Agro-morphological characterization and assessment of variability, heritability, genetic advance and divergence in bacterial blight resistant rice genotypes

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ABSTRACT

Genetic based knowledge of different growth traits including morphological, physiological and developmental plays fundamental role in the improvement of rice. Genetic divergence allows superior recombinants which are essential in any crop development project. Forty-one rice genotypes including bacterial blight (BB) resistant and susceptible checks were assessed for 13 morphological traits. Among the genotypes, almost all the traits exhibited highly significant variation. The higher extent of genotypic (GCV) as well as phenotypic coefficients of variation (PCV) were noticed for number of tillers hill^{-1}, total number of spikelets panicle^{-1}, number of filled grains panicle^{-1}, and yield hill^{-1}. High heritability together with high genetic advance was observed for total number of spikelets panicle^{-1}, number of filled grains panicle^{-1}, and yield hill^{-1} indicating dominant role of additive gene action in the expression of these traits. Number of filled grains panicle^{-1} exhibited positive correlation with most of the traits. Yield hill^{-1} showed a good number of highly significant positive correlations with number of filled grain panicle^{-1}, total number of spikelets panicle^{-1}, 1000 grain weight hill^{-1}, number of panicle hill^{-1}, and panicle length. The UPGMA dendrogram divided all the genotypes in to six major clusters. The PCA showed 13 morphological traits generated about 71% of total variation among all the genotypes under this study. On the basis of 13 morphological traits, genotypes such as IRBB2, IRBB4, IRBB13, IRBB21, and MR263 could be hybridized with genotypes MR84, MR159, MRQ50, MRQ74, PH9 and IR8 in order to develop suitable BB resistant rice genotypes.

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1. Introduction

Rice is an important versatile food crops which feeds over half of the world's population and provides essential food elements, employment opportunity as well as raw materials for different products used by human kind (Zhao et al., 2011; Akinbile et al., 2011; FAO, 2004; Onwueme and Sinha, 1991). The global need of rice has been forecasted to rise by 25% from 2001 to 2025 in order to cope with the increasing population (Maclean et al., 2002). However, the productivity of this vital crop is affected by several biotic and abiotic stresses because of narrow genetic diversity present in the existing rice cultivars. This is believed as consequence of human selection for early maturing and high yielding rice genotypes. Therefore, broadening the genetic base of this key crop by introgressing genes from different sources is a crying need.

Bacterial blight (BB) disease is one of the most detrimental disease in the rice growing regions of the world for both inbred and hybrid rice (Mew, 1987) which is caused by *Xanthomonas oryzae pv. oryzae*. The disease causes yield losses up to 100% under extreme conditions (Zhai and Zhu, 1999). In 1988 and 1994, serious outbreaks were reported in the states of Penang, Kedah, Selangor and Perak of Malaysia where more than 40% of the planted areas were infected with BB disease, causing an estimated yield loss of about 10–50% (Saad et al., 2000). According to Ogawa (1993) using of resistant variety is the most effective way to control BB disease, which eventually minimizes the yield loss.

Genetic diversity plays a fundamental role in plant breeding. Genetic divergence is an efficacious tool for an effective choice of parents for hybridization and breeding program (Vivekananda and Subramanian, 1993). Kwon et al. (2002) suggested that identification of parents based on divergence study for any breeding program would be more effective.

This study assessed the morphological variability of rice genotypes obtained from the International Rice Research Institute (IRRI), Malaysian

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Agricultural Research and Development Institute (MARDI) and Bangladesh Rice Research Institute (BRRI) using quantitative morphological traits. Traditionally, morphological traits are used to determine genetic diversity and classify germplasm. However, this technique is a low-level but powerful taxonomic tool which can be utilized for the preliminary grouping of cultivars prior to their characterization using more robust marker technologies. Moreover, this technique is cost effective, less time consuming, easy to score and it does not need any technical knowledge. According to Din et al. (2010) scientific classification of the plant still relies on morphological traits.

Heritability of a trait is important in determining a cultivar's response to selection (Surek and Beser, 2003). Knowledge of heritability plays key role in the selection based improvement of a crop because it implies the extent of transmissibility of traits into next generations. Therefore, genetic variability is the prerequisite for making progress in crop breeding (Appalaswamy and Reddy, 2004). High genetic advance coupled with high heritability estimates offers the most effective condition for selection (Larik et al., 2000). According to some researchers (Kumar and Shukla, 2002; Ismail et al., 2001) information of association such as genotypic and phenotypic correlation between yield and its component traits is vital for yield improvement through selection programs. To the best of our knowledge this is the first report concerning genetic diversity of near isogenic lines (NIL) and pyramid lines having multiple BB resistant genes developed by the International Rice Research Institute (IRRI). The aims of this study were to assess the nature and magnitude of genetic diversity as well as selection of potential genotypes based on morphological traits from the BB resistant rice genotypes for future utilization in breeding programs.

2. Materials and methods

2.1. Plant material and experimental design

Forty-one rice genotypes (including 35 BB resistant and six susceptible) were obtained from IRRI, MARDI and BRRI (Table 1). The genotypes were grown in the pots at experimental field (No-2) of the Universiti Putra Malaysia. In this experiment, a total of 123 pots were arranged according to randomized complete block design (RCBD) where each replication was formed with 41 pots. Initially, six plants were allowed to grow but at final stage two plants were kept at each pot.

2.2. Data collection

Data for the following 13 morphological traits were recorded from all genotypes at each of the replication : seedling height (SHT, cm), plant height (PHT, cm), number of tillers hill^{-1} (NTH^{-1}, no), days to 50% flowering (DF, day), days to maturity (DM, day), flag leaf length (FLL, cm), number of panicles hill^{-1} (NPH^{-1}, no), panicle length (PL, cm), number of filled grains panicle^{-1}(NFGP^{-1}, no), number of unfilled grains panicle^{-1} (NUFGP^{-1}, no), total number of spikelets hill^{-1} (TNSP^{-1}, no), yield hill^{-1} (YH^{-1}, g) and one thousand grains weight (1000 GWH^{-1}, g) (Table 2).

2.3. Statistical analysis

The most important interaction was expressed by the analysis of variance (ANOVA). Honest significant difference (HSD) or MSD was calculated using Statistical analysis system software (SAS version 9.1, SAS Institute, 2001) (Tables 3a and 3b). In order to identify genetic variation among the genotypes and to determine environmental as well as genetic effects on different traits genetic parameters were estimated. According to Burton (1951), Burton and Devane (1953) and Johnson et al. (1955) the following genetic parameters were worked out.

Table 1
List of different rice genotypes in this study.

Genotype code	Genotype	Genetic constitution	Reaction	Source of collection
G1	IRBB2	Xa2	Resistant	IRRI
G2	IRBB4	Xa4	Resistant	IRRI
G3	IRBB5	xa5	Resistant	IRRI
G4	IRBB7	Xa7	Resistant	IRRI
G5	IRBB10	Xa10	Resistant	IRRI
G6	IRBB11	Xa11	Resistant	IRRI
G7	IRBB13	xa13	Resistant	IRRI
G8	IRBB14	Xa14	Resistant	IRRI
G9	IRBB21	Xa21	Resistant	IRRI
G10	IRBB 54	xa5,Xa21	Resistant	IRRI
G11	IRBB55	Xa13,Xa21	Resistant	IRRI
G12	IRBB57	Xa4,xa5,Xa21	Resistant	IRRI
G13	IRBB58	Xa4,xa13,Xa21	Resistant	IRRI
G14	IRBB59	xa5,xa13,Xa21	Resistant	IRRI
G15	IRBB60	Xa4,xa5,xa13,Xa21	Resistant	IRRI
G16	IRBB61	Xa4,xa5,Xa7	Resistant	IRRI
G17	IRBB64	Xa4,xa5,Xa7,Xa21	Resistant	IRRI
G18	IRBB65	Xa4,Xa7,Xa13,Xa21	Resistant	IRRI
G19	IRBB66	Xa4,xa5,Xa7,Xa13,Xa21	Resistant	IRRI
G20	MR84	Unknown	Susceptible	MARDI
G21	MR159	Unknown	Resistant	MARDI
G22	MR185	Unknown	Resistant	MARDI
G23	MR211	Unknown	Resistant	MARDI
G24	MR219	Unknown	Resistant	MARDI
G25	MR220	Unknown	Resistant	MARDI
G26	MR232	Unknown	Resistant	MARDI
G27	MR253	Unknown	Resistant	MARDI
G28	MR263	Unknown	Resistant	MARDI
G29	MR269	Unknown	Resistant	MARDI
G30	MR220-CL1	Unknown	Resistant	MARDI
G31	MR220-CL2	Unknown	Resistant	MARDI
G32	MRQ50	Unknown	Resistant	MARDI
G33	MRQ74	Unknown	Resistant	MARDI
G34	PH9	Unknown	Resistant	MARDI
G35	Pulut Siding	Unknown	Susceptible	MARDI
G36	IR8	Unknown	Resistant	MARDI
G37	YTM16	Unknown	Resistant	MARDI
G38	Bahagia	Unknown	Susceptible	MARDI
G39	BR28	Unknown	Susceptible	BRRI
G40	BR29	Unknown	Susceptible	BRRI
G41	Purbachi[a]	Unknown	Susceptible	BRRI

(IRGCIS, website: http://www.irgcis.irri.org:81/grc/irgcishome.html and the International Crop Information System (ICIS, website: http://www.iris.irri.org/), MARDI (2008). Bangladesh Rice Research Institute.
[a] Purbachi originated from China but widely cultivated in Bangladesh.

(a) Genotypic variance:

$$\sigma_g^2 = \frac{(MSG - MSE)}{r},$$

where MSG is the mean square of accessions, MSE is mean square of error, and r is number of replications.

(b) Phenotypic variance:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2,$$

where is σ_g^2 genotypic variance and σ_e^2 is mean squares of error.

(c) Phenotypic coefficient of variance (PCV):

$$PCV(\%) = \frac{\sqrt{\sigma_P^2}}{\overline{X}} \times 100$$

where σ_P^2 is the phenotypic variance and $\overline{X}$ is mean of trait.

Table 2
List of 13 morphological traits measured.

Serial no	Trait	Method of measurement
1	Seedling height (SHT, cm)	The average of height from the base to the tip of tallest leaf.
2	Plant height (PHT, cm)	The average of height from the base to the tip of last leaf (Flag leaf).
3	Number of tillers hill^{-1} (NTH^{-1}, no)	Counting of the tillers per hill.
4	Days to flowering (DF, day)	The number of days from cultivation to flowering day.
5	Days to maturity (DM, day)	The number of days from cultivation to maturing day.
6	Flag leaf length (FLL, cm)	From the base to the tip of the flag leaf.
7	Number of panicles hill^{-1} (NPH^{-1}, no)	Counting of the panicles per hill.
8	Panicle length (PL, cm)	From the base (first node) to the tip of last spikelet of panicle.
9	Number of filled grains panicle^{-1} (NFGP^{-1}, no)	By dividing the total filled grains to the total panicle number.
10	Number of unfilled grains panicle^{-1} (NUFGP^{-1}, no)	By dividing the total unfilled grains to the total panicle number.
11	Total number of spikelets panicle^{-1} (TNSP^{-1}, no)	Counting the total spikelet (filled and unfilled grains) per panicle.
12	Yield hill^{-1} (YH^{-1}, g)	Weighing the total grains per hill.
13	1000 grain weight (1000 GWH^{-1}, g)	One thousand seeds were counted and weighed.

(d) Genotypic coefficient of variance (GCV):

$$GCV(\%) = \frac{\sqrt{\sigma_g^2}}{\overline{X}} \times 100$$

where σ_g^2 is genotypic variance and $\overline{X}$ is mean of trait.

(e) Heritability (Broad sense):

$$h_B^2 = \frac{\sigma_g^2}{\sigma_P^2}$$

where σ_g^2 is genotypic variance and σ_P^2 is phenotypic variance.

(f) Expected genetic advance (GA):

$$GA(\%) = K \times \sqrt{\sigma_P^2} \times h_B^2 \times 100.$$

GA as a percent of the mean assuming selection of the superior 5% of genotypes.

$$GA(\%) = K \times \frac{\sqrt{\sigma_P^2}}{\overline{X}} \times h_B^2 \times 100$$

where K is constant, $\frac{\sqrt{\sigma_P^2}}{\overline{X}}$ is phenotypic standard deviation h_B^2 is heritability and $\overline{X}$ is mean of traits.

To assess the relationships among the 13 different traits Pearson's correlation coefficient was analyzed using Statistical analysis system software (SAS version 9.1, SAS Institute, 2001). The standardized morphological data were employed to calculate the Euclidean distances among the 41 rice (*Oryza sativa*) genotypes by NTSYS-pc version 2.1 (Rohlf, 2002). Moreover, unweighted pair group methods of arithmetic mean (UPGMA) algorithm and SAHN clustering were

Table 3a
Mean squares of analysis of variance for six growth traits among 41 rice genotypes.

S.O.V.	df	SHT	PHT	NTH^{-1}	FLL	DF	DM
Blocks (R)	2	47.57**	4.03^{ns}	46.46^{ns}	2.60^{ns}	14.08*	1.53^{ns}
Genotypes (G)	40	24.83**	82.13**	159.67**	22.79**	148.73**	263.50**
Populations (P)	(2)	0.58^{ns}	138.17**	1185.36**	18.69*	1304.57**	2657.33**
G/P	(38)	26.11**	79.18**	105.69**	23.01**	87.90**	137.51**
Error	80	8.54	21.70	35.88	3.89	3.88	3.00

Note: S.O.V—source of variation, df—degree of freedom, SHT—seedling height, PHT—plant height, NTH^{-1}—number of tillers hill^{-1}, FLL—flag leaf length, DF—days to 50% flowering, DM—days to maturity.

* Significant at p ≤ 0.05.
** Significant at p ≤ 0.01.
ns Non-significant.

Table 3b
Mean squares of analysis of variance for seven yield and yield component traits among 41 rice genotypes.

S.O.V.	df	NPH^{-1}	PL	NFGP^{-1}	NUFGP^{-1}	TNSP^{-1}	YH^{-1}	1000 GWH^{-1}
Blocks (R)	2	27.32^{ns}	1.71^{ns}	44.64^{ns}	196.16^{ns}	426.45^{ns}	52.70^{ns}	1.53^{ns}
Genotypes (G)	40	59.58*	4.45**	1962.01**	1354.5**	3579.01**	749.20**	35.61**
Populations (P)	(2)	22.00^{ns}	23.95**	946.67**	157.03^{ns}	1786.18**	1800.73**	102.06**
G/P	(38)	61.56*	3.42**	2015.45**	1417.52**	3673.37**	693.86**	32.11**
Error	80	35.42	1.19	20.08	253.68	315.16	15.37	1.71

Note: S.O.V.—source of variation, df—degree of freedom, NPH^{-1}—number of panicles hill^{-1}, PL—panicle length, NFGP^{-1}—number of filled grains panicle^{-1}, NUFGP^{-1}—number of unfilled grains panicle^{-1}, TNSP^{-1}—total number of spikelets panicle^{-1}, YH^{-1}—yield hill^{-1}, 1000 GWH^{-1} 1000 grain weight.

* Significant at p ≤ 0.05.
** Significant at p ≤ 0.01.
ns Non-significant.

Table 4
Estimation of genetic parameters of 13 quantitative traits among 41 rice genotypes.

Trait	Mean	MSg	Mse	GV	PV	PCV (%)	GCV (%)	h^2_B (%)	GA	GA (%)
NTH^{-1}	29.93	159.67	35.88	41.26	77.14	29.35(h)	21.46(h)	53.49	9.68	32.34
FLL	24.14	22.79	3.89	6.30	10.19	13.23(M)	10.40(m)	61.83	4.07	16.85
SHT	31.59	24.83	8.54	5.43	13.96	11.83(m)	7.38(l)	38.91	3.00	9.49
PHT	84.23	82.13	21.70	20.14	41.84	7.68(l)	5.33(l)	48.14	6.41	7.61
DM	119.05	263.50	3.00	86.83	89.83	7.96(l)	7.83(l)	96.66	18.87	15.34
DF	77.04	148.73	3.88	48.28	52.16	9.37(l)	9.02(l)	92.56	13.77	17.87
TNSP^{-1}	147.59	3579.01	315.16	1087.95	1403.11	25.38(h)	22.35(h)	77.54	59.83	40.70
NFGP^{-1}	99.14	1962.01	20.08	647.31	667.39	26.06(h)	25.66(h)	96.99	51.62	52.06
NUFGP^{-1}	48.46	1354.50	253.68	366.94	620.62	51.41(h)	39.53(h)	59.12	30.34	62.60
PL	19.62	4.45	1.19	1.09	2.28	7.69(l)	5.31(l)	47.73	1.48	7.54
YH^{-1}	48.50	749.20	15.37	244.61	259.98	33.25(h)	32.25(h)	94.09	31.25	64.43
1000 GWH^{-1}	20.37	35.67	1.71	11.32	13.03	17.72(m)	16.51(m)	86.88	6.46	31.71
NPH^{-1}	24.43	59.58	35.42	8.05	43.47	26.99(h)	11.62(m)	18.52	2.52	10.31

Note: NTH^{-1}—number of tillers hill^{-1}, FLL—flag leaf length, SHT—seedling height, PHT—plant height, DM—days to maturity, DF—days to 50% flowering, TNSP^{-1}—total number of spikelets panicle^{-1}, NFGP^{-1}—number of filled grains panicle^{-1}, NUFGP^{-1}—number of unfilled grains panicle^{-1}, PL—panicle length, YH^{-1}—yield hill^{-1}, 1000 GWH^{-1}—1000 grain weight hill^{-1}, NPH^{-1}—number of panicles hill^{-1}, * Significant at p ≤ 0.05, ** Significant at p ≤ 0.01, ns—non-significant, MSg—mean square of genotype, Mse —mean square of error, GV—genotypic variance, PV—phenotypic variance, PCV—phenotypic coefficient of variation, GCV—genotypic coefficient of variation, h^2_B—broad sense heritability, GA—genetic advance in respective traits unit, GA(%)— genetic advance in % of genotypes mean.

also utilized to get the genetic relationships. The Principal component analysis (PCA) of 41 rice genotypes was determined by EIGEN and PROJ modules of NTSYS-pc and Minitab software (version 15).

3. Result

3.1. Variation and genetic parameters among genotypes

Thirteen traits including seedling height, plant height, number of tiller hill^{-1}, flag leaf length, days to 50% flowering, days to maturity, panicle length, number of filled grain panicle^{-1}, number of unfilled grain panicle^{-1}, total number of spikelets hill^{-1}, yield hill^{-1}, 1000 grain weight hill^{-1}, exhibited highly significant variation (P≤0.01) and the remaining number of panicles hill^{-1} showed significant variation (≤0.05) among all the genotypes under this study (Tables 3a, 3b, S1, S2, S3, S4). In this study, most of the yield and yield component traits expressed relatively higher phenotypic coefficient of variation value (PCV) than growth traits in Table 4. The PCV values which ranged from 7.68 for plant height to 51.41% for number of unfilled grains panicle^{-1} were categorized according to Sivasubramanian and Menon (1973). Higher PCV value was observed in number of tiller hill^{-1} (29.35%), total number of spikelets hill^{-1} (25.38), number of filled grain panicle^{-1} (26.06), number of unfilled grain panicle^{-1} (51.41%), yield hill^{-1} (33.25%), and number of panicles hill^{-1} (26.99%). On the other hand, lower value of PCV was

noticed in plant height (7.68%), days to maturity (7.96%), days to 50% flowering (9.37%), and panicle length (7.69%). The genotypic coefficient of variation (GCV) was found to vary from 5.31 for plant height to 39.53% for number of unfilled grain panicle^{-1}. Likewise, most of the yield and yield component traits expressed relatively higher genotypic coefficient of variation (GCV) value than growth traits. The lowest heritability was 18.52% and belonged to number of panicles hill^{-1} whereas it was 96.99% for number of filled grain panicle^{-1}. According to Robinson et al. (1949) heritability was classified. In this analysis, higher heritability was found in flag leaf length (61.83%), days to maturity (96.66%), days to 50% flowering (92.56%), total number of spikelets hill^{-1} (77.54%), number of filled grain panicle^{-1} (96.99), yield hill^{-1} (94.09%) and 1000 grain weight hill^{-1} (86.88%). The rest of the traits showed medium and lower heritability. Genetic advance (GA) was also classified according to Robinson et al. (1949) and it ranged from 7.4 for panicle length to 64.43% for yield hill^{-1} (Table 4).

3.2. Interrelations among the morphological traits

Pearson's correlation coefficient was calculated among 13 morphological traits in 41 rice genotypes (Table 5). Positive correlation was observed among most of the traits. Total number of spikelets panicle^{-1} had significant positive relation with the number of filled grain panicle^{-1}, number of unfilled grain panicle^{-1}, days to 50%

Table 5
Pearson's correlation coefficient among 13 quantitative traits of 41 rice genotypes.

Traits	NFGP^{-1}	NUFGP^{-1}	TNSP^{-1}	YH^{-1}	1000 GWH^{-1}	DF	DM	NTH^{-1}	SHT	PHT	PL	NPH^{-1}	FLL
NFGP^{-1}	1.00												
NUFGP^{-1}	0.09^{ns}	1.00											
TNSP^{-1}	0.74**	0.72**	1.00										
YH^{-1}	0.34**	−0.23**	0.07^{ns}	1.00									
1000 GWH^{-1}	−0.31**	−0.02^{ns}	−0.23**	0.49**	1.00								
DF	0.16^{ns}	0.18*	0.23**	−0.29**	−0.49**	1.00							
DM	0.22*	0.08^{ns}	0.20*	−0.02^{ns}	−0.10^{ns}	0.32**	1.00						
NTH^{-1}	−0.01^{ns}	−0.10^{ns}	−0.08^{ns}	−0.06^{ns}	−0.37**	0.42**	−0.01^{ns}	1.00					
SHT	0.13^{ns}	−0.22*	−0.06^{ns}	0.11^{ns}	0.01^{ns}	−0.15^{ns}	−0.09^{ns}	−0.04^{ns}	1.00				
PHT	0.04^{ns}	0.03^{ns}	0.05^{ns}	0.10^{ns}	−0.05^{ns}	−0.21*	−0.29**	−0.01^{ns}	0.29**	1.00			
PL	0.14^{ns}	0.15^{ns}	0.20*	−0.11^{ns}	−0.05^{ns}	0.31**	−0.19*	0.09^{ns}	0.14^{ns}	0.16^{ns}	1.00		
NPH^{-1}	−0.26**	−0.42**	−0.46**	0.64**	0.26**	−0.20*	−0.15^{ns}	0.19*	0.02^{ns}	0.15^{ns}	−0.21*	1.00	
FLL	0.14^{ns}	−0.18*	−0.02^{ns}	0.28**	0.22*	−0.35**	−0.20*	−0.02^{ns}	0.46**	0.34**	0.17^{ns}	0.10^{ns}	1.00

Note: NFGP^{-1}—number of filled grains panicle^{-1}, NUFGP^{-1}—number of unfilled grains panicle^{-1}, TNSP^{-1}—total number of spikelets panicle^{-1}, YH^{-1}—yield hill^{-1}, 1000 GWH^{-1} —1000 grain weight, DF—days to 50% flowering, DM—days to maturity, NTH^{-1}—number of tillers hill^{-1}, SHT—seedling height, PHT—plant height, PL—panicle length, NPH^{-1}—number of panicles hill^{-1}, FLL—flag leaf length.
 * Significant at p ≤ 0.05.
 ** Significant at p ≤ 0.01.
 ns Non-significant.
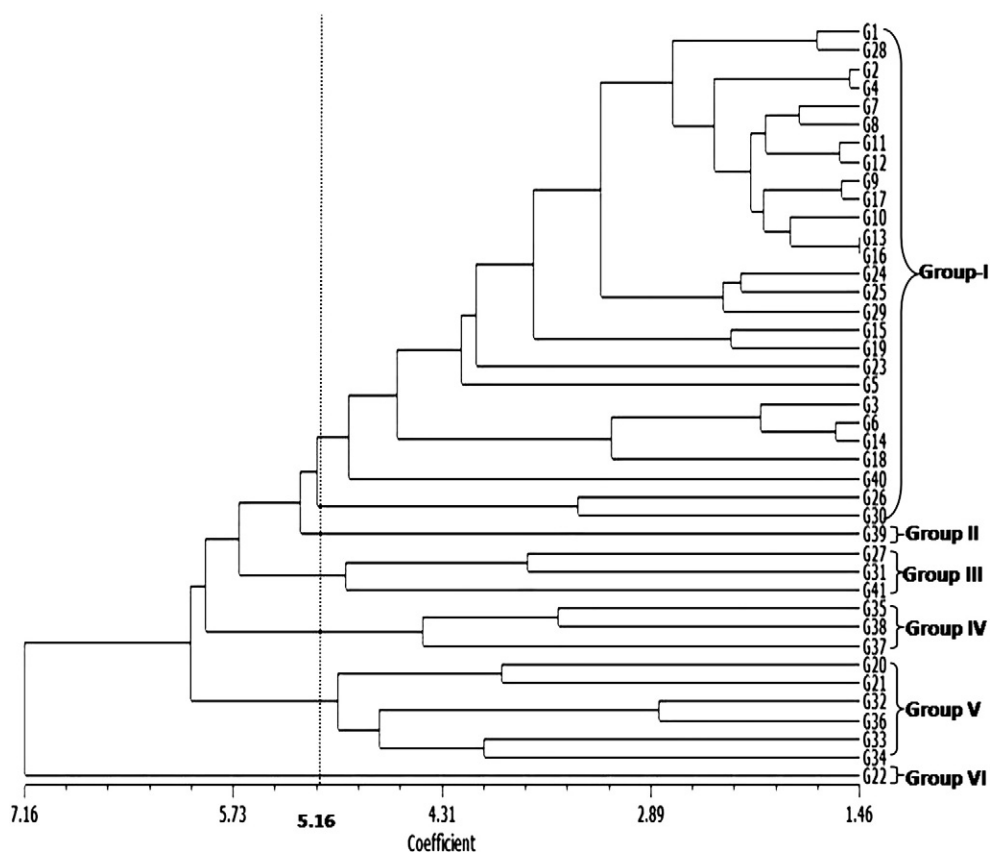
Fig. 1. The dendrogram showing relationship among 41 rice genotypes (*Oryza sativa*) using 13 morphological traits. Note: G1 = IRBB2, G2 = IRBB4, G3 = IRBB5, G4 = IRBB7, G5 = IRBB10, G6 = IRBB11, G7 = IRBB13, G8 = IRBB14, G9 = IRBB21, G10 = IRBB54, G11 = IRBB55, G12 = IRBB57, G13 = IRBB58, G14 = IRBB59, G15 = IRBB60, G16 = IRBB61, G17 = IRBB64, G18 = IRBB65, G19 = IRBB66, G20 = MR84, G21 = MR159, G22 = MR185, G23 = MR211, G24 = MR 219, G25 = MR220, G26 = MR232, G27 = MR253, G28 = MR 263, G29 = MR269, G30 = MR220-CL1, G31 = MR220-CL2, G32 = MRQ50, G33 = MRQ74, G34 = PH9, G35 = Pulut Siding, G36 = IR8, G37 = YTM16, G38 = Bahagia, G39 = BR28, G40 = BR29, G41 = Purbachi.

flowering, days to maturity, and panicle length. Yield hill^{-1} was highly significant (P ≤ 0.01) and positively correlated with the number of filled grain panicle^{-1} (0.34**), 1000 grain weight hill^{-1} (0.49**), flag leaf length (0.28**) and number of panicles hill^{-1} (0.64**).

3.3. Cluster analysis

Thirteen morphological traits clustered 41 rice genotypes in to six major groups. From Fig. 1 and Table 6 it is found that cluster I was the largest (containing 27 genotypes) and cluster II as well as cluster VI (containing one member) were the smallest group. Clusters III, IV and V comprised of three, three and six genotypes, respectively. The fourth group had the highest average compared to other five groups in terms of five traits (Table 7) such as seedling height (34.83 cm), days to maturity (125 days), flag leaf length (25 cm), panicle length (21.86 cm), and number of filled grain panicle^{-1} (139.95). The third group included the

highest average for three traits such as number of panicles hill^{-1} (34.67), yield hill^{-1} (65.82 g), 1000 grain weight hill^{-1} (23.07 g). On the contrary, the second group, revealed the lowest average values in the traits such as number of tiller hill^{-1} (25.67), days to 50% flowering (64.33 days), days to maturity (96.33 days), panicle length (18.13 cm), number of filled grain panicle^{-1} (79.24), number of unfilled grain panicle^{-1} (12.14), and total number of spikelets hill^{-1} (91.38) (Table 7).

3.4. Principal component analysis (PCA)

The PCA mostly confirmed the cluster analysis. In case of distant genotype G22 (MR185) which formed its own group alone both in cluster analysis and PCA analysis (Figs. 1 and 2) gave the evidences. However, genotype G39 (BR28) which was clustered alone in group II of cluster analysis came closer to some other genotypes in PCA and formed group I with other genotypes. According to PCA, the first four principal components accounted for about 70.50% of total variation for all morphological traits and exhibited high correlation among the characteristics analyzed. From the eigenvectors analysis it was found that 23.70, 17.50, 15.70 and 13.70% variation of morphological traits could be explained in respect by the first four principal components (Table 8).

4. Discussion

Among the 41 rice genotypes, all the traits exhibited highly significant (P < 0.01) and significant (P < 0.05) variations. Phenotypic variation has been reported by many rice researchers for different characters in different rice accessions (Ullah et al., 2011; Pandey et

Table 6
Groups of forty-one rice genotypes according to cluster analysis from morphological traits.

Group	Genotype
Group I	IRBB2, MR263, IRBB4, IRBB7, IRBB13, IRBB14, IRBB55, IRBB 54, IRBB58, IRBB61, MR219, MR220, MR269, IRBB60, IRBB5, IRBB11, IRBB59, IRBB65, BR29, MR232, MR220-CL1, IRBB57, IRBB21, IRBB66, IRBB64, MR211, IRBB10
Group II	BR28
Group III	MR253, MR220-CL2, Purbachi
Group IV	Pulut Siding, Bahagia, YTM16
Group V	MR84, MR159, MRQ50, IR8, MRQ74, PH9
Group VI	MR185

Table 7
Mean values of 13 morphological characters for six groups revealed by cluster analysis among 41 genotypes of (*Oryza sativa*).

| Group	SHT (cm)	PHT (cm)	NTH^{-1} (no)	DF (days)	DM (days)	FLL (cm)	NPH^{-1} (no)	PL (cm)	NFGP^{-1} (no)	NUFGP^{-1} (no)	TNSP^{-1} (no)	YH^{-1} (g)	1000 GWH^{-1} (g)
I	32.24	84.63	26.57	74.16	119.33	24.96	24.47	19.48	97.46	49.88	147.34	51.47	21.72
II	31.50	90.50	25.67	64.33	96.33	22.43	23.67	18.13	79.24	12.14	91.38	36.86	19.67
III	29.34	86.56	37.50	72.11	107.11	24.71	34.67	19.77	84.40	32.83	117.24	65.82	23.07
IV	34.83	88.89	32.17	86.67	125.00	25.00	18.67	21.86	139.95	51.56	191.51	40.40	15.06
V	29.64	78.25	40.44	88.39	124.94	20.97	22.89	19.19	97.05	40.51	137.56	33.69	15.45
VI	22.69	82.00	32.33	85.33	116.67	18.37	20.00	20.35	98.62	131.74	230.36	40.99	22.03

Note: SHT—seedling height, PHT—plant height, NTH^{-1}—number of tillers hill^{-1}, DF—days to 50% flowering, DM—days to maturity, FLL—flag leaf length, NPH^{-1}—number of panicles hill^{-1}, PL—panicle length, NFGP^{-1}—number of filled grains panicle^{-1}, NUFGP^{-1}—number of unfilled grains panicle^{-1}, TNSP^{-1}—total number of spikelets panicle^{-1}, YH^{-1}—yield hill^{-1}, 1000 GWH^{-1}—1000 grain weight hill^{-1}.

al., 2011). Highly significant difference among ten varieties of Biroin rice of Bangladesh with ten morpho-physiological traits was observed (Ullah et al., 2011). In another study, using 12 quantitative characters, 40 rice accessions showed highly significant variation among them (Pandey et al., 2011). In two different studies, highly significant divergence was detected among 30 genotypes of rice (Abarshahr et al., 2011) and 57 genotypes of upland rice (Chandra et al., 2007) with 19 as well as 14 quantitative traits, respectively.

In order to design an effective breeding strategy for any crop, knowledge of correlations among different traits is very essential. Directly selections with a view to develop yield may be complicated and time consuming because of some reasons. For example, complex plant characters like yield are quantitatively inherited and influenced by genetic effects as well as by the interaction between genotype and environment. Moreover, the main character is expressed late. Therefore, indirect selection is preferable and it is much easier to measure indirect character than direct character. Thus, it is appropriate to identify and use of highly correlated characters (Ahmadikhah et al., 2008). In the present study, yield hill^{-1} had positive correlation with number of filled grain panicle^{-1}, 1000 grain weight hill^{-1}, number of panicles hill^{-1} and flag leaf length. Positive correlation between 1000-grain weight and grain yield plant^{-1} was reported (Tsuzuki and Umeki, 1990). Moreover, highly significant correlation between grain yield and 1000 grain weight at the phenotypic level

was also observed by some scientists (Mirza et al., 1992; Kennedy and Rangasamy, 1998). Number of filled grain panicle^{-1} was positively as well as significantly correlated to yield hill^{-1} in this study which was also supported by Ullah et al. (2011). Moreover, significant positive correlation was noticed between yield hill^{-1} and number of panicles hill^{-1} which was consistent with the findings of Abarshahr et al. (2011). Another point is that, yield hill^{-1} had significant and positive correlation with flag leaf length which was also supported by Abarshahr et al. (2011). The significant phenotypic association between traits was primarily due to the genetic cause, perhaps owing to the pleiotropic effect rather than linkage between the genes affecting different characters (Singh and Pandey, 2012). Negative correlation was also observed between number of tiller hill^{-1} and 1000 grain weight hill^{-1} and between number of tiller hill^{-1} and total number of spikelets hill^{-1}.

According to Johnson et al. (1955) estimation of heritability and genetic advance is essential for the selection based on phenotypic expression. Thus, high heritability coupled with high genetic advance could be the key target of selection based on morphological traits. In the current study, high heritability together with high genetic advance was obtained for the following four traits namely yield hill^{-1}, number of filled grains panicle^{-1}, 1000 grain weight hill–1 and total number of spikelets panicle^{-1} that indicated preponderance of additive gene action in the expression of the aforesaid traits (Paramsivam et al., 1996). Similar findings were also reported by
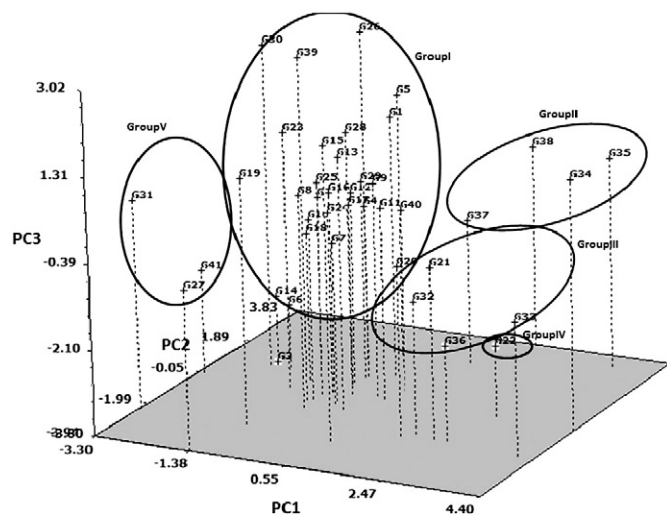
Fig. 2. Three-dimensional plot of PCA showing relationships among 41 rice genotypes using morphological traits. Note: G1 = IRBB2, G2 = IRBB4, G3 = IRBB5, G4 = IRBB7, G5 = IRBB10, G6 = IRBB11, G7 = IRBB13, G8 = IRBB14, G9 = IRBB21, G10 = IRBB54, G11 = IRBB55, G12 = IRBB57, G13 = IRBB58, G14 = IRBB59, G15 = IRBB60, G16 = IRBB61, G17 = IRBB64, G18 = IRBB65, G19 = IRBB66, G20 = MR84, G21 = MR159, G22 = MR185, G23 = MR211, G24 = MR 219, G25 = MR220, G26 = MR232, G27 = MR253, G28 = MR 263, G29 = MR269, G30 = MR220-CL1, G31 = MR220-CL2, G32 = MRQ50, G33 = MRQ74, G34 = PH9, G35 = Pulut Siding, G36 = IR8, G37 = YTM16, G38 = Bahagia, G39 = BR28, G40 = BR29, G41 = Purbachi.

Table 8
Eigenvectors and eigen values of the first four principal components.

Variables	Principal components			
	PC1	PC2	PC3	PC4
Eigen value	3.31	2.44	2.19	1.91
Proportion	0.23	0.17	0.15	0.13
Cumulative	0.27	0.41	0.56	0.70
NFGP^{-1}	−0.01	0.43	0.37	0.01
NUFGP^{-1}	−0.10	0.16	0.29	0.11
TNSP^{-1}	0.23	0.17	0.55	−0.05
YH^{-1}	0.40	0.09	0.38	0.09
1000 GWH^{-1}	0.38	−0.12	−0.10	0.32
DF	−0.43	0.04	0.19	−0.2
DM	−0.22	−0.01	0.23	0.33
NTH^{-1}	−0.20	−0.15	0.18	−0.54
SHT	0.13	0.39	−0.29	−0.12
PHT	0.21	0.29	−0.12	−0.29
PL	−0.09	0.35	−0.10	−0.25
NPH^{-1}	0.36	−0.34	0.16	−0.18
FLL	0.30	0.30	−0.17	−0.16

Note: NFGP^{-1}—number of filled grains panicle^{-1}, NUFGP^{-1}—number of unfilled grains panicle^{-1}, TNSP^{-1}—total number of spikelets panicle^{-1}, YH^{-1}—yield hill^{-1}, 1000 GWH^{-1}—1000 grain weight hill^{-1}, DF—days to 50% flowering, DM—days to maturity, NTH^{-1}—number of tillers hill^{-1}, SHT—seedling height, PHT—plant height, PL—panicle length, NPH^{-1}—number of panicles hill^{-1}, FLL—flag leaf length, PC—principal component.

Chaudhury and Das (1998) for number of filled grain panicle^{-1} and yield hill^{-1}. Therefore, selection might be effective through these characters in segregating generation.

The UPGMA dendrogram broadly clustered the rice genotypes in to six major groups at 5.16 dissimilarity coefficient, which implied a high level of morphological diversity in the rice genotypes. Result of this study unveiled the better resolution power of quantitative traits for grouping of *O. sativa* genotypes. On the basis of 18 morphological traits 58 rice varieties were clustered in to four groups in a study conducted by Ahmadikhah et al. (2008) while Veasey et al. (2008) observed that 23 rice populations were clustered in to 10 different groups based on 20 morphological traits.

The presence of broad morphological differences among genotypes was further confirmed by principal component analysis, which indicated that the overall diversity observed could be elucidated by a few eigenvectors. The first four principal components from morphological traits explained by 70.50% of the total variation, with PC1 explaining 23.70% of the variation, PC2 17.50%, PC3 15.70% and PC4 13.70% of the total variation. The first 10 principal components accounted for 67% of total variation found by Caldo et al. (1996) in his study. This implied a strong correlation among traits which were studied. About 82.7% of total variation among 32 upland rice varieties was also noticed (Lasalita-zapico et al., 2010) where almost 66.9% variation showed by PC1 and 15.87% by PC2.

In the current study, 41 rice genotypes showed notable genetic diversity in terms of morphological traits. Forty-one rice genotypes were divided in to six major groups in cluster analysis. Principal component analysis implied 70.50% of total variation. Cluster analysis and PCA played complementary role to each other with little inconsistencies in respect of composition of cluster. Among these genotypes 35 were BB resistant where 19 genotypes were obtained from IRRI. The IRRI population consisted of near isogenic lines (NIL) along with pyramid lines having multiple BB resistant genes and extensively used as parents in rice growing countries of the world to develop resistant varieties. But genetic diversity among these lines has not yet been studied and this is the first report to the best of our knowledge. High heritability together with high genetic advance was obtained for the following four traits namely yield hill^{-1}, number of filled grains panicle^{-1}, 1000 grain weight hill^{-1} and total number of spikelets panicle^{-1}. Several traits such as plant height, yield hill^{-1}, number of filled grain panicle^{-1}, and total number of spikelets hill^{-1} had positive correlation among each other, which indicated that employing of these traits by selection could develop the desired genotypes. To attain a broad spectrum of variation or heterosis among the genotypes, group I (genotypes IRBB2, IRBB4, IRBB13, IRBB21, MR263) might be hybridized with group V (genotypes MR84, MR159, MRQ50, MRQ74, PH9 and IR8) and group VI (genotype MR185). Moreover, to develop BB tolerant genotypes crossing could be done between (Pulut Siding and IRBB21), (MR84 and IRBB61), (Bahagia and IRBB60), (BR28 and IRBB64), (BR29 and MR185) and (Purbachi and MR159). Similar studies were also reported by Abdullah et al. (2011) and Latif et al. (2011a,b).

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.sajb.2013.01.004.

References

Abarshahr, M., Rabiei, B., Lahigi, H.S., 2011. Assessing genetic diversity of rice varieties under drought stress conditions. Notulae Scientia Biologicae 3 (1), 114–123.

Abdullah, N., Rafii Yusop, M., Ithnin, M., Saleh, G., Latif, M.A., 2011. Genetic variability of oil palm parental genotypes and performance of its' progenies as revealed by molecular markers and quantitative traits. Comptes Rendus Biologies 334, 290–299.

Ahmadikhah, A., Nasrollanejad, S., Alishah, O., 2008. Quantitative studies for investigating variation and its effect on heterosis of rice. International Journal of Plant Production 2 (4), 297–308.

Akinbile, C.O., Abd El-Latif, K.M., Abdullah, R., Yusoff, M.S., 2011. Rice production and water use efficiency for self-sufficiency in Malaysia: a review. Trends in Applied Sciences Research 6 (10), 1127–1140.

Appalaswamy, A., Reddy, G.L.K., 2004. Genetic divergence and heterosis studies of mungbean (*Vigna radiata* (L.) Wilczek). Legume Research 21, 115–118.

Burton, G.W., 1951. Quantitative inheritance in Pearl millet (*P. glaucum*). Agronomy Journal 43, 409–417.

Burton, G.W., Devane, E.M., 1953. Estimating heritability in tall fescue (*Festuca circunelinaceae*) form replicated clonal material. Agronomy Journal 45, 478–481.

Caldo, R., Sebastian, L., Hernandez, J., 1996. Morphology-based diversity analysis of ancestral lines of Philippine rice cultivars. Philippine Journal of Crop Science (Philippines) 21 (3), 86–92.

Chandra, R., Pradhan, S., Singh, S., Bose, L., Singh, O., 2007. Multivariate analysis in upland rice varieties. World Journal of Agricultural Sciences 3 (3), 295–300.

Chaudhury, P.K.D., Das, P.K., 1998. Genetic variability, correlation and path coefficient analysis in deep water rice. Annals of Agricultural Research 19, 120–124.

Din, R., Khan, M.Y., Akmal, M., 2010. Linkage of morphological markers in Brassica. Pakistan Journal of Botany 42 (5), 2995–3000.

FAO (Food and Agriculture Organization of the United Nations), 2004. Rice and us. http://www.fao.org/rice2004/en/aboutrice.htm (Accessed on 20 March 2011).

Ismail, A.A., Khalifa, M.A., Hamam, K.A., 2001. Genetic studies on some yield traits of durum wheat. Asian Journal of Agriculture Science 32, 103–129.

Johnson, H.W., Robinson, H.F., Comstock, R.E., 1955. Estimates of genetic and environmental variability in soybeans. Agronomy Journal 47, 314–318.

Kennedy, V.J.F., Rangasamy, P., 1998. Correlation studies on hybrid rice under low temperature conditions. Madras Agriculture Journal 85, 130–131.

Kumar, P., Shukla, R.S., 2002. Genetic analysis for yield and its attributed traits in bread wheat under various situations. Jawaharlal Nehru Krishi Vishwa Vidyalaya, Research Journal 36, 95–97.

Kwon, S.J., Ha, W.G., Hwang, H.G., Yang, S.J., Choi, H.C., Moon, H.P., Ahn, S.N., 2002. Relationship between heterosis and genetic divergence in 'Tongil'-type rice. Plant Breeding 121, 487–492.

Larik, A.S., Malik, S.I., Kakar, A.A., Naz, M.A., 2000. Assessment of heritability and genetic advance for yield and yield components in *Gossypium hirsutum* L. Scientific Khyber 13, 39–44.

Lasalita-Zapico, F.C., Namocatcat, J.A., Carini-Turner, J.L., 2010. Genetic diversity analysis of traditional upland rice cultivars in Kihan, Malapatan, Sarangani Province, Philippines using morphometric markers. The Philippine Journal of Science 139 (2), 177–180.

Latif, M.A., Rafii Yusop, M., Rahman, M.M., Talukdar, M.B., 2011a. Microsatellite and minisatellite markers based DNA fingerprinting and genetic diversity of blast and ufra resistant genotypes. Comptes Rendus Biologies 5, 361–368.

Latif, M.A., Rahman, M.M., Kabir, M.S., Ali, M.A., Islam, M.T., Rafii, M.Y., 2011b. Genetic diversity analyzed by quantitative traits among rice (*Oryza sativa* L.) genotypes resistant to blast disease. African Journal of Microbiology Research 5, 4383–4391.

Maclean, J.L., Dawe, D.C., Hardy, B., Hettel, G.P., 2002. Rice Almanac. Los Baños (Philippines): International Rice Research Institute, Bouaké (Cote d'Ivoire): West Africa Rice Development Association, Cali (Colombia): International Center for Tropical Agriculture, Rome (Italy): Food and Agriculture Organization (253 pp.).

MARDI (Malaysian Agriculture Research and Development Institute), 2008. Three Decades of Achievements in Research and Development. www.mardi.gov.my.

Mew, T.W., 1987. Current status and future prospects of research on bacterial blight of rice. The Annual Review of Phytopathology 25, 359–382.

Mirza, M.J., Faiz, F.A., Mazid, A., 1992. Correlation studies and path analysis of plant height yield and yield components in rice (*Oryza sativa* L.). Sarhad Journal of Agricultural 8, 647–653.

Ogawa, T., 1993. Methods and strategy for monitoring race distribution and identification of resistance genes to bacterial leaf blight (*Xanthomonas campestris* pv. *oryzae*) in rice. Japan Agricultural Research Quarterly 27, 71–80.

Onwueme, I.C., Sinha, T.D., 1991. Field Crop Production in Tropical Africa. CTA, Ede, The Netherlands (545 pp.).

Pandey, P., Anurag, P.J., Tiwari, D., Yadav, S., Kumar, B., 2011. Genetic variability, diversity and association of quantitative traits with grain yield in rice (*Oryza sativa* L.). Journal of Bio-Science 19, 77–82.

Paramsivam, K., Giridaran, S., Kirubakaran, S.A.P.M., Parthasarathy, P., 1996. Heterosis and combining ability for grain characters in rice. Madras Agricultural Journal 83 (2), 110–114.

Robinson, H.F., Comstock, R.E., Harvey, P.H., 1949. Estimates of heritability and degree of dominance in corn. Agronomy Journal 41, 353–359.

Rohlf, F., 2002. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, 2.1 ed. Department of Ecology and Evolution, State University of NY, Stony Brook.

Saad, A., Habibuddin, H., Alias, I., Othman, O., Azlan, S., Zulkifli, R., 2000. Resistance status of released varieties after MR84 against bacterial blight and the incidence on the disease in Muda irrigation scheme. Proceedings of the Conference on Plant Resource Management, Nov. 22–23, Malaysia, pp. 21–41.

SAS Institute, 2001. SAS/Stat user's guide, Version 9.1. SAS Institute, Cary, NC, USA.

Singh, P., Pandey, A., 2012. Correlation between different traits of aromatic rice (*Oryza sativa* L.) and their cause–effect relationship. Applied Biological Research 14 (1), 47–53.

Sivasubramanian, S., Menon, M., 1973. Heterosis and inbreeding depression in rice. Madras Agricultural Journal 60, 11–39.

Surek, H., Beser, N., 2003. Selection for grain yield and yield components in early generations for temperate rice. Philippine Journal of Crop Science 28 (3), 3–15.

Tsuzuki, E., Umeki, Y., 1990. Studies on the methods of stabilizing and increasing yield in early cultivated rice, correlation between grain yield and characters related with yield. Bulletin faculty Agriculture, Miyazaki University, Japan, 36, pp. 261–269.

Ullah, M.Z., Bashar, M.K., Bhuiyan, M.S.R., Khalequzzaman, M., Hasan, M.J., 2011. Interrelationship and cause–effect analysis among morpho-physiological traits in Biroin rice of Bangladesh. International Journal of Plant Breeding and Genetics 5 (3), 246–254.

Veasey, E.A., Silva, E.F., Schammass, E.A., Oliveira, G.C.X., Ando, A., 2008. Morphoagronomic genetic diversity in American wild rice species. Brazilian Archives of Biology and Technology 51 (1), 94–104.

Vivekananda, P., Subramanian, S., 1993. Genetic divergence in rainfed rice. Oryza 30, 60–62.

Zhai, W.X., Zhu, L.H., 1999. Rice bacterial blight resistance genes and their utilization in molecular breeding. Journal of Chinese Biotechnology 19, 9–15 (In Chinese).

Zhao, K., Chih-Wei, T., Eizenga, G.C., Wright, M.H., Ali, M.L., Price, A.H., Norton, G.J., Islam, M.R., Reynolds, A., Mezey, J., McClung, A.M., Bustamante, C.D., McCouch, S.R., 2011. Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. Nature Communications 2, 467. http://dx.doi.org/10.1038/ncomms1467.