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Genetic Divergence of Rice Genotypes Revealed by Bacterial Blight Disease and Morphological Traits

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ABSTRACT

Bacterial blight is a perilous impediment for rice production. Resistant variety is a sustainable approach to fend off the loss of rice due to bacterial blight disease. In this study, 94 genotypes were screened against bacterial blight disease and its morphological diversity was assessed to find out the resistant donor with desirable morphological characters. Bacterial blight pathogen was inoculated following leaf clipping method for disease scoring. Out of 94 genotypes, 12 showed a resistant reaction, 13 showed moderately resistant reaction and 69 genotypes showed a susceptible reaction. Positive correlation was recorded between yield and most of the morphological characters. Yield hill^{-1} was significantly correlated with the number of tiller hill^{-1} (0.503**), number of effective tiller hill^{-1} (0.538**), total number of spikelets panicle^{-1} (0.595**), number of filled grain panicle^{-1} (0.595**), number of unfilled spikelet panicle^{-1} (0.239*) and 1000 grain weight hill^{-1} (0.843**). Eleven quantitative characters grouped 94 rice genotypes in 16 clusters at coefficient 3.38 and it indicated the presence of great amount diversity among the genotypes. Principal component analysis (PCA) supported the cluster analysis and the first four principal components explained around 70.99% of total divergence for all morphological characters. Principal coordinate analysis (PCoA) demonstrated that the genotypes BR8862-29-1-5-1-3, SVIN301, SVIN321, BR9207-45-2-2, SVIN018, lRBB5, SVIN038, BRRI dhan28 and BRRI dhan29 were placed in distant position from the centroid and it indicated that they were more diverse than the genotypes near the centroid. However, based on disease reaction and genetic diversity analysis crossing could be made between, resistant genotypes such as SVIN317, SVIN017, SVIN316, SVIN313, SVIN315, SVIN314, SVIN038, SVIN307, SVIN302, SVIN304 with the susceptible variety more specifically with BRRI dhan28, BRRI dhan29, BRRI dhan50, BRRI dhan58, BRRI dhan63, BRRI dhan74, BRRI dhan81 and BRRI dhan84 to develop bacterial blight resistant variety.

Key words: Bacterial blight, Correlation, Disease screening, Genetic divergence analysis, Morphological traits, Rice genotypes.

INTRODUCTION

Rice is the primary diet of around half of the Earth's people (Wennberg, 2014). Changing weather parameters of the climate are responsible for several biotic and abiotic stresses which become a threat to rice cultivation in the world (Juroszek and Von Tiedemann, 2011; Zayan, 2018). Rice is threatened by many diseases where bacterial blight is responsible for 20-30% yield loss (Ou, 1985), and the severe attack may cause 80% (Singh *et al*., 1997) to 100% yield loss of rice (Zhai and Zhu, 1999). Bangladesh is an overpopulated country and it is not beyond the effect of climate change and emerging of several pests for crop production. Rice is also a fundamental foodstuff for the population of Bangladesh and here food security is equivalent to rice security (Kabir *et al*., 2015). To date, 32 diseases have been identified in Bangladesh from here blast, tungro, bacterial leaf blight and sheath blight are the severe threats for rice production in Bangladesh (Latif *et al*., 2013). *Xanthomonas oryzae pv oryzae* is the causal organism of bacterial leaf blight disease. The occurrence of this disease was first reported in 1968 in Bangladesh. "Leaf blight" and "Kresek" are the two phases of this disease and the outbreak of these two phases may cause an epidemic in the rice production area (Reddy and Ou, 1976; Ou, 1985). Overdose of nitrogen fertilizer make the rice plant susceptible and favours the outbreak of the disease. Water soak lesion from the leaf margin followed by yellow to white stripes and pale yellow to necrotic symptoms on leaf blades at later stage are the identified symptoms of bacterial leaf blight disease

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(Mizukami and Wakimoto, 1969; Ou, 1985). The word "Kresek" is generated by the Vernacular word of Java, which means "the sound of dead leaves" stroked with one another (Wakimoto, 1969). Kresek was first identified in Indonesia during midcenturies and that time it was considered as a different rice disease but after consecutive studies, it was discovered that the disease kresek occurred by the same pathogen of bacterial leaf blight disease (Mizukami, 1956). The disease kresek generally appears after 1-2 weeks of transplanting and grayish green to whitish leaf blades along with sudden wilting of the plants are the identified symptoms of the kresek disease (Goto, 1992; Watanabe, 1975). Moreover, bacterial ooze in water and rotting smell from the roots are ideal symptoms for kresek disease identification.

As bacterial leaf blight is a bacterial disease, use of antibiotics is the prime solution to control it but due to policy regulation and environment concern adoption of antibiotics in Bangladesh is strictly prohibited. However, no chemicals except antibiotics are effective against bacterial disease. However, use of resistant variety is one and only economic and sustainable environment friendly strategy to tackle up the bacterial disease of rice (Khush *et al.*, 1989; Islam *et al.*, 2017). A resistant variety containing the resistant genes have been released to reduce the rice yield loss by bacterial blight disease (Chen *et al.*, 2002). However, emerging of new races shorten the sustainability of resistant variety and so it is necessary to seek out for the new donors of resistance. To develop bacterial blight resistant high yielding variety there is no alternative of continuous searching of the resistant source to initiate a breeding programme (Islam *et al.*, 2017). Moreover, variation among the parents are also a prime concern to find desirable progeny with superior characters.

Bacterial blight resistant advanced lines of International Rice Research Institute (IRRI) are being used as donor parents in several countries to develop resistant variety. In this study, 94 genotypes were tested against the bacterial blight isolate of Bangladesh to screen out the resistant and susceptible genotypes. Moreover, diversity analysis based on morphological characters were also measured to detect the variation among the genotypes.

MATERIALS AND METHODS

Genotypes collection and plant generation

A total of 94 genotypes were collected from IRRI, Los Banos, Philippines and Bangladesh Rice Research Institute (BRRI), Gazipur-1701, Bangladesh (Table 1). Plants were grownup in the experimental plot of Plant Pathology Division for bacterial blight screening. Genotypes were grown in the seedbed and 25 days aged plants were planted in the plot by implementing randomized complete block design (RCBD) with three replications.

Isolation and purification of pathogens

Bacterial blight infected plants were obtained from rice field for isolation of the pathogen. Infected leaves were cut into small pieces (5mm infected tissue and 5mm of adjacent healthy tissue) and placed in 70% ethanol for 10 seconds, after that the leaves were washed through sterilized water and immersed in 300 µl sterilized water for 15 minutes. A loop was dipped into the water and streaked on PSA (peptone 1.2%, sucrose 1.2%, agar 2%) plates followed by incubation for 3 to 4 days at 30^{o}C for bacterial colony development. The yellow colonies were selected and purified on fresh PSA plates with a sterilized wire loop. The pathogenicity was confirmed according to Koch's postulates on a susceptible variety.

Bacterial culture preparation and disease scoring

After dilution of the bacterial inoculum by distilled water, the concentration was adjusted to 3.3×10^{8} colony forming units per milliliter (cfu/mL), which is a suitable concentration for

Xoo infection in the host. Bacterial culture suspension was inoculated in the plants by clipping methods. Studied genotypes were inoculated at the booting stage. The scissors were dipped in the inoculum and one-fourth of top 3- 4 leaves were clipped by the scissors. After 21 days of inoculation, disease severity and incidence were scored based on following IRRI Standard Evaluation System (IRRI-SES) (IRRI, 2013). Resistant, 1-5% of diseased leaf area (Score 1), moderately resistant 6-12% (Score 3), moderately susceptible 12-25% (Score 5), susceptible 26-50% (Score 7), highly susceptible >50% (Score 9). Later, moderately susceptible, susceptible and highly susceptible were merged into one group as susceptible. However, other groups (resistant and moderately resistant) remain the same as before.

Morphological characters

Morphological characters such as plant height (PHT, cm), number of tillers per hill (NTH^{-1}, no), number of effective tiller per hill (ETH^{-1}, no), days to flowering (DF, no), days to maturity (DM, no), Panicle length (PL, cm), number of filled spikelet per panicle ($NFSP^{-1}$, no), number of unfilled spikelet per panicle ($UFSP^{-1}$), total number of spikelets per panicle ($TNSP^{-1}$), 1000 grain weight (TGW) and yield per hill measured from each replication plot of the respective genotypes.

Data analysis

Descriptive statistics of morphological parameters of the genotypes were calculated by Microsoft excel version 2016. To measure the associations among the 11 morphological characters Pearson's correlation coefficient was done by SPSS software version 20. Euclidean distance of the 94 genotypes was measured based on morphological data by using NTSYS-pc version 2.1 (Rohlf, 1998). Moreover, unweighted pair group methods of arithmetic mean (UPGMA) algorithm and SAHN clustering were applied to determine the relationship among the genotypes. The principal component analysis (PCA) of studied rice lines were revealed by EIGEN and PROJ modules of NTSYS-pc. Moreover, the principal coordinate analysis was done by following the manual instruction of the same software.

RESULTS AND DISCUSSION

Descriptive statistics of morphological parameters of the genotypes

Ninety-four genotypes were screened and out of them 12 showed resistant reaction, 13 showed moderately resistant and 69 showed susceptible reaction against bacterial blight pathogen (Table 1). Table 2 shows the descriptive statistics of morphological parameters of the studied genotypes**.** Among all rice genotypes, the plant height ranged between 64.4 to 105.2 cm with an average of 81.95 cm. The highest (105.2 cm) value for plant height was observed in BR9207-45-2-2 and the lowest (64.4) plant height were observed in genotypes SVIN037 and lRBB8. Besides, number of tiller/hill was ranged from 11 to 33 including an average of 22.87. The maximum and minimum number of tillers belonged to genotypes SVIN311 and BRRI dhan29-SC3-28-16-10-6-HR6 (com)-HR1(GAZ)-P11(Hbj) respectively. The number of effective tillers $hill^{-1}$ varied between 8 to 32 with an average of 20.96. The highest number of effective tillers $hill^{-1}$ was obtained in BRRI dhan74. The lowest number of that tillers was found in BRRI dhan29-SC3-28-16-10-6-HR6 (com)-HR1(GAZ)-P11(Hbj) and BR8862-29-1-5-1-3. Days to flowering varied from 103 to 118 days with an average of 110.43 days. In case of days to maturity, the value was ranged from 133 to 148 with a mean value of 140.43. The line SVIN039 showed the highest panicle length (29.4 cm) and BR8862-8-3-4-4-1 showed the lowest panicle length (17.6 cm), whereas the average panicle length was 23.94 cm. The highest (141) number of spikelets $panicle^{-1}$ was recorded in genotype SVIN301 while that of

the lowest (54) was recorded in genotype IRBB10. The average number of spikelet panicle^{-1} was 87.48. The range of filled grains panicle^{-1} was 45 to 106 with an average value of 67.24. The highest (106) filled grain was found in BR8862-29-1-5-1-3 and that of the lowest (45) was recorded in IR99285-1-1-1-P1. The maximum (40) number of unfilled grains was recorded IR99285-1-1-1-P2 and that of the lowest (4) number was found in BRRI dhan63 with an average value of 18.24. The range of 1000-seed weight was 13 to 28 g with an average value of 21.69 g. The minimum value for TGW was observed in SVIN304 and the highest value was found in BR8862-8-3-4-4-1. Whereas the grain yield hill^{-1} ranged from 12.56 to 80.80 g with the average value of 30.74 g. The maximum yield per hill was recorded in BR9207-45-2-2 and that of the lowest was found in SVIN020.

Table 1. List of 94 genotypes and their reactions to bacterial blight disease.

Designation	Code	DR	Designation	Code	DR	Designation	Code	DR
SVIN317	G1	R	SVIN308	G33	S	lRBB64	G65	S
SVIN017	G2	R	SVIN296	G34	S	lRBB8	G66	S
SVIN316	G3	R	SVIN010	G35	S	Purbachi	G67	S
SVIN313	G4	R	SVIN035	G36	S	IR99056-B-B-15	G68	S
SVIN315	G5	R	SVIN012	G37	S	BR-8938-30-2-4-2-1	G69	S
SVIN314	G6	R	SVIN045	G38	S	BR8904-28-1-2-2-2	G70	S
SVIN038	G7	R	SVIN301	G39	S	KARJAT-5	G71	S
SVIN307	G8	R	SVIN050	G40	S	BR9675-68-5-1	G72	S
SVIN302	G9	R	SVIN287	G41	S	BR8562-11-2-6-1-1-1	G73	S
SVIN304	G10	R	SVIN037	G42	S	BRRI dhan29-SC3-28-16-10-6-HR6(com)-HR1(GAZ)-P8(Hbj)	G74	S
SVIN291	G11	MR	SVIN306	G43	S	BRRI dhan29-SC3-28-16-10-6-HR6(com)-HR1(GAZ)-P11(Hbj)	G75	S
SVIN017	G12	MR	SVIN029	G44	S	BR8862-29-1-5-1-3	G76	S
SVIN046	G13	MR	SVIN296	G45	S	BR8862-8-3-4-4-1	G77	S
SVIN045	G14	MR	SVIN003	G46	S	BR8995-2-5-5-2-1	G78	S
SVIN305	G15	MR	SVIN020	G47	S	BR9205-10-1-5-3	G79	S
SVIN039	G16	MR	SVIN001	G48	S	BR8590-5-2-5-2-1	G80	S
SVIN018	G17	MR	SVIN041	G49	S	BR8590-5-2-5-2-2	G81	S
SVIN312	G18	MR	SVIN321	G50	S	BR9207-45-2-2	G82	S
SVIN318	G19	MR	SVIN024	G51	S	IR99285-1-1-1-P2	G83	S
SVIN046	G20	MR	SVIN047	G52	S	BR(Bio) 9777-26-4-3	G84	S
SVIN311	G21	MR	SVIN299	G53	S	BRRI dhan28	G85	S
lRBB27	G22	MR	SVIN026	G54	S	BRRI dhan29	G86	S
IR99285-1-1-1-P1	G23	MR	SVIN306	G55	S	BRRI dhan50	G87	S

Table 1. Continued.

Designation	Code	DR	Designation	Code	DR	Designation	Code	DR
SVIN301	G24	S	SVIN038	G56	S	BRRI dhan58	G88	S
SVIN048	G25	S	lRBB10	G57	S	BRRI dhan63	G89	S
SVIN020	G26	S	lRBB11	G58	S	BRRI dhan74	G90	S
SVIN008	G27	S	lRBB13	G59	S	BRRI dhan81	G91	S
SVIN040	G28	S	lRBB2	G60	S	BRRI dhan84	G92	S
SVIN001	G29	S	lRBB24	G61	S	IRBB60	G93	R
SVIN319	G30	S	lRBB3	G62	S	IRBB65	G94	R
SVIN033	G31	S	lRBB4	G63	S			
SVIN298	G32	S	lRBB5	G64	S			

Note: DR-Disease reaction, R-Resistant, MR-Moderately resistant, S-Susceptible.

Table 2. Descriptive statistics of 11 traits of 94 rice genotypes.

	PHT	NTH^{-1}	ETH^{-1}	DF	DM	PL	NSP^{-1}	FGP^{-1}	$UFGP^{-1}$	TGW	YH^{-1}
Max	105.2	33	32	118	148	29.4	141	106	40	28	80.80
Min	64.4	11	8	103	133	17.6	54	45	4	13	12.56
Mean	81.95	22.87	20.96	110.44	140.44	23.94	87.00	67.24	18.24	21.69	30.74
CV (%)	12.35	23.74	25.81	5.30	4.17	10.09	23.31	21.41	44.37	13.87	40.57

Note: PHT-Plant height, NTH^{-1} – Number of tiller per hill, ETH^{-1}- Effective tiller per hill, DF-Days to flowering, DM- Days to maturity, PL-Panicle length, $TNSP^{-1}$-Total number of spikelet per panicle, FSP^{-1}-Number of filled spikelet per panicle, $UFSP^{-1}$-Number of unfilled spikelet per panicle, TGW-1000 grain weight, YH^{-1}-Yield per hill.

Correlation study of morphological characters

Pearson's correlation coefficient was measured from the data of 11 morphological characters of 94 rice genotypes (Table 3). Maximum traits showed a positive relationship after correlation analysis. Total number of spikelets panicle^{-1} displayed significant ($p \leq 0.01$) positive relationship with the number of filled grain panicle^{-1} (0.895**), number of unfilled grain panicle^{-1} (0.593**), TGW (0.389**) and Yield hill^{-1} (0.595**). Yield hill^{-1} had highly significant and positive correlation with the number of tiller hill (0.503**), number of effective tiller hill^{-1} (0.538**), total number of spikelets panicle^{-1} (0.595**), number of filled grain panicle^{-1} (0.595**), number of unfilled spikelet panicle^{-1} (0.239*) and TGW hill^{-1} (0.843**). Many rice scientists reported that the relationship between grain yield and TGW is highly significant (Mazid *et al.*, 2013; Xu *et al.*, 2015; Li *et al.*, 2019). Morphological traits are highly influenced by environmental conditions so it is better to select highly correlated traits for the breeding programme.

Cluster analysis

Eleven morphological characters grouped the studied genotypes in sixteen principal groups at coefficient 3.38 and it indicated the presence of diversity among the genotypes. Based on 18 quantitative characters, 58 rice genotypes were clustered into four groups (Ahmadikhah *et al.*, 2008) whereas Mazid *et al.*, 2013 stated six groups in terms of 13 morphological characters of 41 accession of rice. Melchinger (1993) suggested that multivariate statistical techniques i.e. cluster and PCA could be applied to study the variation among the samples perfectly. Table 4 depicted that Cluster 2 was the largest (containing 24 genotypes) followed by Cluster-6 (21 genotypes),

Table 3. Correlation studies of 11 morphological characters of 94 rice genotypes.

Traits	PHT	NTH-1	ETH-1	DF	DM	PL	NSP-1	FGP-1	UFGP-1	1000 GW	YH-1
PH	1										
NTH-1	-0.029	1									
ETH-1	-0.017	0.953**	1								
DF	0.123	0.070	0.081	1							
DM	0.123	0.070	0.081	1.000**	1						
PL	-0.157	-0.124	-0.230	0.040	0.040	1					
NGP-1	-0.181	0.025	0.017	0.052	0.052	0.0880	1				
FGP-1	-0.128	0.003	-0.003	0.11	0.110	0.1160	0.895**	1			
UFGP-1	-0.169	0.051	0.043	-0.084	-0.084	-0.0150	0.593**	0.172	1		
1000 GW	-0.130	0.171	0.209*	0.143	0.143	-0.1900	0.389**	0.372**	0.187	1	
YH-1	-0.114	0.503**	0.538**	0.117	0.117	-0.1720	0.595**	0.595**	0.239*	0.843**	1

Note: PHT-Plant height, NTH-1 – Number of tiller per hill, ETH-1- Effective tiller per hill, DF-Days to flowering, DM- Days to maturity, PL-Panicle length, TNSP-1-Total number of spikelet per panicle, FSP-1-Number of filled spikelet per panicle, UFSP-1-Number of unfilled spikelet per panicle, TGW-1000 grain weight, YH-1-Yield per hill.
** indicates significant at the 0.01 level, * indicates significant at the 0.05 level.

Cluster-5 (9 genotypes), Cluster-4 (8 genotypes), Cluster-1 (7 genotypes), Cluster-8 (5 genotypes), Cluster-7 (4 genotypes), Cluster-3 and Cluster-10 (3 genotypes each), Cluster-9, Cluster-11 and Cluster-12 (2 genotypes per cluster) and Cluster-13, Cluster-14, Cluster-15, and Cluster-16 (each contains a single genotype). Moreover, genotypes having resistant, moderately resistant and susceptible phenomena were placed in the same cluster and this happened because they might be originated from the same ancestors having similar morphological characters. The cluster-14 had the highest average value for the three characters (Table 5) those are plant height (102.67 cm), days to flowering (118) and days to maturity (148). Cluster-16 contained the maximum number spikelets per panicle (132) and filled grain per panicle (106). The highest (32.63) average number of tiller was found in cluster-7 while the highest (30.33) number of effective tiller per hill was found in cluster-3. The highest mean panicle length (27.20 cm) and yield per hill (61.51 g) were found in cluster-13 whereas the maximum mean value (29 g) for TGW was found in cluster-12. On the contrary, cluster-16, showed the lowest mean value for number of tiller hill^{-1} (14.33), number of effective tiller per hill (8.67), TGW (18.00 g) and yield per hill (16.54 g). Average lowest value for number of tiller per hill (14.33), number of effective tiller per hill (12.83) and TGW (19.50 g) were found in cluster-9. Moreover, the lowest value for plant height (69.50), days to flowering (105.05), days to maturity (134.25), panicle length (22.48 cm) and yield per hill (19.10) was found in cluster-11, cluster-6, clster-4, cluster-8 and cluster-5, respectively.

Table 4. Cluster wise distribution of genotypes.

Cluster	No. of genotype	Genotype	Reaction
Cluster-1	7	SVIN317, SVIN315, SVIN291, SVIN305, SVIN039, SVIN046, IR99285-1-1-1-P1	R+MR
Cluster-2	24	SVIN048, SVIN008, SVIN001, SVIN319, SVIN035, SVIN012, SVIN287, SVIN003, lRBB11, lRBB13, lRBB4, Purbachi, BR8904-28-1-2-2-2, BR9675-68-5-1, BR8562-11-2-6-1-1-1, BR8862-8-3-4-4-1, BR9205-10-1-5-3, BR8590-5-2-5-2-1, BR8590-5-2-5-2-2, BRRI dhan58, BRRI dhan81, BRRI dhan84, IRBB60, IRBB65.	S+ R
Cluster-3	3	lRBB27, lRBB8, BRRI dhan74	MR+ S
Cluster-4	8	SVIN313, SVIN314, SVIN038, SVIN302, SVIN304, SVIN017, SVIN045, SVIN312	R+MR
Cluster-5	9	SVIN301, SVIN020, SVIN296, BR-8938-30-2-4-2-1, KARJAT-5, BRRI dhan28, BRRI dhan29, BRRI dhan50, BRRI dhan63	S
Cluster-6	21	SVIN040, SVIN033, SVIN298, SVIN308, SVIN010, SVIN045, SVIN050, SVIN306, SVIN029, SVIN296, SVIN001, SVIN041, SVIN024, SVIN047, SVIN299, SVIN306, lRBB2, lRBB24, lRBB3, BR8995-2-5-5-2-1, BR(Bio) 9777-26-4-3	S
Cluster-7	4	SVIN311, SVIN026, SVIN038, lRBB10	MR+S
Cluster-8	5	SVIN017, SVIN316, SVIN307, SVIN018, SVIN318	R+MR
Cluster-9	2	BRRI dhan29-SC3-28-16-10-6-HR6(com)-HR1(GAZ)-P11(Hbj), IR99285-1-1-1-P2	S
Cluster-10	3	SVIN301, SVIN037, SVIN321	S
Cluster-11	2	lRBB5, lRBB64	S
Cluster-12	2	IR99056-B-B-15, BR9207-45-2-2	S
Cluster-13	1	SVIN020	S
Cluster-14	1	SVIN046	MR
Cluster-15	1	SVIN305	S
Cluster-16	1	BR8862-29-1-5-1-3	S

Note: R-Resistant, MR-Moderately resistant, S-Susceptible.

Table 5. Cluster wise mean of respective characters.

Cluster	PHT	NTH^{-1}	ETH^{-1}	DF	DM	PL	$TNSP^{-1}$	FSP^{-1}	$UFSP^{-1}$	TGW	YH^{-1}
Cluster-1	85.76	21.05	19.52	117.00	147.00	24.94	81.14	62.00	19.14	20.86	25.12
Cluster-2	85.47	22.51	20.53	115.17	145.17	24.07	82.42	66.58	15.21	21.88	30.02
Cluster-3	70.33	31.50	30.33	117.33	147.33	22.67	76.67	66.33	10.33	20.00	40.40
Cluster-4	76.71	22.92	20.63	104.25	134.25	23.48	75.00	59.75	15.25	20.13	24.53
Cluster-5	88.78	15.00	13.33	106.00	136.00	24.84	78.78	67.89	10.89	21.00	19.10
Cluster-6	77.80	22.44	20.86	105.05	135.05	23.00	88.67	64.71	22.48	21.67	29.24
Cluster-7	79.96	32.63	29.42	106.25	136.25	26.45	63.00	51.75	11.25	23.25	35.87
Cluster-8	81.13	28.33	26.07	112.40	142.40	22.48	112.40	85.20	27.20	22.80	50.88
Cluster-9	74.50	14.33	12.83	112.00	142.00	25.10	127.50	91.00	36.50	19.50	22.44
Cluster-10	73.67	27.22	25.22	107.00	137.00	24.50	132.00	74.00	26.00	24.00	44.33
Cluster-11	69.50	30.42	29.58	118.00	148.00	20.00	72.50	58.00	14.50	24.00	41.21
Cluster-12	101.33	25.33	23.83	117.00	147.00	24.80	111.00	82.50	28.50	29.00	59.22
Cluster-13	88.67	32.00	29.00	103.00	133.00	27.20	123.00	101.00	22.00	21.00	61.51

Table 5. Continued.

Cluster	PHT	NTH^{-1}	ETH^{-1}	DF	DM	PL	TNSP^{-1}	FSP^{-1}	UFSP^{-1}	TGW	YH^{-1}
Cluster-14	102.67	19.67	17.33	118.00	148.00	23.60	100.00	77.00	23.00	20.00	26.69
Cluster-15	88.67	19.33	14.00	112.00	142.00	25.00	80.00	64.00	16.00	19.00	17.02
Cluster-16	71.20	14.33	8.67	112.00	142.00	27.00	132.00	106.00	26.00	18.00	16.54

Note: PHT-Plant height, NTH^{-1} – Number of tiller per hill, ETH^{-1}- Effective tiller per hill, DF-Days to flowering, DM- Days to maturity, PL-Panicle length, TNSP^{-1}-Total number of spikelet per panicle, FSP^{-1}-Number of filled spikelet per panicle, UFSP^{-1}-Number of unfilled spikelet per panicle, TGW-1000 grain weight, YH^{-1}-Yield per hill.

Principal component analysis (PCA)

Correlation matrix of the samples were used to compute the principal components and the first component had the maximum variance. However, the PCA typically supported the cluster analysis. Some of the genotypes did not actually follow the clustering pattern in PCA and they grouped with another cluster. The first four principal components of PCA explained about 70.99% of overall divergence for all quantitative characters. From the eigenvectors study, it was found that 23.33%, 19.81%, 17.59% and 10.26% variation could be revealed by the first four principal components (Table 6 and Fig. 1). Caldo *et al.* (1996) reported 67% of the total divergence of quantitative characters from 10 principal components while Lasalita-Zapico *et al.* (2010) reported 82.7% of total variation from 32 rice accesions.

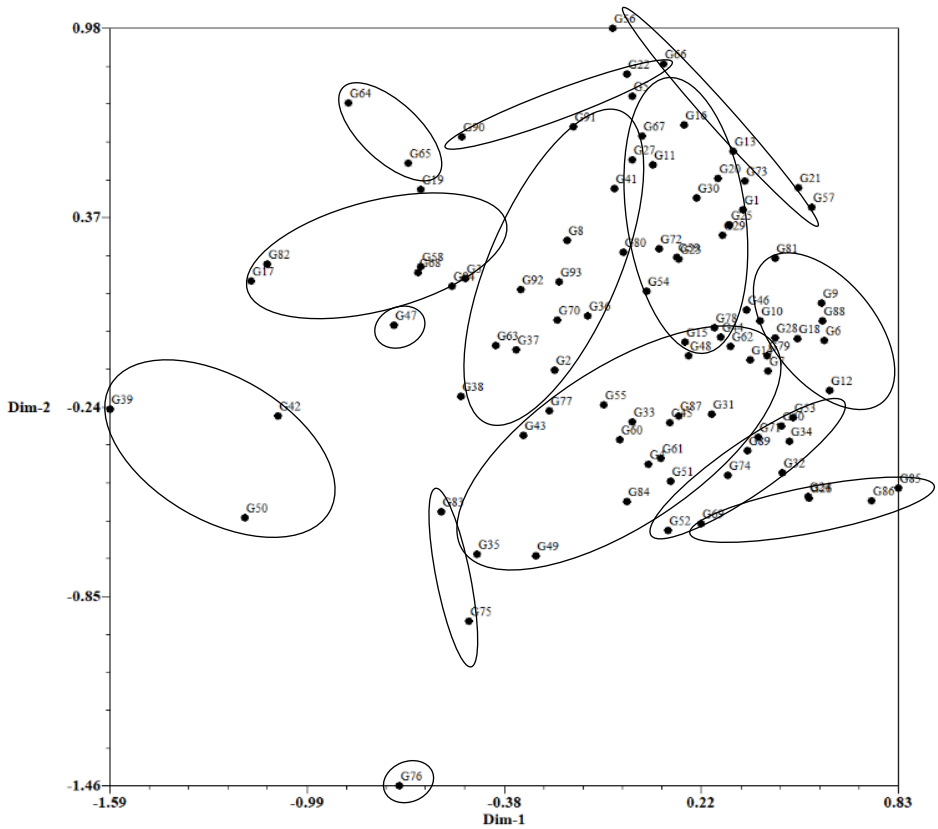

Fig. 1. Two-dimensional plot of principal component analysis portrayed the relationships of 94 genotypes by utilizing the data of 11 morphological characters.

Table 6. Eigenvectors and eigen values of the first four principal components.

Variable	Principal component			
	PC1	PC2	PC3	PC4
Eigen value	2.566	2.179	1.936	1.129
Percent	23.329	19.811	17.596	10.261
Cumulative	23.329	43.141	60.737	70.998
PHT	0.226	0.283	0.182	0.515
NTH^{-1}	-0.333	0.624	-0.625	-0.193
ETH^{-1}	-0.340	0.647	-0.634	-0.104
DF	-0.346	0.618	0.686	-0.051
DM	-0.346	0.618	0.686	-0.051
PL	0.005	-0.263	0.312	-0.686
NSP^{-1}	-0.866	-0.413	0.028	-0.032
FGP^{-1}	-0.780	-0.317	0.140	-0.024
$UFGP^{-1}$	-0.504	-0.339	-0.193	-0.027
TGW	-0.642	0.035	-0.089	0.376
YH^{-1}	-0.151	-0.267	0.084	0.442

Note: PHT-Plant height, NTH^{-1} – Number of tiller per hill, ETH^{-1}- Effective tiller per hill, DF-Days to flowering, DM- Days to maturity, PL-Panicle length, $TNSP^{-1}$-Total number of spikelet per panicle, FSP^{-1}-Number of filled spikelet per panicle, $UFSP^{-1}$-Number of unfilled spikelet per panicle, TGW-1000 grain weight, YH^{-1}-Yield per hill.
PC1-Principal component-1, PC2-Principal component-2, PC3- Principal component-3. PC4- Principal component-4

Principal coordinate analysis (PCoA)

PCoA described the spatial distribution of the studied genotypes based on morphological traits. Two-dimensional graph of PCoA demonstrated that the genotypes BR8862-29-1-5-1-3 (G76), SVIN301 (G39), SVIN321 (G50), BR9207-45-2-2 (G82), SVIN018 (G17), lRBB5 (G64), SVIN038 (G56), BRRI dhan28 (G85) and BRRI dhan29 (G86) were placed in distant position from the centroid and remaining genotypes situated at near to the centroid (Fig. 2). Results of the PCoA plot described that the genotypes which were situated in distant position from the central point were more diverse and genotypes near to the central point were less diverse. Strong heterosis is expected to be found by crossing between the parents having low similarity (Abubakar *et al*., 2011; Nihad *et al*., 2021).

CONCLUSION

In this study 94 genotypes were screened for disease reaction and morphological diversity. Out of 94 genotypes, 12 showed resistant reaction, 13 showed moderately resistant reaction and rest of the genotypes showed susceptible reaction. Yield $hill^{-1}$ had high and significant positive correlation with the number of tiller $hill^{-1}$, number of effective tiller $hill^{-1}$, total number of spikelets $panicle^{-1}$, number of filled grain $panicle^{-1}$, number of unfilled spikelet $panicle^{-1}$ (0.239*) and 1000 grain weight $hill^{-1}$. Moreover, based on 11 morphological characters, 94 rice genotypes were clustered into sixteen major groups and it indicates the presence of diversity among the genotypes. However, the PCA and PCoA mostly confirmed the cluster analysis. The first four principal components explained around 70.99% of total divergence for all quantitative

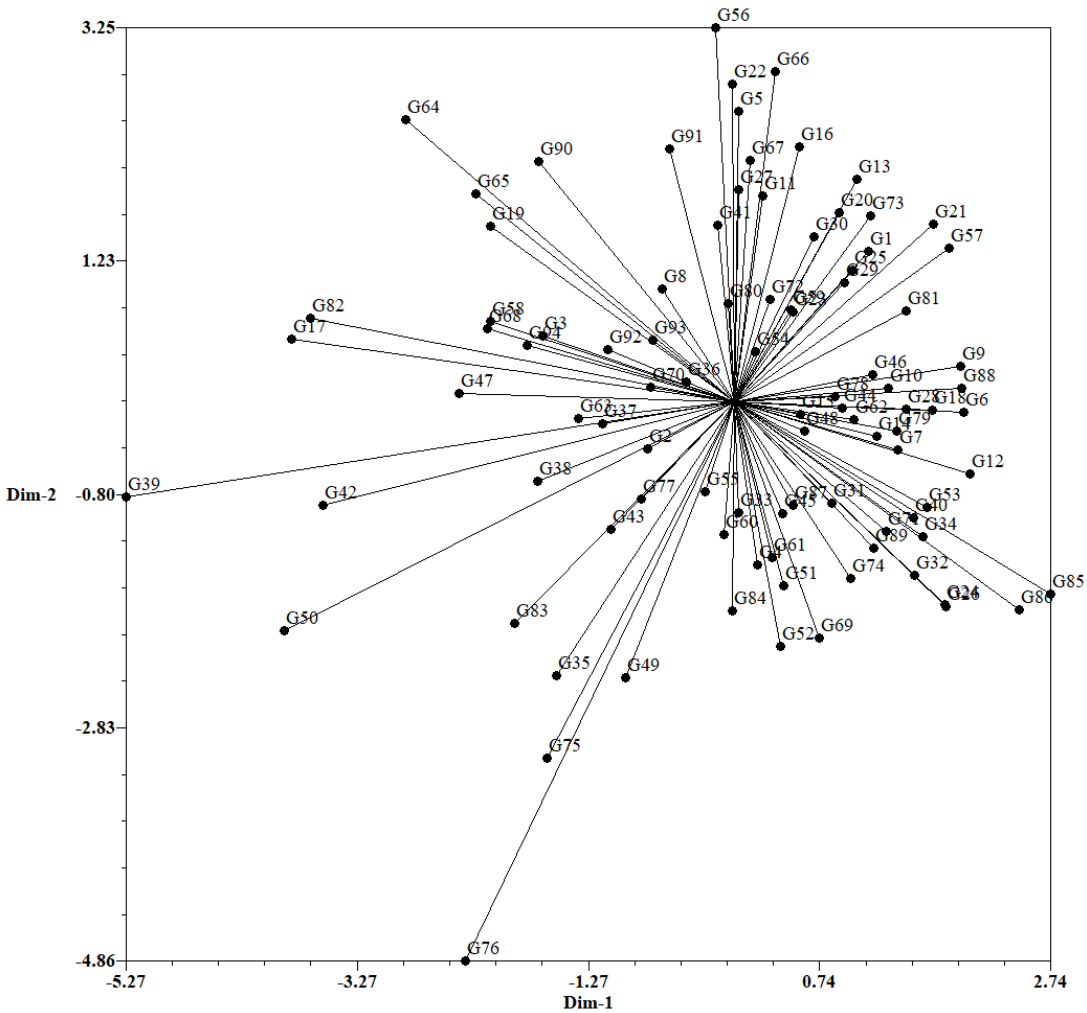

Fig. 2. Two-dimensional plot of principal coordinate analysis depicted the relationships of 94 rice genotypes by using the data of 11 morphological characters.

characters. However, based on disease reaction and genetic diversity analysis crossing could be done between genotype of two distant clusters, resistant genotypes such as SVIN317, SVIN017, SVIN316, SVIN313, SVIN315, SVIN314, SVIN038, SVIN307, SVIN302, SVIN304 with the susceptible lines or variety more specifically with BRRI dhan28, BRRI dhan29, BRRI dhan50, BRRI dhan58, BRRI dhan63, BRRI dhan74, BRRI dhan81 and BRRI dhan84 to develop bacterial blight resistant variety.

AUTHORS' CONTRIBUTION

SAIN: Conceptualization, data curation, formal analysis, investigation, methodology, resources, software, validation, writing - original draft, writing - review and editing. **AA**: Conceptualization, methodology, data curation. **MMR**: Methodology, investigation. **MAIH**: Formal analysis. **MAIK**: Formal analysis, visualization. **MAL**: Conceptualization, resources, funding acquisition, supervision, writing – review and

editing. All authors read and approved the final manuscript.

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DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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