tadukan have been reported to be resistant to moderately resistant (Satija *et al.,* 2005; Mizobuchi *et al.,* 2016). In northern Iran, BS is one of the important diseases of improved rice varieties in seedling and heading stages (Padasht-Dehkaei and Izadyar, 1998). The reduction of water resources and the occurrence of drought (Madani, 2014) are going to enhance BS severity in rice-growing regions of Iran. Hence, host plant resistance is an important tool for rice BS disease control and has played a key role in sustaining rice productivity in this country. However, there is no comprehensive study on identifying sources of disease resistance in Iran. Resistance to BS is of quantitative nature and is subject to genotype-by-environment interaction. New sources of resistance could be incorporated into rice genotypes to expand the existing gene pool for BS resistance. The development of DNA-based markers in rice provides a powerful tool for the dissection of quantitative traits, which has resulted in the designation of 26 QTLs for resistance to BS (Sato *et al.,* 2008; Mizobuchi *et al.,* 2016; Mandal *et al.,* 2017; Matsumoto *et al.,* 2017).

Therefore, the present study was undertaken to (i) identify rice genotypes resistant to BS disease at field under conditions of water and fertilizer stress, in order to facilitate breeding programs to improve BS resistance in rice genotypes and find out the yield potential amongst them, (ii) discover association BS severity on leaf level with morphological traits for identification of desirable traits in order to help rice breeders define their selection strategy to manipulate morphological traits and reduce disease incidence, (iii) assess the association of leaf BS with grain BS and discover their effect on yield-attributing parameters.

Materials and Methods

Plant material and study site

Ninety-five rice genotypes (Table 1) were evaluated for resistance to BS disease in a research field at Rice Research Institute of Iran (RRII, Rasht, Iran, 37.16° N, 49.36° E) during the years 2017 and 2018. Iranian genotypes were chosen from important cultivated Iranian genotypes. Tetep, Khazar, Usen, IR50, IR64 are resistant to moderately resistant, while IR36 is susceptible to BS (Satija *et al.,* 2005; Banu *et al.,* 2008; Mizobuchi *et al.,* 2016).

Table 1 List of rice genotypes used in this study, their pedigree and country of origin.

No.	Genotypes	Pedigree/Parentage	Origin
1	Sadri	Landrace	Iran
2	Domsiyah	Landrace	Iran
3	Domsiyah-Soleiman-Darab	Landrace	Iran
4	Hasan saraei	Landrace	Iran
5	Hasan saraei-Atashgah	Landrace	Iran
6	Hasan saraei-Pichide ghalaf	Landrace	Iran
7	Binam	Landrace	Iran
8	Hashemi	Landrace	Iran
9	Domsefid	Landrace	Iran
10	Domsorkh	Landrace	Iran
11	Domzard	Landrace	Iran
12	Gharib	Landrace	Iran
13	Gharib-Siyah-Reihani	Landrace	Iran
14	Anbarbu	Landrace	Iran
15	Ali-Kazemi	Landrace	Iran
16	Hasani	Landrace	Iran
17	Salari	Landrace	Iran
18	Abjibo-Ji	Landrace	Iran
19	Rashti-Sard	Landrace	Iran
20	Ghasrodashti	Landrace	Iran
21	Sange-Jo	Landrace	Iran
22	Ghashangeh	Landrace	Iran
23	Champa-Budar	Landrace	Iran
24	Gerdeh	Landrace	Iran
25	Dashti	Landrace	Iran
26	Mehr	Moosa-Tarom	Iran
27	Ahmad-Jo	Landrace	Iran
28	Shahpasand	Landrace	Iran
29	Zireh	Landrace	Iran
30	Zireh-Bandpey	Landrace	Iran
31	Tarom-Mahalli	Landrace	Iran
32	Tarom-Amiri	Landrace	Iran
33	Tarom-Pakotah	Landrace	Iran
34	Tarom-Mantaghe	Landrace	Iran
35	Sange-Tarom	Landrace	Iran
36	Ahlami-Tarom	Landrace	Iran
37	Mir-Tarom	Landrace	Iran
38	Moosa-Tarom	Landrace	Iran
39	Deilamani	Landrace	Iran
40	Anburi	Landrace	Iran
41	Khazar	TNAU7456/IR2071-625-1-52	Iran
42	Gilaneh	Abjibo-Ji/Saleh	Iran
43	Gohar	Pusa1238-1/pusa1238-81-6	Iran
44	Sepidrud	Domsiyah /IR28//Garme-Sadri	Iran
45	Dorfak	Salari/ Sepidrud	Iran
46	Bejar	Domsiyah /IR28//IR28	Iran
47	Saleh	Khazar/IR39385-20-1-2-1-2	Iran
48	Kadus	IR64669-153-23	Iran
49	Gil1	Moosa-Tarom/Ansitku	Iran
50	Nemat	Amol3/Sange-Tarom	Iran
51	Neda	Sange-Tarom/ Hasan saraei//Amol3	Iran
52	Dasht	Amol1/IR24	Iran
53	Amol1	Tarom-Firozkandeh/ Taichong Native 1	Iran
54	Amol2	IR28	Iran
55	Amol3	GEB24/TN1	Iran
56	Koohsar	HSCSS	Iran
57	Fajr	IR62781-175-1-10	Iran
58	Keshvari	IR66233-169-3-3	Iran
59	Sahel	Basmati	Iran
60	Shirudi	Khazar/Deilamani	Iran
61	Pardis	Sange-Jo/Sepidrud//Sange-Jo//Sange-Jo	Iran
62	Pazhoohesh	Sange-Jo/Sepidrud//Sange-Jo//Sange-Jo	Iran
63	Tarom-Jolodar	Landrace	Iran
64	Mohammadi-Chaparsar	Landrace	Iran
65	Tabesh	Mutant line drived from Tarom-Mahalli	Iran
66	Shafagh	IR67015-94-2-3	Iran
67	Zayandehrud	Nogeran Lenjan	Iran
68	Sazandegi	Nogeran Lenjan	Iran
69	Ghaem	Sange-Jo/Sepidrud//Sange-Jo//Sange-Jo	Iran
70	Danial	LD183	Iran
71	Hooveizeh	Landrace	Iran
72	IR28	IR833-6-2-1-1/ IR1561-149-1//IR24*4/*O. nivara*	Philippines
73	IR30	IR1541-102-6-3/IR20*4/*O. nivara*	Philippines
74	IR36	IR1561-228-1-2/IR1737 CR94-13	Philippines
75	IR50	IR2153-14-1-6-2/IR28 IR36	Philippines
76	IR60	IR4432-53-33/PTB33 IR36	Philippines
77	IR64	IR5657-33-2-1/ IR2061-465-1-5-5	Philippines
78	Line 120	Introduction	Philippines
79	Line 213	Introduction	Philippines
80	Line 338	Introduction	Philippines
81	Line 830	Introduction	Philippines
82	Line 833	Introduction	Philippines
83	Line 834	Introduction	Philippines
84	Line 835	Introduction	Philippines
85	Line 839	Introduction	Philippines
86	Usen	Introduction	Egypt
87	Dcl	Introduction	Egypt
88	CY	Introduction	Egypt
89	Dular	Landrace	USA
90	KMP41	Introduction	India
91	NP125	Introduction	India
92	Norin 22	KINK115/NORIN6	Japan
93	Kanto51	GIN BOZU/TO TO	Japan
94	Tetep	Landrace	Vietnam
95	Zenith	Introduction	USA

Experimental design and crop establishment

The experiment was designed in a randomized complete block design with three replicates. Forty-day-old seedlings were transplanted on 28 May 2017 and 21 May 2018. The individual experimental unit (plot) was 0.6 m^2 (60 × 100 cm). Plant-to-plant spacing was 20 cm with three seedlings per hill for all genotypes. The experimental field had no residue from the previous rice crop. A single manual weeding was carried out at 30 days after transplanting. Due to the effect of water and nutrient deficiency on increasing disease severity (Ou, 1985; Datnoff *et al.,* 1997), field resistance to BS disease in rice genotypes was evaluated under water and fertilizer stress. Irrigation water at the time of transplanting was maintained at a depth of 3-4 cm. Three weeks after transplanting a constant water depth of 5 cm was maintained to keep the field continuously flooded and then irrigation was withdrawn for the next one month, until the appearance of the cracking in the field bed and wilting symptoms in rice genotypes. Then the crop was given light irrigation to keep the soil wet and the irrigation was suspended during rainy days. Soil test was performed before transplanting for determining fertility levels and the recommended fertilizer rate was 80: 120: 40 kg N: P: K per hectare. In order to create fertilizer stress, fertilization was not carried out in the process of preparation of the field and the growth period.

Data collection

Data was recorded on the central two rows of each plot, by leaving two rows from each side to avoid border effect. In each plot, 10 tillers were selected randomly from two central rows and disease scoring on leaf level was assessed using the standard evaluation system for rice (IRRI, 2013), where 0 refers to no incidence, 1 = less than 1% leaf area covered, 2 = 1–3%, 3 = 4–5%, 4 = 6–10%, 5 = 11–15%, 6 = 16–25%, 7 = 26–50%, 8 = 51–75% and 9 = 76–100% leaf area covered by the disease. The genotypes scoring 0 and 1 were considered to be highly resistant (HR); 2 as resistant (R); 3 as moderately resistant (MR); 4–6 as moderately susceptible (MS); 7 as susceptible (S) and 8 and 9 as highly susceptible (HS). Disease scoring was estimated four times at 14-day intervals after the appearance of the first disease symptom. Morphological traits were measured for each plot 10-15 days after heading. The traits investigated included the number of productive tillers (TN), flag leaf length (FLL), flag leaf width (FLW), flag leaf angle (FLA), second leaf length (SLL), second leaf width (SLW), second leaf angle (SLA), third leaf length (TLL), third leaf width (TLW) and third leaf angle (TLA) (IRRI, 2013). At the stage of physiological maturity (IRRI, 2013), observations of yield contributing traits were recorded on 10 randomly selected panicles per genotype per replication for the traits i.e. the severity of discolored grains (GD), the number of filled grains (FG), the number of unfilled grains (UFG) and the hundred seed weight (HSW). Discoloration severity on the grains of each panicle per genotype was scored using a 0-7 scale, modified from IRRI (2013) as follows: 0 = no disease symptoms, 1 = less than 1%, 2 = from 1.1 to 5%, 3 = from 5.1 to 10%, 4 = from 10.1 to 25%, 5 = from 25.1 to 50%, 6 = 50.1 to 75%, and 7 = more than 75% of the grains surface with disease symptoms.

Data analysis

The values obtained from the grade scale in leaf and grain levels were used to calculate the disease index, according to the formula suggested by McKinney (1923). The percent disease index (PDI) was calculated using the following formula:

$$\text{PDI} = \frac{\text{Sum of all the numerical rating}}{\text{Number of observations} \times \text{Maximum disease grade}} \times 100$$

The effects of disease severity on rice genotypes along a given time period can be evaluated using the area under the disease progress curve (AUDPC). The disease rating data on leaf level were used to calculate the total AUDPC. The total AUDPC is calculated from all the four ratings at different times thus leading to a more accurate phenotypic

evaluation. AUDPC was calculated following the equation developed by Shanner and Finney (1977), which is given by:

$$AUDPC = \sum_{i=1}^{n}\left[\left(\frac{Y_{i+1}+Y_i}{2}\right)\times\left(t_{i+1}-t_i\right)\right]$$

Where, y_i = disease score at the i^{th} observation, t_i = time at the i^{th} observation and n = total number of observation.

The apparent infection rate is an estimate of the rate of progress of a disease, based on proportional measures of the extent of infection at different times. The apparent infection rate was estimated in terms of disease severity recorded on genotypes to assess the highest and least infection periods (Van der Plank, 1963).

$$\text{Apparent infection rate} = \left(\frac{log\, x_2 - x_1}{\left(t_1 - t_2\right)}\right)\times 2.303$$

Where, x_1 and x_2 are the disease scores at time t_1 and t_2, respectively.

The data obtained from the disease screening and yield attributing parameters were analyzed using RCBD combined analysis in year. A combined analysis of variance was performed following a test of homogeneity of variances. The model included: genotype, block, year, genotype-year interaction. Analysis of variance (ANOVA) was calculated using the generalized linear model procedure (PROC GLM) in SAS (version 9.1 for Windows). The mean comparison was carried out by MSTAT-c software. To describe the magnitude of the relationships among agronomic traits and disease severity index, Pearson's correlation coefficients (*r*) were calculated using SPSS version 16.

Results

Leaf brown spot disease

Table 2 summarizes the results from the combined analysis of variance for epidemiological parameters. A highly significant effect was observed for the final brown spot index, total AUDPC and apparent infection rate. The effect of year on epidemiological parameters was not significant. Results indicated a separate response of genotypes for BS including R, MR, MS and S. Data showed a discrepancy in the values of resistance within parameters and genotypes. The response of rice genotypes to BS was similar in the two study years. Among the genotypes tested, 10 (10.53%) genotypes exhibited resistant responses, 12 (12.63%) moderately resistant, 51 (53.68%) moderately susceptible and 22 (23.16%) were found to be susceptible. Highest and lowest values of final BSI were observed on Mohammadi-Chaparsar and Nemat varieties i.e. 67.14% and 40.86%, respectively. The highest total AUDPC was found on NP125 (163.33) while Nemat, Shirudi, Neda, Amol3, IR60, Kanto51 and Gharib-Siyah-Reihani (56.00) had a significantly lower total AUDPC. The apparent infection rate ranged between 0.0055 and 0.0418. The highest values of apparent infection rate was observed in Domsiyah (0.0418) and the lowest was found on Nemat, Shirudi, Neda, Khazar, Amol3, IR60, Kanto51 and Gharib-Siyah-Reihani (0.0055). In general, 10 rice genotypes, i.e. Nemat, Shirudi, Neda, Khazar, Amol3, IR60, Kanto51, Gharib-Siyah-Reihani, Usen and Shafagh were marked as possessing high levels of resistance based on the three parameters in both growing seasons (Table 3).

Kernel discoloration severity

ANOVA showed there was a highly significant difference in the severity of grain discoloration among rice genotypes (Table 2). Data in Table 3 shows the severity of grain discoloration ranged from 6.55 to 73.03 percent. Among the seed samples collected from different genotypes, the highest grain discoloration was observed in Line 120 with an average severity of 73.03 percent followed by Usen (70.89%), Sepidrud (63.33%) and IR50 (61.13%). The minimum severity of grain discoloration was observed in Hasan saraei-Pichide ghalaf (4.50%) followed by Domsefid (5.79%), Hashemi (6.09%), Bejar (6.52%) and Gerdeh (6.55%).

Table 2 Mean square value of epidemiological and yield attributing parameters of 95 genotypes during 2017 and 2018 growing seasons at Rice Research Institute, Rasht, Iran.

Source of variation	df	GD	Epidemiological parameters				Yield-attributing parameters				
			df	BSI	AUDPC	Apparent infection rate	df	FG	UFG	HWS	TN
Genotype	93	13.78**	94	196.19**	1093.68**	0.000150**	94	1084.52**	1112.12**	0.47**	41.66**
Year	1	45.64**	1	199.20^{ns}	21234^{ns}	0.000785^{ns}	1	269.45^{ns}	90.80^{ns}	0.42**	15.83^{ns}
Error (Year)	4	1.12	4	684.03	3894.73	0.000134	4	24.86	11.28	0.04	86.05
Genotype*Year	93	0.36^{ns}	94	18.47^{ns}	162.37**	0.000034413**	94	0.11^{ns}	0.10^{ns}	0.03**	5.89^{ns}
Error (Total)	372	0.47	376	21.66	103.89	0.000015	376	13.42	8.54	0.015	2.35
CV (%)	-	14.58	-	8.91	7.79	11.40	-	7.70	11.43	5.25	17.70

GD, severity of grain discoloration; BSI, final brown spot index (70 DAT); AUDPC, total area under disease progress curve; FG, number of filled grains per panicle; UFG, number of unfilled grains per panicle; HSW, hundred seed weight; TN, number of productive tillers; df, degree of freedom; CV, coefficient of variance; **, P < 0.0001 level; ^{ns}, non-significant.

Table 3 Mean of epidemiological and yield attributing parameters of 95 genotypes during 2017 and 2018 growing season at Rice Research Institute, Rasht, Iran.

Genotype	Epidemiological parameters			GD	Yield parameters				Reaction type
	BSI	AUDPC	Apparent infection rate		FG	UFG	HSW	TN	
Mohammadi-Chaparsar	67.14	151.67	0.0398	20.93	33.37	23.83	2.33	6	S
Deilamani	62.05	143.50	0.0405	27.59	56.53	22.50	2.28	8	S
IR36	61.98	157.50	0.0373	13.43	50.47	20.98	2.44	12	S
Hasan saraei	61.03	151.67	0.0345	12.46	49.43	18.30	2.15	7	S
Sazandegi	60.96	149.33	0.0398	34.07	60.63	13.43	2.17	7	S
Dular	60.82	156.33	0.0398	15.76	51.83	20.57	2.27	7	S
Domzard	60.30	142.33	0.0398	8.48	44.53	18.98	2.11	10	S
Line 120	60.27	150.50	0.0403	73.03	8.68	36.32	2.13	7	S
Ahmad-Jo	60.05	141.17	0.0405	19.43	72.83	18.03	2.38	6	S
Hasani	59.98	130.67	0.0396	18.04	53.93	8.97	2.97	7	S
Salari	59.66	148.17	0.0371	12.67	37.80	10.83	2.66	6	S
IR28	59.57	156.33	0.0383	44.35	46.55	20.52	2.47	8	S
Hashemi	58.87	156.33	0.0383	6.09	44.47	7.30	2.44	8	S
Sange-Tarom	58.72	147.00	0.0380	9.31	69.67	18.05	2.47	7	S
Koohsar	58.40	126.00	0.0396	42.65	24.47	32.45	2.51	7	S
Line 833	58.36	138.83	0.0405	20.89	50.77	28.27	2.38	6	S
Gharib	58.32	141.17	0.0387	36.20	57.15	16.43	2.67	10	S
Domsiyah	58.32	148.17	0.0418	7.88	35.53	12.57	2.41	8	S
NP125	57.23	163.33	0.0412	17.87	51.60	31.90	1.73	11	S
Gil1	56.79	142.33	0.0398	20.74	64.33	35.45	2.56	6	S
Abjibo-ji	56.20	156.33	0.0382	7.52	44.93	12.67	2.45	8	S
Sadri	56.17	161.00	0.0412	13.94	64.87	14.90	2.38	6	S
Domsorkh	55.92	127.17	0.0373	13.53	47.37	18.77	2.63	7	MS
Line 338	55.58	142.33	0.0382	14.44	46.80	27.87	2.50	8	MS
Ali-Kazemi	55.51	136.50	0.0374	8.01	62.03	12.33	2.99	7	MS
Zireh	55.48	131.83	0.0357	13.61	50.67	7.13	2.55	6	MS
Hasan saraei-Atashgah	55.28	120.17	0.0374	7.20	45.20	13.97	2.56	7	MS
Ahlami-Tarom	55.19	145.83	0.0389	17.26	41.60	14.53	2.10	8	MS
Champa-Budar	55.11	140.00	0.0328	22.00	57.93	20.10	2.80	7	MS
IR30	55.04	151.67	0.0364	14.94	58.73	11.18	2.26	7	MS
Binam	55.01	131.83	0.0357	8.70	68.97	8.73	2.83	8	MS
Tarom-Pakotah	54.77	136.50	0.0336	17.83	54.47	20.30	2.43	8	MS
Line 213	54.69	151.67	0.0383	55.65	51.20	26.98	1.94	9	MS
Moosa-Tarom	54.42	130.67	0.0366	15.31	52.47	35.65	2.35	7	MS
Mehr	54.36	138.83	0.0336	11.83	61.47	13.87	2.32	9	MS
Tarom-Mantaghe	54.14	130.67	0.0363	11.28	50.67	13.43	2.49	14	MS
Hooveizeh	54.06	149.33	0.0380	28.15	30.47	19.35	2.00	6	MS
Ghaem	54.00	129.50	0.0373	40.56	52.63	19.82	2.26	7	MS
Rashti-Sard	53.98	138.83	0.0389	14.15	52.20	16.27	2.34	7	MS

Table 3 continued

Tarom-Mahalli	53.70	138.83	0.0336	19.83	63.13	26.63	2.32	6	MS
Saleh	53.47	126.00	0.0366	39.52	54.70	38.85	2.15	8	MS
Domsiyah-Soleiman-Darab	53.46	137.67	0.0307	17.37	25.87	23.07	2.27	8	MS
Zireh-Bandpey	53.12	133.00	0.0345	51.93	50.07	16.57	1.95	9	MS
Zayandehrud	52.80	122.50	0.0302	36.20	43.23	41.47	2.23	7	MS
Norin22	52.78	147.00	0.0361	38.24	49.57	16.60	2.36	9	MS
Danial	52.78	144.67	0.0348	19.76	56.37	27.72	1.72	10	MS
Mir-Tarom	52.68	119.00	0.0345	9.30	61.43	16.50	2.10	9	MS
Hasan saraei-Pichide ghalaf	52.07	138.83	0.0354	4.50	44.20	7.30	2.50	9	MS
Line 835	51.96	138.83	0.0369	40.46	41.93	35.73	1.94	7	MS
Pardis	51.90	122.50	0.0311	20.17	34.20	21.77	2.38	8	MS
Sange-Jo	51.81	135.33	0.0366	11.02	51.13	17.33	2.31	8	MS
Gerdeh	51.74	109.67	0.0252	6.55	63.80	13.17	2.54	10	MS
KMP41	51.60	133.00	0.0325	25.65	64.60	24.07	2.14	8	MS
CY	51.60	135.33	0.0348	47.54	19.07	38.13	2.18	9	MS
Line 839	51.60	145.83	0.0369	23.96	59.80	43.47	2.53	8	MS
Ghashangeh	51.55	117.83	0.0339	19.72	43.73	13.93	2.55	9	MS
Line 830	51.02	141.17	0.0316	19.76	62.87	7.53	2.31	8	MS
Dashti	50.85	131.83	0.0316	10.39	52.47	9.97	2.54	5	MS
Zenith	50.76	128.33	0.0341	45.87	30.00	19.37	2.49	12	MS
Line 834	50.76	133.00	0.0364	51.65	46.13	37.30	2.43	11	MS
Shahpasand	50.7	117.83	0.0273	15.93	25.27	18.55	3.53	9	MS
Anburi	49.59	141.17	0.0355	13.83	58.83	16.02	2.62	8	MS
Bejar	49.56	120.17	0.0336	6.52	23.60	64.45	2.29	7	MS
Sepidrud	49.5	119.00	0.0325	63.33	32.57	78.97	2.51	11	MS
Domsefid	49.36	119.00	0.0348	5.79	43.30	31.83	2.17	11	MS
Fajr	48.63	138.83	0.0373	24.44	45.43	47.17	1.96	9	MS
Anbarbu	48.37	126.00	0.0280	22.83	74.10	12.87	2.66	7	MS
Gilaneh	48.33	117.83	0.0316	39.72	46.83	38.47	2.40	7	MS
Tarom-Amiri	48.06	112.00	0.0280	17.02	43.90	26.03	2.23	8	MS
Dorfak	47.72	117.83	0.0334	12.78	38.87	33.42	2.40	9	MS
Ghasrodashti	47.44	136.50	0.0316	13.60	70.63	24.22	2.69	7	MS
Gohar	47.13	127.17	0.0334	19.70	53.77	26.27	2.29	7	MS
Dcl	47.06	138.83	0.0357	53.30	37.00	24.57	2.10	11	MS
IR64	46.66	107.33	0.0197	30.37	65.03	22.12	2.54	7	MR
Dasht	46.34	99.17	0.0181	28.57	12.93	35.37	2.20	10	MR
Kadus	46.33	95.67	0.0197	15.96	56.10	35.70	2.34	7	MR
Pazhoohesh	45.88	91.00	0.0197	24.33	45.60	19.62	2.26	16	MR
Tarom-Jolodar	45.74	91.00	0.0197	7.82	23.77	21.73	2.36	10	MR
Tabesh	45.69	95.67	0.0197	24.54	37.90	42.60	2.47	9	MR
Tetep	45.65	95.67	0.0197	28.70	56.00	24.37	2.44	8	MR
Keshvari	45.31	79.33	0.0165	12.22	56.53	44.55	2.48	12	MR
IR50	44.65	88.67	0.0165	61.13	38.97	31.77	2.30	11	MR
Amol1	44.58	102.67	0.0165	37.06	69.50	50.02	2.00	11	MR
Sahel	44.33	72.33	0.0197	30.96	36.80	59.98	2.52	15	MR
Amol2	44.27	94.50	0.0209	47.48	24.73	50.43	2.33	9	MR
Shafagh	43.82	58.33	0.0110	47.39	33.53	47.02	2.13	13	R
Usen	43.54	58.33	0.0110	70.89	53.50	11.07	1.67	20	R
Gharib-Siyah-Reihani	43.53	56.00	0.0055	-*	43.20	16.13	3.06	5	R
Kanto51	43.35	56.00	0.0055	23.56	47.03	14.90	2.23	10	R
IR60	43.15	56.00	0.0055	35.76	27.83	44.88	1.95	13	R
Amol3	43.04	56.00	0.0055	33.54	41.50	44.67	2.27	15	R
Khazar	42.25	60.67	0.0055	22.69	51.07	41.00	2.33	8	R
Neda	41.62	56.00	0.0055	31.37	38.13	28.85	2.61	14	R
Shirudi	40.95	56.00	0.0055	28.89	39.27	33.70	2.25	14	R
Nemat	40.86	56.00	0.0055	33.63	47.80	25.17	2.69	11	R
LSD	11.63	26.46	0.0083	9.49	10.89	11.95	0.46	3	-

BSI, final brown spot index (70 DAT); AUDPC, total area under disease progress curve; GD, severity of grain discoloration; FG, number of filled grain per panicle; UFG, number of unfilled grain per panicle; HSW, hundred seed weight; TN, number of productive tillers; R, Resistant; MR, Moderately resistant, MS, Moderately susceptible, S, Susceptible, LSD, least significant difference; *, color seed coat Gharib-Siyah-Reihani is black.

Yield parameters

The analysis of variance for 95 rice genotypes revealed significant variations for grain yield-related parameters (Table 2). Filled grains per panicle ranged from 8.68 to 74.10. The maximum number of filled grains per panicle was observed in Anbarbu (74.10) followed by Ahmad-Jo and Ghasrodashti where 72.83 and 70.63 filled grains were recorded, respectively. The minimum number of filled grains per panicle was recorded in Line 120 (8.68) followed by Dasht (12.93). The maximum number of unfilled grains per panicle was produced by Sepidrud (78.97) followed by Bejar (64.45). The smallest figure in this index was produced by Zireh (7.13) followed by Hashemi, Hasan saraei-Pichide ghalaf (7.30) and Line 830 (7.53). The highest grain weight was found in Shahpasand (3.53) and lowest in Usen (1.67). The number of productive tillers ranged from 5 to 20. The maximum number of productive tillers was observed in Usen. The minimum was observed in Dashti and Gharib-Siyah-Reihani varieties (Table 3).

Correlation analysis

Pearson's correlation coefficients were estimated among 4 epidemiological parameters and the severity of grain discoloration (Table 4). A positive correlation was found between DR and the final BSI and the total AUDPC with a strong *r*-value *i.e.*, 0.827 and 0.877, respectively. This relationship was positive, however of the weaker nature, with apparent infection rate (r = 0.157, $P < 0.01$). Furthermore, DR showed a weak and negative correlation with GD (r = -0.246, $P < 0.01$). Table 5 summarizes correlation coefficients (r) describing the degree of correlations among measured yield-attributing parameters and disease indices (total AUDPC and GD). The correlations between GD, FG, UFG, HSW, and TN were significant. GD was negatively correlated to FG and HSW (r = -0.295, $P < 0.01$ and r = -0.277, $P < 0.01$, respectively). On the other hand, GD was weak and positively correlated to UFG and TN (r = 0.362, $P < 0.01$ and r = 0.274, $P < 0.01$, respectively). The total AUDPC had non-significant correlations with FG and HSW and UFG. The total AUDPC showed medium significant and negative correlation (r = -0.452, $P < 0.01$) with TN. Pearson's correlation coefficients were estimated among all the 9 morphological traits and disease progress (Table 6). Total AUDPC had positive and significant correlations ($r < 0.3$, $P < 0.05$) with all traits under stressed condition.

Table 4 Pearson's correlation coefficients (r) describing the relationship between disease indices of 95 rice genotypes evaluated under water and fertilizer stress conditions.

Index	DR	BSI	AUDPC	Apparent infection rate	GD
DR	1	0.827**	0.877**	0.157**	-0.246**
BSI		1	0.690**	0.088*	-0.196**
AUDPC			1	0.100*	-0.229**
Apparent infection rate				1	0.093*
GD					1

DR, disease rating; BSI, final brown spot index (70 DAT); AUDPC, total area under disease progress curve; GD, severity of grain discoloration; *, $P < 0.05$ level (2-tailed); **, $P < 0.01$ level (2-tailed).

Table 5 Pearson's correlation coefficients (r) describing the relationship between the area under disease progress curve and the severity of grain discoloration with yield-attributing parameters of 95 rice genotypes evaluated under water and fertilizer stress conditions.

Index	AUDPC	GD	FG	UFG	HSW	TN
AUDPC	1	-0.229**	-0.202ns	0.319ns	-0.030ns	-0.452**
GD		1	-0.295**	0.362**	-0.277**	0.274**
FG			1	-0.335**	0.088*	-0.160**
UFG				1	-0.213**	0.204**
HSW					1	-0.197**
TN						1

AUDPC, total area under disease progress curve; GD, severity of grain discoloration; FG, number of filled grain per panicle; UFG, number of unfilled grain per panicle; HSW, hundred seed weight; TN, number of productive tillers; *, $P < 0.05$ level (2-tailed); **, $P < 0.01$ level (2-tailed); ns, non-significant.

Table 6 Pearson's correlation coefficients (*r*) describing of the relationship between the area under disease progress curve and the morphological traits of 95 rice genotypes evaluated under water and fertilizer stress conditions.

Index	AUDPC	TLA	TLW	TLL	SLA	SLW	SLL	FLA	FLW	FLL
AUDPC	1	0.152**	0.144**	0.202**	0.094*	0.142**	0.224**	0.109**	0.125**	0.087*
TLA		1	0.159**	0.405**	0.808**	0.221**	0.362**	0.771**	0.220**	0.017^{ns}
TLW			1	0.414**	0.157**	0.912**	0.467**	0.137**	0.813**	0.319**
TLL				1	0.356**	0.405**	0.799**	0.309**	0.369**	0.404**
SLA					1	0.161**	0.306**	0.762**	0.177**	0.003^{ns}
SLW						1	0.471**	0.155**	0.891**	0.411**
SLL							1	0.252**	0.430**	0.612**
FLA								1	0.155**	-0.146**
FLW									1	0.372**
FLL										1

AUDPC, total area under disease progress curve; FLL, flag leaf length; FLW, flag leaf width; FLA, flag leaf angle; SLL, second leaf length; SLW, second leaf width; SLA, second leaf angle; TLL, third leaf length; TLW, third leaf width; TLA, third leaf angle; *, $P < 0.05$ level (2-tailed); **, $P < 0.01$ level (2-tailed); ^{ns}, non-significant.

Discussion

In this study, a range of Iranian and foreign rice genotypes from the Rice Research Institute of Iran was evaluated under water and fertilizer stress conditions for BS resistance, in order to identify potentially useful disease tolerance donors for future breeding programs. The present study revealed the existence of a highly significant difference between genotypes for disease indices and yield parameters.

The delayed rates of disease development and lower total AUDPC were observed in resistant genotypes indicating a higher level of resistance. No genotype was found completely resistant (with disease rating 0) to BS in this experiment. The 53.68% of genotypes in present experiment fell under MS category which could be due to the emergence of more aggressive pathogen races under favorable environmental conditions for BS disease in this area. However, no information about pathogen races is available from Iran. Nemat, Shirudi, Neda, Khazar, Amol3, IR60, Kanto51, Gharib-Siyah-Reihani, Usen and Shafagh genotypes showed a high level of resistance followed by Amol2, Sahel, Amol1, IR50, Keshvari, Tetep, Tabesh, Tarom-Jolodar, Pazhoohesh, Kadus, Dasht and IR64. These sources of resistance identified from among these genotypes can be exploited for future rice breeding programs to develop promising resistant lines in management of the BS. Tetep, Khazar, Usen, IR64 and IR50 have been previously reported to be resistant to BS (Satija *et al.,* 2005; Mizobuchi *et al.,* 2016). The field-based assessment of BS resistance was assessed through DR, the final BSI, the total AUDPC and the apparent infection rate. DR is the most used parameter for this purpose. In our study, an attempt was made to elucidate the association between these parameters. In this study, parameters used to identify resistance to BS were strongly and positively correlated, except apparent infection rate ($r < 0.2$, $P < 0.01$). This may be due to the fact that apparent infection rate is a regression DR with a larger error variance. Overall, DR, final BSI, and total AUDPC were equally powerful to compare genotypes based on disease development.

Grain discoloration has been considered as one of the important problems which directly affect the quality of the produce (Marchetti and Petersen, 1984; Soave *et al.,* 1984). It has been prevalent in most rice-growing regions of the world because of the unavailability of resistant varieties combined with good yield characters for cultivation (Narain, 1992). *B. oryzae* can attack at any stage of development, but the damage is worse at end of the cycle because it drastically decreases the yield and quality (Soave *et al.,* 1984; Ou, 1985). Hasan saraei-Pichide ghalaf, Domsefid, Hashemi, Bejar, Gerdeh, Hasan saraei-Atashgah, Abjibo-Ji, Tarom-Jolodar, Domsiyah,

Ali-Kazemi, Domzard, Binam, Mir-Tarom and Sange-Tarom genotypes showed low levels of grain discoloration (%GD < 10). Leaf infection by *B. oryzae* did not significantly affect the number of filled and unfilled grain per panicle and hundred seed weight (r = -0.202, r = 0.319 and r = -0.030, P > 0.05, respectively), which corroborated with the findings of Prabhu *et al.,* (1980). The given data of grain weight reflects the overall genetic potential of experimental genotypes which may or may not be directly related to disease conditions on the leaf. On the other hand**,** a significant negative correlation was observed between the total AUDPC and the number of tillers (r = -0.452, P < 0.01), indicating that leaf infection caused a decline in yield by decreasing the number of productive tillers (Lee, 1992). Yield losses due to infection of BS on rice leaves need to be further investigated. A significant negative correlation was observed between GD with FG and HSW, indicating that grain's infection affects seed development and may cause loss in weight. As a consequence of paddy grain discoloration, weight was reduced significantly and weight loss depended on the level of discoloration (Table 5).

A significant negative correlation (r = -0.229, P < 0.01) was observed between leaf and grain infection (Table 4). Leaf resistance among rice genotypes was not related to their growing period. Frequent and heavy rainfalls particularly near the harvest season make the wet panicles more prone to invasions by fungal species. Genotypes with a long growing period seem more prone to grain infection than genotypes with either a short or an average period of development. This may be attributed to a higher incidence of rainfall from the flowering stage to the grain maturity stage. Discolored rice grains are observed in both dry and wet seasons but the severity is higher in the wet season (Reddy *et al.,* 2004). In northern parts of Iran, *B. oryzae* may infect the glumes, causing dark brown to black oval spots. What is more, rainfall at maturity stage results in development of conidiophores and conidia on the spots which give the seeds a velvety appearance. In some cultivars, the fungus causes panicle neck rot. These results indicate that infection by *B. oryzae* during the period from flowering to the ripening stage can have greater effect on reducing the number of filled grain per panicle and hundred weight seed than that caused by leaf infection, which corroborates the finding of Prabhu *et al.* (1980).

Information on the correlation between BS severity and morphological traits is limited. Leaf orientation in rice genotypes may influence dew or moisture deposition on the leaf surface essential for the germination of spores of the BS pathogen (Ou, 1985; Percich *et al.,* 1997). Thus, it may influence the response of rice genotypes to BS disease, especially in the warmer and humid growing regions of the world. Association of different morphological traits with BS resistance is not well elucidated. In this study, the majority of morphological traits studied showed a weak correlation with the progression of the disease in field (Table 6). The evaluation of the relationship of the leaf angle and BS severity showed that genotypes having erect or semi-erect leaf angles generally show lower disease severity than those with horizontal or recurved leaves. However, both high and low AUDPC types were seen in erect and horizontal leaf angle genotypes, which indicate the absence of complete genetic linkage. For this reason, the resistance of rice genotypes to BS disease cannot be definitively attributed to leaf erectness. Gangopadhyay and Chattopadhyay (1974) reported that leaf angle was associated with disease incidence, and that BS infection increased with an increase in the leaf angle. In other study, Joshi and Chand (2002) reported that a positive correlation between leaf angle and AUDPC further indicated a positive influence of leaf erectness on severity to spot blotch disease in wheat. The infection efficiency of pathogen increases with an increase in temperature, humidity, and moisture. The low mean AUDPC of erect leaf genotypes might partly be due to the fact that erect leaves hold less free water that is essential for germination of pathogen spores. Prolonged leaf wetness periods in rice canopy generally lead to increased lesion densities (Percich *et al.,* 1997; Barnwal *et al.,* 2013). Additionally, successful inoculation by conidia required a relative humidity of > 89% and

infection was favored by free water on leaf surface (Ou, 1985). Differences among resistant and susceptible genotypes for the length and width of the leaf as well as flag leaf anatomy have been observed (Table 6).

Conclusion

Our results emphasize the important effects of BS disease on rice growth and productivity under water and fertilizer stress conditions. The present study reveals that the genotypes have enough diversity regarding resistance to BS in North of Iran. None of the genotypes was marked as immune. Shirudi, Khazar, IR60, Kanto51, Shafagh, Amol2, IR50, Keshvari, Tetep, Tabesh, Tarom-Jolodar, Kadus and Dasht genotypes which have much higher levels of resistance but lower yield-attributing parameters may be used in breeding programs to transfer their better resistance character on leaf and grain level. Nemat, Neda, Amol3, Gharib-Siyah-Reihani, Usen, Sahel, Amol1, Pazhoohesh and IR64 genotypes which also have higher yield-attributing parameters, compared to other genotypes resistant to BS, could be recommended for cultivation and further breeding utilization. We conclude that manipulation of leaf angle by rice breeders can be effective in reducing disease incidence.

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Conflict of interest

The authors declare that they have no conflict of interest.

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غربال‌گری مقاومت به بیماری لکه قهوه‌ای در ژنوتیپ‌های برنج همراه با شاخص‌های عملکرد دانه و ارتباط آن با شاخص‌های مورفولوژیک

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چکیده: بیماری لکه قهوه‌ای با عامل قارچی *Bipolaris oryzae*، یکی از بیماری‌های مخرب برنج بوده که موجب خسارت عملکرد در اغلب نواحی برنج‌کاری جهان می‌شود. اصلاح ژنوتیپ‌های برنج برای صفت مقاومت به بیماری، از استراتژی‌های ترجیحی در مدیریت لکه قهوه‌ای محسوب می‌شود. از این‌رو، شناسایی منابع مقاومت و متعاقباً توسعه مقاومت به بیماری لکه قهوه‌ای در ژنوتیپ‌های برنج ضروری می‌باشد. مقاومت مزرعه‌ای ۹۵ ژنوتیپ برنج به بیماری لکه قهوه‌ای تحت تنش آبی و کودی در دو سال زراعی ۱۳۹۶ و ۱۳۹۷ ارزیابی شد. سطح مقاومت به بیماری در ژنوتیپ‌های برنج از طریق تیپ واکنش (درجه بیماری) و شاخص‌های اپیدمیولوژیکی از قبیل درصد شاخص بیماری، سطح زیر منحنی پیشرفت و نرخ آلودگی ظاهری ارزیابی شد. شاخص‌های درجه بیماری، درصد شاخص بیماری لکه قهوه‌ای و سطح زیر منحنی پیشرفت بیماری تفاوت در پاسخ ژنوتیپ‌های برنج به بیماری را تحت شرایط مزرعه آشکار کردند که می‌توان از این شاخص‌ها جهت ارزیابی مقاومت به بیماری لکه قهوه‌ای استفاده کرد. در بین ژنوتیپ‌های مورد بررسی، ۲۲ ژنوتیپ واکنش مقاوم تا نیمه‌مقاوم را نشان دادند (۲۳/۱۶٪)، در حالی‌که واکنش اکثر ژنوتیپ‌های مورد بررسی به بیماری لکه قهوه‌ای نیمه‌حساس تا حساس بود (۷۶/۸۴٪). ارتباط مثبت بین زاویه برگ و سطح زیر منحنی پیشرفت بیماری، اشاره به تأثیر کاهش زاویه برگ بر کاهش شدت بیماری لکه قهوه‌ای داشت. نتایج نشان داد که آلودگی برگ تأثیر معنی‌داری روی تعداد دانه پر در هر خوشه یا وزن صد دانه ندارد، اما از طریق کاهش تعداد پنجه بارور موجب کاهش عملکرد برنج می‌گردد. اما، آلودگی ژنوتیپ‌های برنج طی مرحله گلدهی تا رسیدن دانه موجب کاهش تعداد دانه پر در هر خوشه و وزن دانه شد. ژنوتیپ‌های مقاوم شناسایی شده در این مطالعه می‌توانند در برنامه‌های اصلاحی برنج برای توسعه لاین‌های مقاوم امیدبخش در مدیریت بیماری لکه قهوه‌ای به‌کار روند.

واژگان کلیدی: مقاومت مزرعه‌ای، *Bipolaris oryzae*، مقاومت دانه، زاویه برگ، *Oryza sativa*، شاخص‌های عملکرد.