Journal of Plant Physiology and Breeding
2018, 8(1):111-124 ISSN: 2008-5168

Genetic diversity of rice mutant genotypes using multivariate methods

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Received: November 30, 2015 Accepted: May 21, 2017
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Abstract

Genetic diversity among 64 rice genotypes including 56 M5 mutants and 8 check varieties was studied using multivariate analysis. The experimental materials were evaluated during growing season of 2013-14 at the experimental field of Rice Research Institute of Iran (RRII), Rasht, Iran. The field experiment was arranged in a randomized complete block design with three replications. With respect to the positive and significant partial regression coefficients and direct effects of number of panicles per plant and number of spikelet per panicle, it could be stated that increasing the amount of these traits will cause an increase in grain yield. The dendrogram from cluster analysis divided all 64 rice genotypes into three main groups. Maximum distance existed between clusters II and III, therefore the genotypes selected from these clusters could be used in hybridization programs. The first principal component included plant height, internode length, number of panicles per plant, panicle length, panicle weight, number of filled grains, grain productivity, 100 grain weight, grain width and grain yield. Second principal component included days to flowering, number of panicles per plant and number of unfilled grains. Two-dimensional plot based on the first two principal components indicated the existence of differences among rice genotypes under study. The presence of vast diversity among 64 rice genotypes by cluster analysis was also confirmed partly by the three-dimensional graph of three principal components. In conclusion, the studied genotypes represent a rich source of genetic diversity and could be useful in rice breeding programs. The crosses G53 × G39 and G62 × G11 will be useful for hybridization, because the parental genotypes were identified as being most divergent.

Keywords: Cluster analysis; Genetic diversity; Mutant; Principal component analysis; Regression analysis; Rice

Citation: Sharifi P, 2018. Genetic diversity of rice mutant genotypes using multivariate methods. Journal of Plant Physiology and Breeding 8(1): 111-124.

Introduction

Rice is a widely consumed food source for over half of the world’s population and feeds over half of the global population. Grain yield of rice is a quantitative trait influenced by other agronomic traits and environmental factors. Hence, rice breeders are interested in developing cultivars with improved yield and other desirable agronomic traits. Genetic variability for agronomic traits is the key component of breeding programs for broadening the gene pool of rice (Garg *et al.* 2011).

Mutagenesis is a useful technique to increase genetic variability in plant populations. Rice mutation breeding could be successful in obtaining new cultivars and broadening the genetic base of this crop. New varieties have been developed either directly after mutagenic treatment or through crosses involving mutant mutant lines (Domingo *et al.* 2007). Success of hybridization and subsequent selection of desirable segregates depends largely on the selection of parents with high genetic distance for different traits. The more diverse the parents, within overall limits of fitness, the higher chance of obtaining larger amount of heterotic expression in F_1 and wide range of variability in segregating generations (Singh and Bains 1968). Crossing between moderately diverse parents also has shown maximum heterosis (Chauhan and Singh 1982). Several reports have emphasized the importance of genetic variation in rice genotypes for selection of desirable parents (Bose and Pradhan 2005; Akter *et al.* 2009; Kiani 2013; Kim *et al.* 2016).

When a number of accessions are assessed for several characters of agronomic, morphological and physiological importance, multivariate analyses are useful for characterization, evaluation and classification of plant genetic resources. If measurements are bivariate rather than multivariate, the biological relationships among the characters may become different (Peeters and Martinelli 1989). Different types of multivariate analyses such as cluster and principal component analysis (PCA) have been used for identifying groups of genotypes with beneficial traits and to recognize relationships among genotypes (Cowen and Frey 1987). PCA is one of the popular clustering methods for exploratory data analysis requiring no knowledge of data structures (Goodacre *et al.* 2004). Some researchers used PCA for grouping of rice genotypes (Lasalita-Zapico *et al.* 2010; Worede *et al.* 2014; Khatun *et al.* 2015; Kim *et al.* 2016). There are also studies on rice diversity and clustering using agronomic traits (Sabesan *et al.* 2009; Chun-Hong *et al.* 2010; Kiani 2012; Rabara *et al.* 2014). Many researchers have used several multivariate techniques for explanation and classification of rice genotypes by morphological, biochemical or molecular variables (Habib *et al.* 2005; Sarawgi and Bisne 2007; Khalequzzaman *et al.* 2008; Sabesan *et al.* 2009; Elyasi *et al.* 2015).

The aims of the present study were to assess the genetic diversity in a collection of 56 rice mutant lines and eight check varieties using principal component and cluster analyses based on morphological, yield and quality traits and to identify the potential genotypes for future utilization in rice breeding programs. Multiple regression analysis was also performed to determine the effects of morphological and agronomic traits on grain yield. The novelty of present study is the use of mutant genotypes, which can be utiized for developing the new varieties directly or through their crosses as parents.

Material and Methods

Experimental field

The rice genotypes used in this study were four local landraces (Hashemi, Tarom, Alikazemi, Sangjo), four high yielding cultivars (Khazar, Fajr, H.P, Sephidrod) and 56 M5 mutant lines (Table 1) that were derived from above eight genotypes. H.P. is considered as a check variety having high quality. The genotypes were grown during the spring of 2013-14 at the research field of Rice Research Institute of Iran (RRII), Rasht, Iran. The field is situated between 49 ˚E longitude and 37 ˚N latitude at an altitude of 7 m below the sea level in the north of Iran. Seeds of each genotype were exposed to 300 Gy, {Gy= Grey (1 Grey= 10 krad)}, gamma rays from Cobalt 60 source at Iranian Agricultural Atomic Research Institute, Tehran, at equilibrium moisture content of eight percent during 2010. Following irradiation treatment of seeds of eight genotypes, M_1 generation was grown in the field. Surviving

Table 1. Name of the rice mutants and check varieties, their grain yield and cluster number.

Genotype number	Genotype code	Cluster number	GY (kg/ha)	Genotype number	Genotype code	Cluster number	Grain yield (kg/ha)
1	TM6-220-10-4-1	3	5599.50	33	HM5-250-12-1	3	4710.50
2	TM6-230-VE-7-5-1	3	5312.50	34	HM5-250-23-2	1	3750.50
3	TM6-230-VE-8-4-1	3	4991.00	35	HM5-250-26-1	1	3491.50
4	TM6-250-10-7-1	3	5078.50	36	HM5-250-34-1	1	3885.50
5	TM6-250-15-5-1	3	5790.50	37	HM5-250-41-1-E	1	3426.50
6	TM6-B-2-1-E	3	5341.50	38	HM5-250-41-2-E	1	3722.00
7	TM6-B-7-1	3	5861.00	39	HM5-250-42-1-E	1	4053.50
8	TM6-B-19-1-E	3	5243.50	40	HM5-300-E-1	3	5308.50
9	TM6-B-19-2	3	4913.00	41	HM5-300-3-1	3	5161.00
10	TM6-230-1-1	1	3980.00	42	HM5-300-3-2	1	4179.00
11	TM6-230-1-2	3	4313.00	43	HM5-300-5-1	1	3237.00
12	TM6-300-5-1	1	3560.00	44	HM5-300-5-3	1	3588.50
13	TM6-250-11-5	1	3425.50	45	HM5-300-6-E	2	2300.50
14	FM6-200-E6	3	5528.50	46	HM5-300-16-1	1	3660.50
15	FM6-200-E5	3	4837.50	47	KM5-200-4-2-E	1	3401.00
16	HM5-250-7-4-2	3	5173.50	48	KM5-200-17-1	1	4187.50
17	HM5-250-7-4-3	2	2739.50	49	KM5-200-19-1	1	4317.50
18	HM5-250-7-5-2	2	4221.50	50	K5-200-19-2	1	3602.50
19	HM5-250-7-7-2	1	3506.00	51	KM5-200-21-1	2	2269.00
20	HM5-250-25-1-3	1	3533.00	52	KM5-200-24-1	2	2807.00
21	HM5-250-26-1-E-1	1	3345.00	53	KM5-200-43-1-E	2	2857.50
22	HM5-250-42-1-E-1	1	3474.50	54	KM5-250-2-2-E	2	3109.50
23	HM5-250-E-1-1	2	5506.50	55	KM5-250-3-1-E	2	3050.50
24	HM5-250-E-3-2	2	5487.00	56	KM5-250-5-1	3	4909.50
25	HM5-250-E-5-2	3	4744.00	57	HASHEMI	1	3686.50
26	HM5-250-E-18-1	1	3828.00	58	KHAZAR	1	4227.50
27	HM5-250-2-4	2	3078.00	59	FAJR	3	5112.00
28	HM5-250-3-1	1	3422.50	60	TAROM	1	3646.50
29	HM5-250-5-1	2	2328.50	61	H.P	1	3756.00
30	HM5-250-6-6	1	3587.50	62	SEPIDROUD	1	4540.00
31	HM5-250-7-1	1	3889.50	63	ALI KAZEMI	1	3586.50
32	HM5-250-7-6	3	5621.50	64	SANGJO	1	3370.00

plants were selfed to get M_2 seeds and consequently were selfed for several times to obtain M_3, M_4 and M_5 generations.

The seeds of M_5 mutant lines and parental genotypes (as check varieties) were sown in a nursery on 10 May 2014, and 25-day old seedlings were transplanted to the field. The field experiment was arranged as a randomized complete block design with three replications, using 25 × 25 cm spacing, in four-row plots of three m long. Recommended doses of nitrogen (200 kg ha^{-1}) and phosphorous (100 kg ha^{-1}) were applied. One-third of nitrogen and whole phosphorous was applied as a basal dose at the time of transplanting the seedlings. The remaining two-third of nitrogen was applied in two split doses 30 days after transplanting and at the time of panicle initiation. Two hand weedings were done 30 and 50 days after transplanting. The permanent flood water level was maintained at 10 cm.

Measured characters

The following traits were measured based on Standard Evaluation System for Rice (SES, 2002): Grain yield (GY), plant height (PH), number of panicles per plant (NPP), panicle length (PL), number of spikelets per panicle (SNP), internode length (IL), number of filled grains per panicle (FG), grain productivity (GP), 100 grain weight (HGW), grain length (GL), grain width (GW), amylose content (AC) and gelatinization temperature (GT).

Statistical analyses

Principal component, multiple regression and cluster analyses were carried out by SPSS 17 (SPSS 2007). Stepwise regression was used in order to remove characteristics that are not effective on grain yield (Leilah and Al-Khateeb 2005). In order to identify the variation patterns of the mutant genotypes, principal component analysis (PCA) was conducted. Principal components (PCs) with eigenvalues greater than one were selected (Jeffers 1967). The first two and three principal components (PCs) were used to show two-dimensional and three-dimensional scatter plots, respectively. The scatter plots were drawn by Minitab 16 (Minitab 2010).

Clustering of genotypes into groups was also performed using Ward’s hierarchical algorithm based on squared Euclidean distance. The pseudo F statistic and the pseudo T2 statistic (Jobson 1992) were examined to determine the numbers of clusters. The generalized genetic distance between clusters was calculated using the generalized Mahalanobis D^2 statistics equation (Mahalanobis 1936):

$$D^2_{ij}= (x_i-x_j)\ s^{-1}\ (x_i-x_j)$$

where, D^2_{ij} is the squared distance between two clusters of i and j, X_i and X_j are the vectors of traits for the i^{th} and j^{th} genotypes, and S^{-1} is the inverse of pooled variance covariance matrix.

Results

Multiple regression analysis

Multiple regression analysis of variance indicated a highly significant regression model (p= 0.000). The grain yield was considered as dependent variable and number of panicles per plant, 100 grain weight, number of spikelets per panicle and grain length were considered as casual (independent) variables:

$$GY= 2395.56 + 168.83\ NPP + 486.98\ HGW + 300.91\ SNP - 410.57\ GL$$

The partial regression coefficients (direct effects) of number of panicles per plant (0.44), number of spikelets per panicle (0.29) and 100 grain weight (0.15) were positive and significant, while grain length had significant negative effect (-0.250) on grain yield.

Cluster analysis

At the distance of 5, 64 rice genotypes were clustered into three main groups (Figure 1). First cluster included 32 genotypes. All of the parental and check genotypes, except Fajr,7 and some mutants derived from Tarom, Hashemi and Khazar were included in this cluster. The second and third clusters contained 12 and 20 genotypes, respectively. The second cluster was comprised of other mutants derived from Hashemi and Khazar. The remaining mutants that were obtained from Tarom, Hashemi and Khazar and two mutants from Fajr, were included in the third cluster. The cluster means showed a wide range of variation for all of the traits under study (Table 2). Cluster I was characterized with high mean values for plant height, internode length, panicle length and grain width. Cluster II had high means for days to flowering, panicle weight, spikelet number per panicle, number of unfilled grains, grain length, gelatinization temperature and grain length to width ratio. Cluster III exhibited a low mean for number of unfilled grains and days to flowering. This cluster had also higher means for number of panicles per plant, number of filled grains, grain productivity, 100 grain weight, grain yield and amylose content. The highest grain yield was observed for TM6-B-7-1 and TM6-250-15-5-1 mutant lines with 5861 and 5790.5 kg.ha^{-1}, respectively and the lowest values were obtained for KM5-200-21-1 (2269 kg.ha^{-1}) and HM5-300-6-E (2300.5 kg.ha^{-1}) (Table 1).

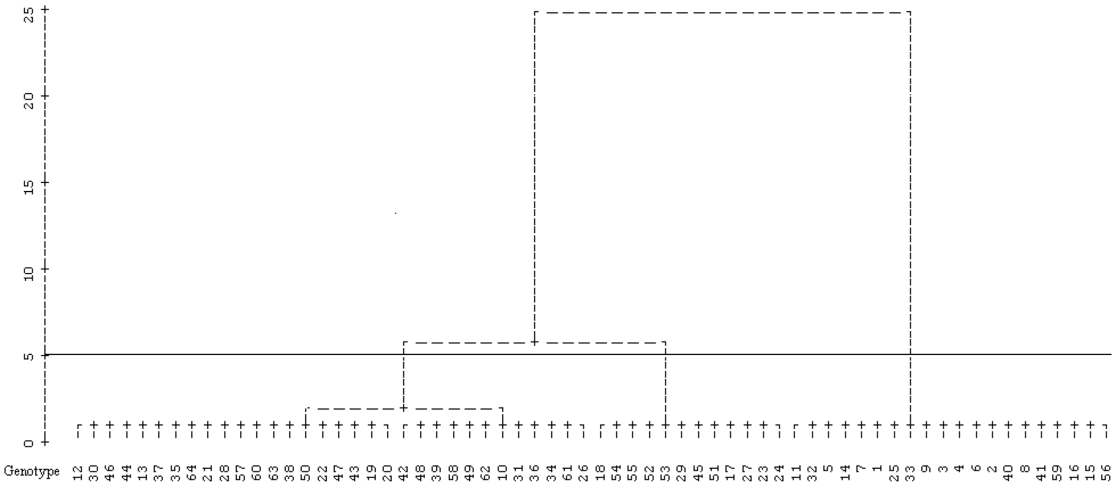

Figure 1. Grouping of 64 rice genotypes based on 17 morphological and quality traits using Euclidean distance and Ward clustering; the name of genotypes are given in Table 1.

Inter-cluster distances are presented in Table 2. The inter-cluster distances indicated the presence of diversity among groups. The inter-cluster distances ranged from 2.01 to 5.21 and indicated a high degree of genetic diversity among the genotypes. Cluster III showed highest genetic distance (5.21) from cluster II followed by cluster I (3.55). The minimum inter-cluster diversity was observed between clusters I and II (2.01) indicating the closeness of the genotypes of these clusters.

PCA

PCA was carried out to describe better the sources of variance among rice check and mutant genotypes. This procedure was also used for grouping of genotypes based on 17 studied traits. Five PCs accounted for most of the variability observed among the rice genotypes from different locations (Table 4). First principal component (PC1) accounted for 32.8% of the variation and was loaded on plant height (0.348), internode length (0.228), number of panicles per plant

(0.032), panicle length (0.111), panicle weight (0.061), number of filled grains (0.016), grain productivity (0.367), 100 grain weight (0.293), grain width (0.113) and grain yield (0.068). Other characters contributed negatively to the first component. Second principal component (PC2) accounted for 14.1% of the variation and was loaded on days to flowering (0.014), number of panicles per plant (0.243) and number of unfilled grains (0.033). The third principal component (PC3) accounted for 10.4% of the total variation. Grain length to width ratio contributed the highest (0.519), while spikelet number per panicle (0.045) contributed the lowest to PC3. Other characters that contributed to the variation of this component included plant height (0.215), internode length (0.314), number of panicles per plant (0.269), panicle length (0.226), number of filled grains (0.116), grain productivity (0.124), grain yield (0.055) and gelatinization temperature (0.143). The fourth principal component (PC4) contributed 9.4% to the total variation. Characters that included in this component were days to flowering (0.081), plant height (0.017), internode length (0.144), panicle length (0.242), grain productivity (0.063), grain length (0.148), grain width (0.098) and grain length to width ratio (0.083). The fifth principal component (PC5) accounted for 6.9% of the total variation. Number of filled grains had the highest load (0.356), followed by panicle weight (0.075), spikelet number per panicle (0.077), grain productivity

Table 2. Means of clusters with respect to different rice characters.

Cluster	I	II	III
DF	83.97±0.67	86.00±2.04	83.15±1.05
PH (cm)	122.56±1.52	111.81±4.29	118.74±2.68
IL (cm)	45.88±0.82	40.22±1.35	42.08±0.96
NPP	12.65±0.37	11.82±0.58	14.07±0.49
PL (cm)	27.00±0.32	26.86±0.70	26.39±0.38
PW (g)	2.28±0.05	2.45±0.15	2.38±0.07
SNP	8.29±0.14	8.51±0.21	8.33±0.20
FG	94.63±2.21	92.03±4.36	94.88±3.27
UnG	12.28±1.59	17.19±4.01	12.18±2.38
GP	88.93±1.28	85.07±3.11	89.31±1.64
HGW (g)	2.60±0.04	2.47±0.06	2.63±0.07
GL (mm)	10.46±0.08	10.48±0.13	10.12±0.14
GW (mm)	2.66±0.12	2.51±0.04	2.54±0.06
GL/W	4.14±0.06	4.19±0.07	4.05±0.15
GY (kg/ha)	3714.61±47.63	3312.92±330.74	5177.53±86.92
AC (%)	22.11±0.17	22.18±0.34	22.31±0.16
GT (score)	4.29±0.08	4.42±0.10	4.28±0.05

DF: days to flowering; PH: plant height; IL: internode length; GY: grain yield; NPP: number of panicles per plant; PL: panicle length; PW: panicle weight; SNP: spikelet number per panicle; FG: number of filled grains; UnG: number of unfilled grains; GP: grain productivity; HGW: 100 grain weight; GL: grain length; GW: grain width; AC: amylose content; GT: gelatinization temperature.

Table 3. Inter-cluster distances using Mahalanobis D^2 for the 64 rice genotypes under study.

Cluster	I	II	III
I	0	2.01	3.55
II		0	5.21
III			0

Table 4. Results of principle component analysis of 64 rice genotypes.

Traits	Principal components				
	1	2	3	4	5
DF	-0.339	0.014	-0.210	0.081	-0.146
PH	0.348	-0.079	0.215	0.017	-0.138
IL	0.228	-0.054	0.314	0.144	-0.435
NPP	0.032	0.243	0.269	-0.542	-0.078
PL	0.111	-0.137	0.226	0.242	-0.343
PW (g)	0.061	-0.538	-0.152	-0.161	0.075
SNP	-0.208	-0.459	0.045	-0.080	0.077
FG	0.016	-0.504	0.116	0.001	0.356
UnG	-0.381	0.033	-0.100	-0.053	-0.088
GP	0.367	-0.136	0.124	0.063	0.173
HGW	0.293	-0.126	-0.167	-0.200	-0.196
GL	-0.304	-0.204	0.278	0.148	-0.105
GW	0.113	-0.179	-0.466	0.098	-0.320
GL/W	-0.258	-0.004	0.519	0.083	0.144
GY	0.068	-0.046	0.055	-0.656	0.025
AC	-0.278	-0.068	-0.089	-0.190	-0.181
GT	-0.182	-0.208	0.143	-0.189	-0.515
Eigenvalues	5.58	2.4013	1.7755	1.6013	1.1653
% of variance	32.8	14.1	10.4	9.4	6.9
% of cumulative variance	32.8	47.0	57.4	66.8	73.7

DF: days to flowering; PH: plant height; IL: internode length; GY: grain yield; NPP: number of panicles per plant; PL: panicle length; PW: panicle weight; SNP: spikelet number per panicle; FG: number of filled grains; UnG: number of unfilled grains; GP: grain productivity; HGW: 100 grain weight; GL: grain length; GW: grain width; GL/W: grain length to width ratio; AC: amylose content; GT: gelatinization temperature.

(0.173), grain length to width ratio (0.144) and grain yield (0.025). Amylose content had weak or no discriminatory power. Cumulatively, these five principal components determined 73.7% of the total variation.

Diversity of evaluated genotypes in terms of 17 traits could be explanted with few numbers of new components that have no correlation with each other. This matter can be presented in two or three dimensional spaces (Moghaddam *et al.* 1994). As mentioned before, the first three components accounted for 32.8%, 14.1% and 10.4% of the total variation, respectively. Two-dimensional plot based on first two principal components (Figure 2) showed the diversity of rice genotypes under study on the scatter plot.

This plot was divided into four categories A, B, C, D) on the basis of the two first principal components. Group A consisted of maximum number of genotypes (21) with positive values of the first and second principal components. Genotypes which were placed in the area B, such as G10, G52, G53, G54 and G56 showed negative values of PC1 and positive values of PC2. Genotypes located in C had negative values of first the two principal components and included G14, G15, G47, G48, G51, G58, G59 and G62. On the other hand, genotypes that were placed in D area, had positive and negative values of the first and second principal components, respectively which included 21 genotypes. Genotypes of this area were characterized with important traits such as grain yield, panicle length, panicle weight, grain width, 100 grain weight, grain productivity, internode length and plant height. The dispersion of genotypes in four sections of 2-D plot indicated the presence of fair amount of genetic diversity among the mutant genotypes under investigation.

The cosine of the angle between the vectors of any two traits approximates the correlation coefficient between the traits (Yan and Kang 2003). Thus, the scatter plot suggested close associations among number of unfilled grains, days to flowering, grain length to width ratio, amylose content, grain length, gelatinization temperature and spikelet number per panicle. There were positive associations among grain yield, panicle length, grain width 100 grain weight, grain productivity, internode length and plant height. Grain productivity was positively associated with panicle weight. Grain yield showed a positive relationship with plant height, internode length, grain productivity, 100 grain weight, panicle length and grain width (Figure 2). The genotypes that were located on the right side of the two-dimensional plot, had larger grain yield and associated traits. On the other hand, the genotypes that were located on the left side, had greater values for number of unfilled grains, days to flowering, grain length to width ratio, amylose content, grain length, gelatinization temperature and spikelet number per panicle. Some discrepancies between the two-dimensional plot predictions and original data were expected because the first two PCs accounted for 46.9% of the total variation.

The positions of 64 genotypes on the three-dimensional (3-D) scatter plot with respect to the first three principal components are shown showed in Figure 3. The samples fell into three groups. The first group consisted of one genotype (G62). The second group included 14 members (G14, G15, G47, G48, G49, G50, G51, G52, G53, G54, G55, G56, G58, G59). Group III included the maximum number of genotypes (49), which consisted of 76.56% of all genotypes under study, mostly originating from the Hashemi and Tarom varieties (Figure 3).

Discussion

Stepwise regression is a semi-automated process of building a model by successively adding or removing variables based on the t-statistics of

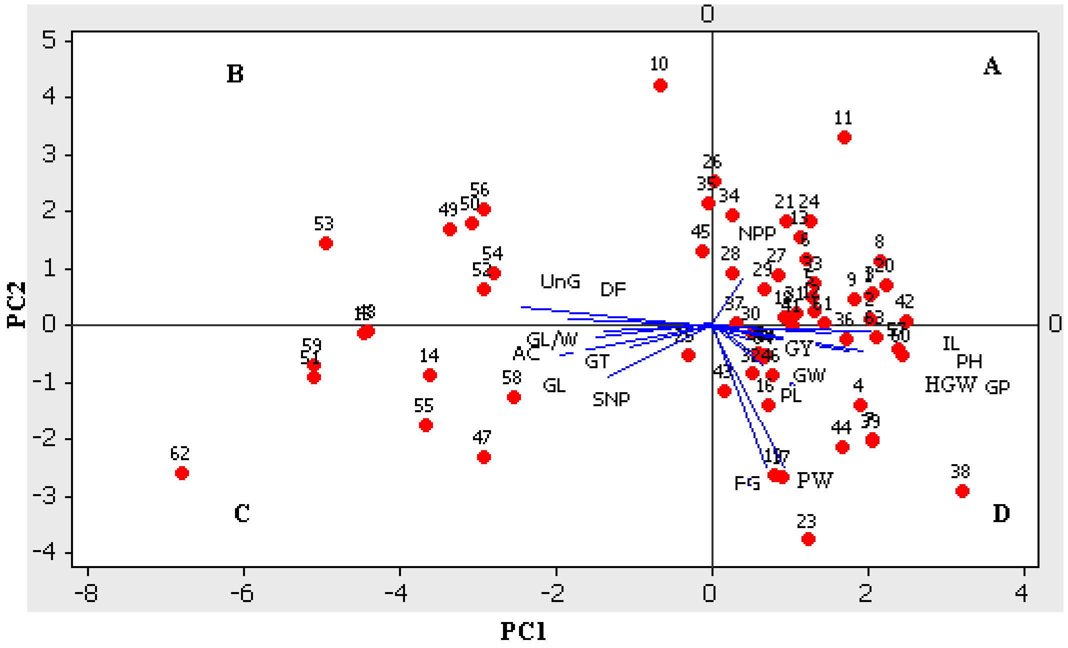

Figure 2. Two-dimensional graph of 64 rice genotypes based on morphological, physiological, yield and quality characters using principal component analysis.

their estimated coefficients (Leilah and Al-Khateeb 2005). According to regression analysis number of panicles per plant, 100 grain weight, number of spikelets per panicle and grain length contributed greatly to the rice grain yield and could be used as selection criteria in rice breeding programs. With respect to the positive and significant regression coefficients of number of panicles per plant and number of spikelets per panicle, it could be stated that increasing the amount of these traits will cause an increase in the grain yield. These results are in agreement with findings by Sabouri *et al.* (2008), Sharifi *et al.* (2013) and Li *et al.* (2014).

Cluster analysis of the rice genotypes indicated that genotypes in each cluster had some specific characteristics. Considering the importance of genetic distance, relative contributions of characters to total divergence and yield potential of genotypes, the present investigation suggests that genotypes could be selected from cluster I for plant height, internode length, panicle length and grain width, cluster II for slender grains, earliness, panicle weight, spikelet number per panicle and gelatinization temperature, and cluster III for 100 grain weight, number of panicles per plant, number of filled grains, grain productivity, grain yield and amylose content. Selection of genotypes with desired traits could be made from these clusters to be used in cross breeding programs to attain higher hybrid vigor. Regarding to inter-cluster distance, cluster

III had highest genetic distance from other clusters. Two high yielding mutants, G7 (TM6-B-7-1) and G5 (TM6-250-15-5-1), belonged to cluster III. It appears that crosses between the genotypes of cluster II and cluster III would exhibit higher heterosis for yield potential and could be used in the hybridization programs, since hybridization between divergent parents is likely to produce wider variability and transgressive segregation. Similarly, Habib *et al. (*2005) and Khalequzzaman *et al.* (2008) reported large differences between clusters of rice genotypes under study. Ahmadikhah *et al.* (2008) and Khatun *et al.* (2015) grouped rice varieties into four clusters based on morphological characters. Rahman *et al.* (2011) divided 21 rice varieties into five clusters based on 14 physiological traits.

The principal component analysis in our study revealed that the first five principal components accounted for 73.7% of the total variation. The genotypes closer to each other had little or no differences with respect to traits under study. Genotypes far from origin exhibited more variability for quantitative traits and could be utilized as diverse parents in broadening the genetic base of rice through hybridization (Yan and Kang 2003). Different characters contributed differently to the total variation as indicated by their weight and loading on the different principal axes. Khatun *et al.* (2015) selected the first three principal components as the most important components to reflect the variation pattern among genotypes and suggested to use the characters highly associated with these, in differentiating the accessions under study.

The clustering of scores among PC axes suggested the existence of some relationships among individuals within a cluster. The main components which discriminated between genotypes for PC1 (X-axis) were plant height, internode length, number of panicles per plant, panicle length, panicle weight, number of filled grains, grain productivity, 100 grain weight, grain width and grain yield, and for PC2 (Y-axis) were day to flowering, number of panicles per plant and number of unfilled grains (Figure 2). Generally, G62 had low ratings for most of the components. On the other hand, G11 had high ratings for the two components.

The 64 rice genotypes were clustered into three groups based on the dendrograms of cluster analysis and three- dimensional PCA graph. By grouping the genotypes on the basis of three first components, it is seen that genotypes get special position on the basis of weights of traits in each component. The results of PCA partly supported the findings from cluster analysis that there were large differences among 64 rice. Khatun *et al.* (2015) also reported a similar clustering pattern from hierarchical cluster analysis and principal component analysis for 24 rice genotypes under investigation.

Diversity analysis can help in the selection of genetically divergent parents, which can produce new recombinants with suitable traits when crossed to each other. Several reports have emphasized the importance of genetic variation for selection of desirable parents (Bose and Pradhan 2005; Akter *et al.* 2009; Kiani 2012; Mazid *et al.* 2013). The results of present study

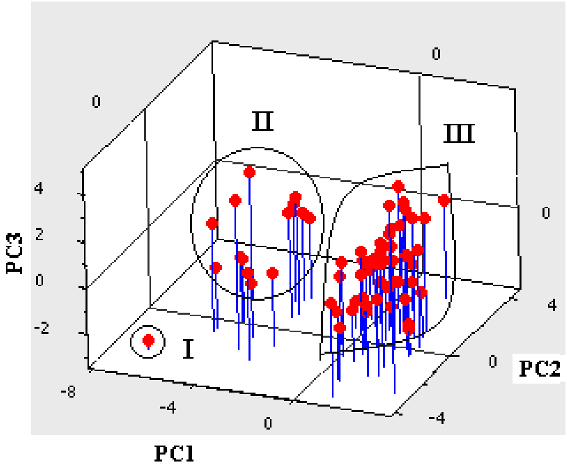

Figure 3. Three- dimensional graph of 64 rice genotypes based on morphological, physiological, yield and quality characters using principal component analysis.

indicated that there was high genetic diversity for morphological, agronomical and quality traits in rice check varieties and mutant lines. The genotypes represent a rich source of genetic diversity and could be useful in rice breeding programs.

The clustering pattern revealed that the parental genotypes from different sources and mutants derived from different parental genotypes clustered together, indicating that there was no association between original distribution of the genotypes and genetic divergence. The possible reason for grouping of the genotypes of different origin in one cluster could be the free exchange of germplasm among the breeders of different regions, or unidirectional selection practiced by breeders in tailoring the promising cultivars for different regions (Verma and Mehta 1976).

Conclusion

Cluster analysis and three-dimensional PCA classified the genotypes into three groups based on morphological, agronomical and quality traits. The present results suggested that induced mutation is governed by the genetic architecture of the material used. Stepwise regression indicated that number of panicles per plant, spikelet number per panicle and 100 grain weight had the highest impact on grain yield. The crosses involving parents belonging to maximum divergent clusters were expected to manifest wide genetic variability. Thus, a higher heterosis could be produced from the crosses between genetically distant parents. The results of PCA were mainly comparable to the cluster analysis. Based on our results, the genotypes in cluster II that were characterized with panicle weight, spikelet number per panicle, number of unfilled grains, grain length, grain length to width ratio and gelatinization temperature and the genotypes within cluster III that were distinguished for lower values for number of unfilled grains and days to flowering and higher value for number of panicles per plant, number of filled grains, grain productivity, 100 grain weight, grain yield and amylose content might be selected to be utilized in future breeding programs. The genotypes with economically important traits could be combined to pool the desired traits in one line with the broad genetic base. The crosses G53 × G39 and G62 × G11 would be useful in the hybridization programs because the pairs were identified as being the most divergent.

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تنوع ژنتیکی در ژنوتیپ‌های موتانت برنج با استفاده از برخی از تجزیه‌های چند متغیره

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چکیده

تنوع ژنتیکی ۶۴ ژنوتیپ برنج از جمله ۵۶ ژنوتیپ جهش‌یافته نسل M5 و ۸ رقم شاهد با استفاده از تجزیه چند متغیره به صورت طرح بلوک‌های کامل تصادفی با سه تکرار بررسی شد. مواد آزمایشی در فصل رشد ۱۳۹۳-۱۳۹۲ در مزرعه آزمایشی موسسه تحقیقات برنج ایران (RRII) در رشت، ارزیابی شدند. با توجه به ضرایب رگرسیون جزء و اثرات مستقیم مثبت و معنی‌دار تعداد خوشه در بوته و تعداد خوشه‌چه در خوشه می‌توان اظهار داشت که افزایش این صفات منجر به افزایش عملکرد دانه خواهد شد. دندروگرام تجزیه خوشه‌ای ۶۴ ژنوتیپ برنج را در سه گروه اصلی قرار داد. حداکثر فاصله بین دسته دوم و سوم وجود داشت. بنابراین، ژنوتیپ‌های انتخاب شده از این گروه‌ها می‌توانند در برنامه‌های دورگ‌گیری مورد استفاده قرار گیرند. در تجزیه به مولفه‌های اصلی، اولین مؤلفه اصلی صفات ارتفاع بوته، طول میانگره، تعداد خوشه در بوته، طول خوشه، وزن خوشه، درصد باروری دانه، وزن صد دانه، عرض دانه و عملکرد دانه را در بر گرفت. مؤلفه اصلی دوم شامل تعداد روز تا گل‌دهی، تعداد خوشه‌چه در خوشه و تعداد دانه‌های پوک در خوشه بود. نمودار دو بعدی بر اساس دو مؤلفه اصلی اول اختلاف‌های ژنتیکی بین ژنوتیپ‌ها را نشان داد. تفاوت‌های زیاد بین ۶۴ ژنوتیپ با استفاده از تجزیه کلاستر، توسط نمودار سه بعدی حاصل از سه مؤلفه اصلی اول نیز تا حدودی تأیید شد. در مجموع، ژنوتیپ‌های مورد مطالعه یک منبع غنی از تنوع ژنتیکی را نشان دادند که می‌توانند برای برنامه‌های اصلاح برنج مفید باشند. تلاقی‌های ۵۳ × ۳۹ و ۶۲ × ۱۱ با توجه به تفاوت زیاد بین والدهای آن‌ها، برای دورگ‌گیری مناسب هستند.

واژه‌های کلیدی: برنج؛ تجزیه خوشه‌ای؛ تجزیه رگرسیون؛ تجزیه به مؤلفه‌های اصلی؛ تنوع ژنتیکی؛ موتانت