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Assessment phenotypic diversity of rice (*Oryza sativa* L.) genotypes by multivariate analysis

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ABSTRACT

An efficient way to achieve superior productivity is to determine the genetic variation of the various rice genotypes. This research was aimed at estimating potential variations between rice genotypes and identifying each trait contribution in total variation and classifying superior genotypes. The experiment was performed at the Rice Research and Training Centre, Sakha, Kafr El-Sheik, Egypt. Twenty-two genotypes of rice were analyzed using seven agronomic traits. Multivariate approaches were utilized including principal components and cluster analysis. Results showed that PC1 and PC2 represented 66.1% of the variation between studied genotypes, mainly 48% because of grain yield per plant variation in PC1 followed by the characteristics of its components i.e., number of panicles per plant, number of filled grains per panicle, and 1000-grain weight. The three Egyptian rice genotypes Giza 181, Giza 178, and Giza 177 were the best genotypes for grain yield. Cluster results revealed that the majority of genotypes originated from one source (except for Indian variety IET1444) or belonged to one classification were clustered together. Multivariate analytical approaches are ideal instruments for providing information on agronomic character variations. Consequently, the results of the current study should be taken into account when developing new rice varieties.

KEYWORDS: Rice (*Oryza sativa* L.), Agronomic traits, Multivariate analysis, Phenotypic diversity

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the Gramineae family crops, the daily meal of over two-thirds of the world's population and provides approximately 20% of a human's calories consumed (Wogu *et al.*, 2010). World rice production amounts are 782 million tons over the total area of approximately 167 million hectares (FAO, 2018). It is cultivated commercially in several countries in the world. It is particularly important in Asia (90.7%), America (5.2%), Africa (3.4%), Europe (0.6%) and Oceania (0.1%) as a commercial crop (FAO, 2018). China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil, Japan are the top ten rice producers worldwide in 2018 (FAO, 2018). In Egypt, it has a production area of 4280 metric tons at an area of 0.445 million/ha and a productivity of 9.6 tons/ha. There are still a lot of challenges to achieving food security worldwide. Therefore, there is a need to improve high-yielding varieties. Adequate knowledge of genetic variation in different genotypes is a preliminary

step in breeding programs for the selection and production of new varieties (Kumbhar *et al.*, 2015; Ahmed *et al.*, 2016). Rice genotype diversity is an essential method of transmission of genetic information (Nambara & Nonogaki, 2012; Martínez-Andújar *et al.*, 2012). The recent method, the biplot technique, provides breeders with a complete visual representation of all aspects of variables by producing a biplot that simultaneously represents both mean efficiency and stability (Yan & Holland, 2010; Gedif & Yigzaw, 2014; Yan & Frégeau-Reid, 2018; Bányai *et al.*, 2020). Multivariate analysis is a sufficient measurement of the degree of difference between genotypes. Principle component analysis (PCA) and cluster analysis are both used to assess variation as multivariate methods (Maji & Shaibu, 2012; Tiwari *et al.*, 2020). PCA is used to study diversity and to determine commitment to several specific characteristics. Cluster analysis is used when we need to classify genotypes according to genetic or agronomic traits into different groups (Shabir *et al.*, 2013). Also, Nachimuthu *et al.* (2014) used PCA to assess variation between rice genotypes derived from various

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countries. They studied many characteristics like filled grains number per panicle, grain yield per plant, panicle length and plant height. They found that there was a high coefficient of variation for the number of filled grains per panicle and grain yield per plant and when using PCA. They defined traits that had an impact on variation. However, (Chakravorty *et al.*, 2013) assessed the variability of the rice genotypes by PCA. Results showed that the number of grains per panicle affected the variety of genotypes studied. Also, Sanni *et al.* (2012) used cluster analysis to evaluate the variability of 434 rice genotypes in 10 agronomic traits under upland conditions. Their results showed that seven groups were derived from ten agronomic and botanical traits. They stated that certain clusters in breeding programs are valuable in determining parental genotypes. The objective of this research was to (i) estimate the possible diversity between the rice genotypes studied by multivariate analysis, (ii) define the contribution of each studied trait to the total variation under the irrigated condition and (iii) determine the best genotypes for the breeding of new high yielding cultivars under irrigated condition in future.

MATERIAL AND METHODS

Plant Materials

On the Rice Research and Training Center (RRTC) experimental, Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt, twenty-two rice (*Oryza sativa* L.) genotypes originating from different countries were evaluated for various quantitative traits (Table 1). The germplasm included commercial Egyptian varieties, imported varieties, Egyptian landraces, Egyptian advanced lines and accessions from the collections of the International Rice Research Institute (IRRI), Los Banos, Philippines and National Small Grains, USDA, ARS, USA. Seeds were kindly provided by the International Rice Research Institute (IRRI), Los Banos, Philippines; USDA-ARS,

National Small Grains Collection, USA and Rice Department, Agriculture Research Center (ARC), Giza, Egypt.

Field experiments

The research was conducted at the Rice Research and Training Center (RRTC) experimental farm, Sakha, Kafr El-Sheikh, Egypt, in the two-rice successive cultivated seasons 2017 and 2018. The seeds of the twenty-two rice genotypes were grown in the nursery and transplanted in four rows after 30 days from sowing. The experiment was arranged in a randomized complete block design (RCBD) with three replicates. Each replicate consists of three rows, 5 m long, 20 cm in length between rows, comprising 25 hills each of a single plant. The recommended cultural rice practices have been applied as usual for the ordinary rice field in the region. A specific dose of N: P: K (10: 18: 18) was applied during ground preparation at a rate of 80 kg/ha. Urea was applied at a rate of 80 kg/ha, first at tillering and second dose at booting. Hand-weeding was done regularly to minimize the infestation of weeds.

Data Collection

Seven quantitative traits were determined using the rice (*O. sativa*) descriptor methods (IRRI, 1980). The variables included in the descriptive and multivariate analysis were phenological (for each plot the number of days to heading) and agro-quantitative (plant height (cm), panicle length (cm), number of panicles per plant, number of filled grains per panicle, 1000-grain weight (g) and grain yield per plant (g)). The data were calculated based on ten randomly selected guarded plants from each plot. Data were recorded on a single plant-dependent basis for each genotype.

Statistical Analysis

Mean averaged data were obtained over two years and used to measure mean values. The twenty-two genotypes were divided into groups using the method of unweighted pair group system of average linkage (UPGMA) and studied traits were analyzed using principal component analysis (PCA). The datasets were first tested with normality tests for normality and then subjected to variance analysis using an appropriate model (Anderson & Darling, 1952). PCA was used to determine the degree of characteristic variance between genotypes based on (Everitt & Dunn, 1992). While biplot analysis was used to select the best performing genotype. PCA as one of the factor analysis methods is a reduction of a large number of variables correlated with smaller sets of variables called factors or components (Cattell, 1965). The group array, the amount of variance expressed by the combined variables, was estimated by the highest correlation coefficient in each array, as suggested by Seiller & Stafford (1985). The experimental results were statistically analyzed using Genstat version 12.0 software for multivariate analysis. The UPGMA was performed using the statistical method of numerical taxonomic and multivariate analysis methods (NTSYS version 2.1 software package (Rohlf, 1998).

Table 1: List of 22 rice genotypes used in this study

No	Genotype	Origin	Source of seed	Subspecies group
1	IR 20	Philippines	IRRI	Indica
2	IR 22	Philippines	IRRI	Indica
3	IR 24	Philippines	IRRI	Indica
4	IR 50	Philippines	IRRI	Indica
5	IR 64	Philippines	IRRI	Indica
6	IR 74	Philippines	IRRI	Indica
7	Bala	India	IRRI	Indica
8	IET 1444	India	IRRI	Indica
9	Arabi	Egypt	USDA, USA	Japonica
10	Agamy M1	Egypt	USDA, USA	Japonica
11	Nahda	Egypt	USDA, USA	Japonica
12	Yabani M1	Egypt	USDA, USA	Japonica
13	Yabani M7	Egypt	USDA, USA	Japonica
14	Yabani 15	Egypt	USDA, USA	Japonica
15	Yabani lulu	Egypt	USDA, USA	Japonica
16	Giza 14	Egypt	USDA, USA	Japonica
17	Giza 171	Egypt	ARC, Egypt	Japonica
18	Giza 172	Egypt	ARC, Egypt	Japonica
19	Giza 177	Egypt	ARC, Egypt	Japonica
20	Giza 178	Egypt	ARC, Egypt	Indica/Japonica
21	Giza 181	Egypt	ARC, Egypt	Indica
22	Gz 1386-5-4	Egypt	ARC, Egypt	Indica

RESULTS

Rice Genotypes Performance Under Irrigated Condition

Table 2 presents the sum of values, mean, minimum and maximum, standard deviation and coefficient of variance (CV) for the various traits. The largest variation with CV 20.71 was observed in GYP, followed by PH, NPP, PL and DH with CV = 15.31, 14.06, 10.57 and 9.53%. TGW and NFGP had the lowest variation (CV = 7.91 and 4.87, respectively). Table 2 shows that DH ranged from 93 days for the earliest rice genotype Giza 177 to 135 days for the latest rice genotypes IR 20 with an average of 111.5 and 42 days for both mean and range, respectively. For PH the range varies from 80 cm for the shortest genotype IR 24 to 140 cm for the tallest genotype Arabi with an average of 106.8 and 60 cm for both the mean and range, respectively. Besides, PL ranged from 18.32 cm for the Agamy M1 rice genotype to 27.1 cm for the rice genotype IR 64 with 22.32 cm and 8.78 for the mean and range, respectively. Furthermore, the NPP range varies from 15 panicles per plant for the lowest rice genotype Nahda to 28 panicles per plant for the highest rice genotype Giza 178 with 20.86 and 13 panicles per plant for both the mean and range, respectively. Moreover, the NFGP varied from 111 for the lowest rice genotypes IR 20 to 139 for the highest rice genotypes Giza 178 with 123.7 and 28 for the mean and range, respectively. For TGW the range varies from 18.98 g for the lowest rice genotype Bala to 26.45 g for the highest rice genotype Giza 177 with 21.98 and 7.47 for both the mean and range, respectively. Finally, for GYP varied from 24.63 g for the lowest rice genotypes Nahda to 44.87 g for the highest rice genotype Giza 178 with 30.05 and 20.24 g for the mean and range, respectively. The variation between the genotypes studied was mainly due to the highest variation in grain yield per plant followed by plant height then in the number of panicles per plant with a coefficient of variation reached (20.71, 15.31 and 14.06) for each of them, respectively (Table 2). Diverse frequency distribution models for the characters studied resulted when the Scree histogram was applied (Figure 1).

Principle Component Analysis

PCA is a method used to identify character contribution in variation among different genotypes which could be a valuable selection tool for rice improvement. The PCA result has demonstrated the rice collection's genetic diversity. Proper values measure the importance and contribution of each component to total variance, while each value indicates the degree of contribution of each original variable associated with each main component. Seven PCA were collected for the seven characteristics, but only the first two PCA (PC1 and PC2) were approved to show the variation in the studied characteristics as they reported an eigenvalue greater than 1.0 (3.3575, 1.2647), respectively (Figure 2), while the other components were rejected because they were less than one resulting in an eigenvalue. In the principal component analysis of the relationships between the measured parameters, under irrigated replications, the eigenvalue of two principle components was greater than one, which together represented 66.1% of the total variance in which the PC1 had the highest variability (48%)

followed by PC2 (18.1%) (Figure 2). Whereas other major components were neglected (Table 3).

The first principal component PC1 is related to the contribution of five characteristics, $r = 0.504$ for GYP, $r = 0.478$ for NPP, $r = 0.415$ for NFGP, $r = 0.401$ for TGW and $r = -0.321$ for DH ($P < 0.01$) (Figure 3). Meanwhile, the second principal component PC2 correlated only with two traits, $r = -0.693$ for PH and $r = 0.499$ for PL (Table 4) (Figure 4).

Identifying Superior Rice Genotypes Under Irrigated Conditions

As a result, the traits in PC1 traits had the greatest influence on the total variation of the genotypes studied followed by traits in PC2 (Table 5). This revealed varying yield output among the rice genotypes. To visualize the relationship between genotypes, the origin of the plot was drawn to connect. So, in breeding programs, they could be used significantly to identify superior rice genotype. Figure 5 presents the plot distribution of both genotypes and PCA traits. This revealed that the number of panicles per plant is the closest correlated trait with grain yield per plant since they have a small angle between them, followed by 1000-grain weight and then filled grains number per plant. All traits were positively correlated with PC1 because they had scored more than zero except for days to heading and plant height. Genotypes distributed in the four quarters of the biplot revealed that both Giza 178 and Giza 181 rice genotypes were the most representative of variation as their scores were positive and more than other PC1 varieties followed at the same time by the genotype Giza 177. These three varieties were associated with grain yield per plant and the number of panicles per plant (Figure 5).

Cluster Analysis

Cluster analysis results according to dissimilarity (Table 6) showed five main cluster groups, four of which contained subclusters. The first major group included three varieties, the indica rice genotype Giza 181, the indica/japonica rice genotype Giza 178 and the japonica rice genotype Giza 177, all of which derive from Egypt. The second main group included only one rice genotype Giza 171 which is japonica type from Egypt. The third major group consisted of seven varieties, six of which were japonica genotypes (Yabani lulu, Yabani 15, Yabani M7, Giza 14, Yabani M1 and Giza 172), originating in Egypt and the indica rice genotype IET 1444 originating in India. In addition, the fourth major group consisted of seven indica genotypes (IR 74, IR 64, Gz 1386, IR 50, Bala, IR 24 and IR 22) all originating in the Philippines and indica rice genotype, except for Bala and Gz 1386 originating in India and Egypt, respectively. The fifth and last main group included four varieties, four of which are japonica genotypes (Agamy M1, Nahda and Arabi) from Egypt and the indica genotype IR 20 from the Philippines (Figure 6).

DISCUSSION

The variation between the genotypes studied was mainly due to the highest variance in grain yield per plant followed by

Table 2: Descriptive statistics for the estimated variables in 22 rice genotypes

Trait	Sum	Mean	Min.		Max.		Range	SD	CV
			Value	Genotypes	Value	Genotypes			
HD	2453	111.5	93	Giza177	135	IR 20	42	10.63	9.535
PH	2349	106.8	80	IR24	140	Arabi	60	16.35	15.31
PL	490.9	22.32	18.32	AgamyM1	27.1	IR 64	8.78	2.358	10.57
PNP	459	20.86	15	Nahda	28	Giza178	13	2.933	14.06
FGNP	2721	123.7	111	IR 20	139	Giza178	28	6.035	4.879
TGW	483.5	21.98	18.98	Bala	26.45	Giza177	7.47	1.740	7.918
GYP	661.0	30.05	24.63	Nahda	44.87	Giza178	20.24	6.222	20.71

The trait and statistics codes are HD: days to heading; PH: plant height; PL: panicle length; PNP: number of panicles per plant; FGNP: number of filled grains per panicle; TGW: 1000-grain weight; GYP: grain yield per plant.; SD: standard deviation; CV: coefficient of variance

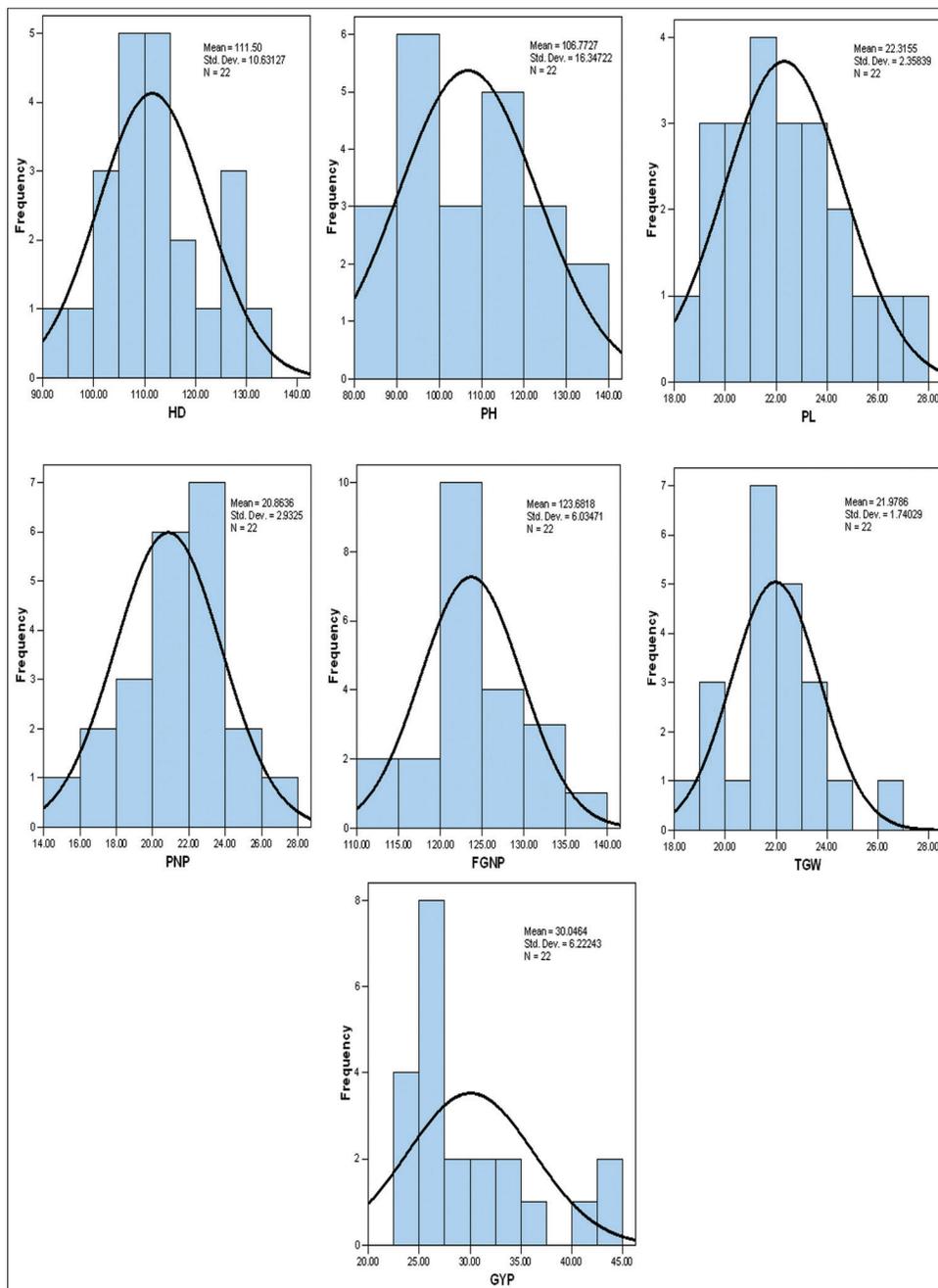


Figure 1: Frequency screen histogram of studied traits with the normal curve. The trait codes are HD: days to heading; PH: plant height; PL: panicle length; PNP: number of panicles per plant; FGNP: number of filled grains per panicle; TGW: 1000-grain weight; GYP: grain yield per plant

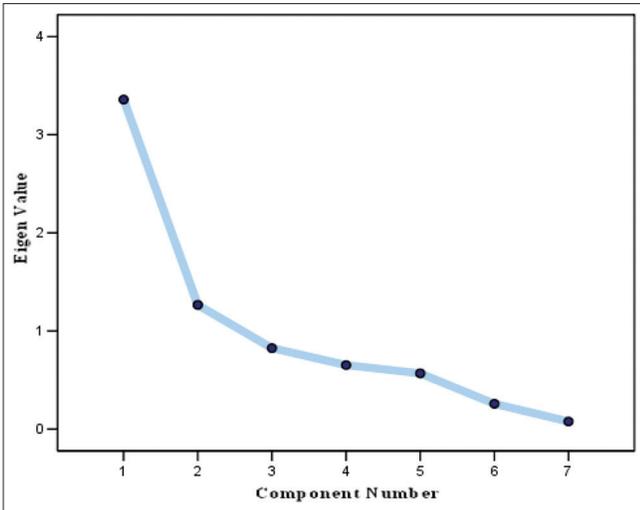


Figure 2: Scree plot diagram of Eigenvalues constructed on seven studied traits recorded in 22 rice genotypes

plant height then in the number of panicles per plant with a coefficient of variation reached (20.71, 15.31 and 14.06) for each of them. This agrees with the findings of Nachimuthu *et al.* (2014) who found that the coefficient of variation (CV) for both grain yield per plant and plant height was 23.19 and 18.82, respectively. We followed in this study (Clifford & Stephenson, 1975), which improved by Guei *et al.* (2005) who mentioned that (PC1, PC2, PC3) are usually more effective in symbolizing models of variability significantly between genotypes and that the traits correlated with them are more useful in distinguishing genotypes. According to this criterion, the first two main components were gathered, which means that these characteristics will play a major role in providing a wide variation for rice improvement. This agrees with Guei *et al.* (2005) who confirmed that traits in PC1, PC2 reflect variation in rice genotypes. Days to heading were found in one principle component with yield and features of its components, while plant height in another PC meaning that the days to heading had more effect in the variation than plant height. This is in

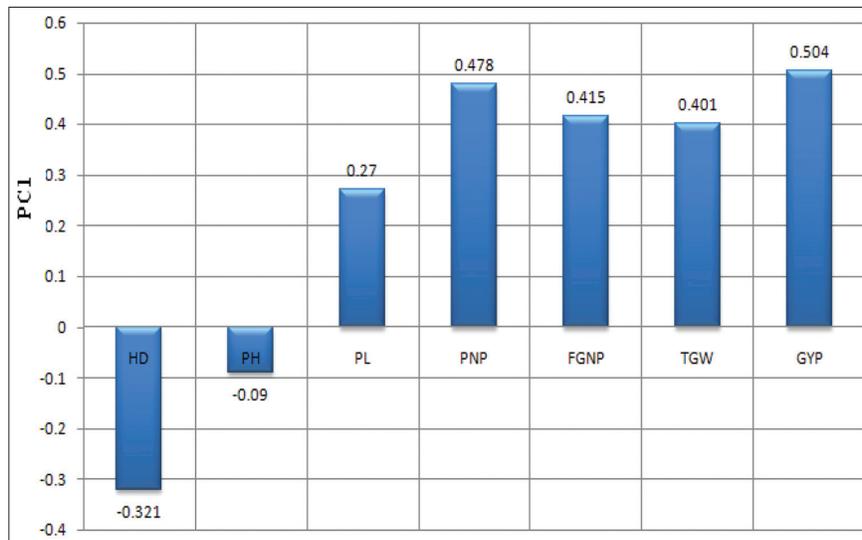


Figure 3: Contribution of studied traits in the first principle component (PC1). The trait codes are HD: days to heading; PH: plant height; PL: panicle length; PNP: number of panicles per plant; FGNP: number of filled grains per panicle; TGW: 1000-grain weight; GYP: grain yield per plant

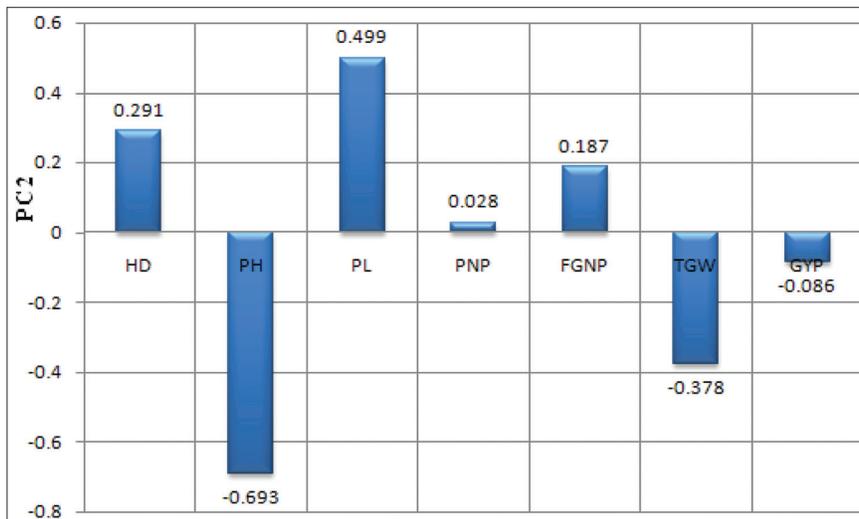


Figure 4: Contribution of studied traits in the second principal component (PC2). The trait codes are HD: days to heading; PH: plant height; PL: panicle length; PNP: number of panicles per plant; FGNP: number of filled grains per panicle; TGW: 1000-grain weight; GYP: grain yield per plant

Table 3: Eigenvalue, proportion and cumulative variation of analyzed components

Principal components	Eigen value	The proportion of variation %	Cumulative variation %
PC1	3.3575	48.0	48
PC2	1.2647	18.1	66
PC3	0.8246	11.8	77.8
PC4	0.6519	9.3	87.1
PC5	0.5674	8.1	95.2
PC6	0.2571	3.7	98.9
PC7	0.0768	1.1	100

Table 4: Principle components for studied traits in 22 rice genotypes

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7
HD	-0.321	0.291	0.512	-0.646	0.309	0.091	0.168
PH	-0.090	-0.693	0.595	0.283	0.062	0.267	0.044
PL	0.270	0.499	0.491	0.234	-0.592	0.174	0.046
PNP	0.478	0.028	-0.229	-0.094	0.254	0.730	0.335
FGNP	0.415	0.187	0.249	0.348	0.572	-0.470	0.249
TGW	0.401	-0.378	0.020	-0.475	-0.381	-0.370	0.434
GYP	0.504	-0.086	0.166	-0.304	0.115	0.002	-0.778

HD: days to heading; PH: plant height; PL: panicle length; PNP: number of panicles per plant; FGNP: number of filled grains per panicle; TGW: 1000-grain weight; GYP: grain yield per plant

Table 5: Traits in resulted principle components in 22 rice genotypes

PC1	PC2
Days to heading	Plant height
Number of panicles per plant	Panicle length
Number of filled grains per panicle	
1000-grain weight	
Grain yield per plant	

Table 6: Matrix of dissimilarity according to Euclidean square based on studied traits in rice genotypes

*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0																					
2	2.43	0																				
3	3.30	1.70	0																			
4	5.11	3.46	2.52	0																		
5	4.28	3.21	2.65	2.74	0																	
6	4.07	2.88	2.27	2.78	1.02	0																
7	3.86	2.74	2.46	2.77	3.92	3.77	0															
8	3.89	3.93	3.94	4.75	3.67	4.20	4.18	0														
9	3.46	3.32	2.62	3.48	3.34	3.62	3.16	2.41	0													
10	4.76	4.33	3.97	3.08	2.95	3.36	3.90	3.74	3.26	0												
11	2.79	2.89	3.08	3.94	4.32	4.39	3.13	3.52	2.18	3.68	0											
12	3.93	3.88	3.82	3.39	3.67	4.07	3.23	3.36	2.65	1.91	2.43	0										
13	2.87	3.05	3.23	3.44	3.35	3.42	2.66	3.72	2.99	2.59	2.61	1.70	0									
14	3.68	2.87	2.88	2.47	3.43	3.38	1.84	4.20	3.24	2.60	2.75	2.04	1.50	0								
15	3.65	3.05	2.87	2.41	3.06	3.17	2.53	3.80	2.70	1.83	2.38	1.40	1.47	1.05	0							
16	4.51	3.38	2.78	1.66	1.93	2.11	2.95	4.02	3.24	1.80	3.79	2.62	2.52	2.02	1.73	0						
17	4.29	3.52	3.50	2.81	3.82	4.09	2.26	3.59	3.00	2.46	2.69	1.57	2.20	1.48	1.53	2.46	0					
18	4.84	3.61	4.01	3.71	3.94	4.05	4.25	4.32	4.25	3.17	3.69	3.57	3.96	3.32	3.00	3.28	3.10	0				
19	4.12	3.92	4.19	3.78	3.55	3.79	3.71	3.74	3.73	1.63	3.43	1.92	2.10	2.25	1.87	2.49	2.21	2.77	0			
20	6.71	5.81	5.61	4.04	4.53	4.50	5.57	6.81	6.14	3.34	5.99	4.56	4.04	3.82	3.30	4.60	4.30	3.66	0			
21	7.71	6.17	5.86	4.41	4.57	4.61	6.23	6.80	6.66	4.38	7.00	5.91	6.11	5.33	5.13	3.99	5.47	4.08	4.84	3.20	0	
22	5.82	4.71	4.55	3.70	3.30	3.18	5.14	5.57	5.27	2.96	5.42	4.48	4.39	3.94	3.60	2.74	4.40	3.07	3.24	2.36	2.24	0

* Code numbers of varieties are listed in Table 1

agreement with Rashid *et al.* (2008) who found that days to heading and grain yield are located in PC1 and with Ogunbayo *et al.* (2005) who declared that plant height was found in PC2. Grain yield per plant and yield traits (1000-grain weight, number of filled grains per panicle and panicle number per plant) affect positively the PC1 on the contrary of days to heading that hurt the same PC1. This indicates the increase in grain yield per plant and yield traits (panicle number per plant, number of filled grains per panicle, 1000-grain weight) as a result of early heading. To be sure, improving a given yield trait will direct the improvement of other yield traits collected in the same PC as long as they have the same positive effect. In PC2, the plant height had a negative effect while the panicle length had a positive effect, suggesting that smaller plants have smaller panicles. These findings are consistent with other studies (Caldo

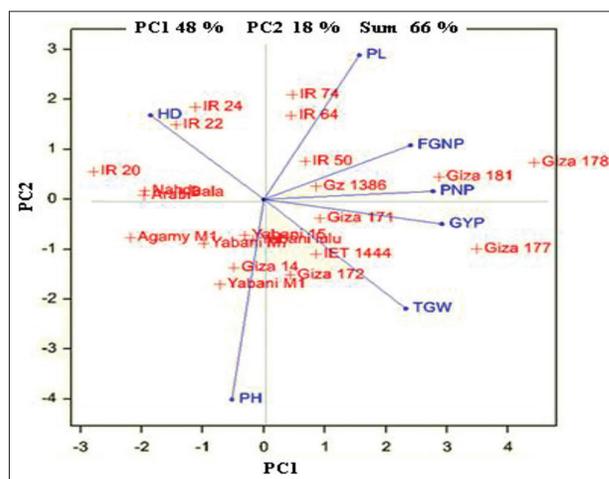


Figure 5: Biplot distribution of 22 rice genotypes and studied traits depending on principal component axes PC1 and PC2

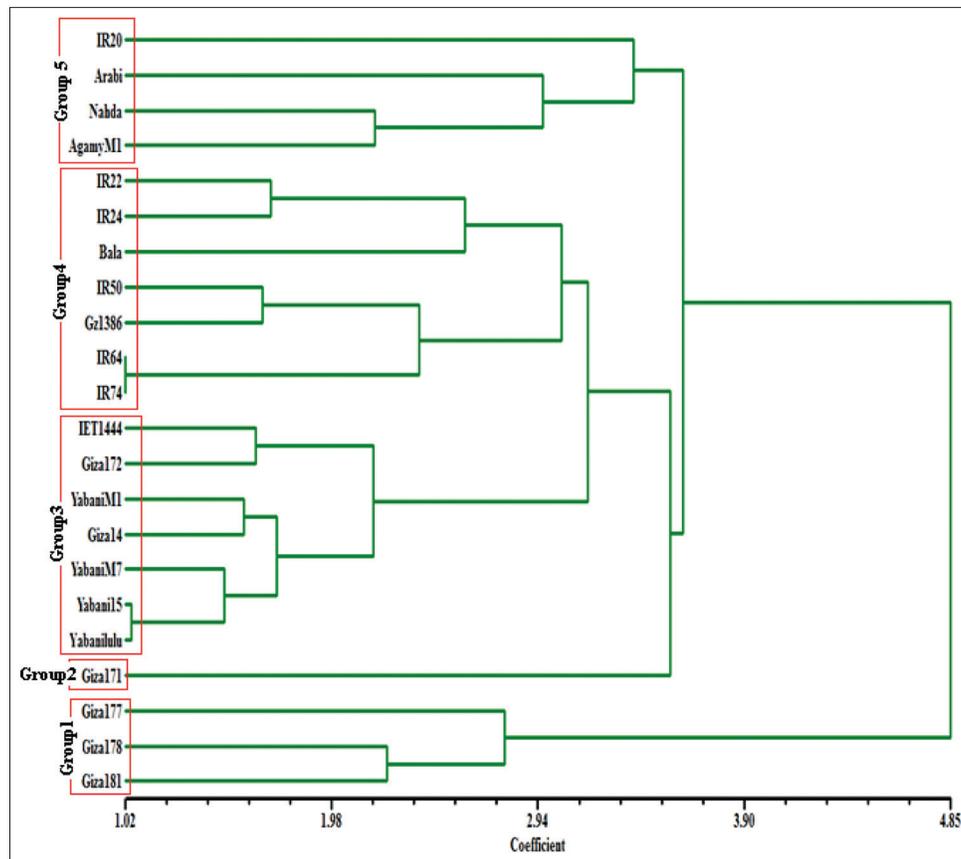


Figure 6: Dendrogram of 22 rice genotypes based on studied traits

et al., 1996; Lasalita-Zapico *et al.*, 2010; Maji & Shaibu, 2012; Sanni *et al.*, 2012; Sinha & Mishra, 2013; Chakravorty *et al.*, 2013; Nachimuthu *et al.*, 2014).

Concerning cluster results, it was evident that most varieties derived from a similar source of origin (except for the Indian variety IET 1444) or belong to the same classification collected in clusters that reflect the effect of the original environment and the variation of taxonomy within the rice genotypes. This is in agreement with Hien *et al.* (2007) who mentioned that genotypes found in one cluster from one ancestor and some genotypes from many sources of origin could be collected in the same cluster. This finding is also consistent with the previous study by Pagi *et al.* (2017) that this technique can effectively reveal the efficacy and stability of the genotype. Clustering according to the traits studied could be a useful tool when defining parents for the breeding program (Sanni *et al.*, 2012; Lei *et al.*, 2018; Bányai *et al.*, 2020).

CONCLUSION

The studied rice genotypes showed considerable variability for most of the traits that could be exploited for crop improvement. Analysis of clusters based on morphology traits suggested the genotypes could be grouped all together. These groupings are useful for breeders to classify possible genotypes that can be used as parents in breeding for some of the agronomic characteristics that have been studied.

Both the principal component and the cluster methods are effective tools for providing information on variability in agronomic traits. Use multiple trait profiles evaluated under the target environment may help boost improvements in selection in variable environments. So, when developing new rice varieties, it is recommended to consider the findings of this study.

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