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Grouping of rice mutant lines based on morphological and agronomical traits under different moisture conditions using multivariate statistical methods

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Abstract

To study the morphological and agronomical traits and determine heterotic patterns at the reproductive stage of 96 mutant rice genotypes (M_2), two separate experiments were carried out under drought stress and flooding conditions at Gonbad Kavous University, Iran, in 2016 using a randomized complete block design with three replications. A factor analysis was conducted to reduce the number of variables to fewer independent factors, and three and four factors were identified under the flooding and drought conditions, explaining 68.3% and 76.05% of the total variance, respectively. Cluster analysis using the selected factors by Ward's minimum variance method and Euclidean distance led to the grouping of the mutant rice genotypes. The best cutting points of the dendrograms were determined by the discriminant function analysis and four clusters were identified at both irrigation conditions, which were also significantly different. Genotypes of the first and second clusters had the highest yield and its components under the flooding condition. In contrast, under the drought condition, genotypes of the third and fourth clusters were higher-yielding and more tolerant to the drought stress. Mutants No. 4, 18, 25, 28, 29, 43, 44, 48, 53, 72, 79, 88, 90, 91, 94 and 95 were present in the first and second clusters of the flooding condition, and in the third and fourth clusters of the drought-stress condition, indicating their superiority over other mutants in terms of grain yield and drought tolerance. The results obtained from this study can be used for selecting suitable parents for hybridization from different clusters to produce new rice cultivars.

Keywords: Cluster analysis; Discriminant function analysis; Drought; Factor analysis; Mutant; Rice.

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Introduction

Rice, after wheat, is considered as the most important food crop in the world. In 2018, 580 thousand hectares went under rice cultivation in Iran. In the same year, average rice hulls production in Iran was 3431 kg/ha (FAO 2018). Water scarcity and drought stress are the leading issues limiting rice production (Pandey and Shukla 2015). Increasing tolerance to salinity and drought stress by breeding tolerant cultivars is regarded as an economic solution to overcome food shortages in the world. One of the most important breeding tools for improving desirable characteristics amongst commercial cultivars is mutation induction (Senthamizh Selvi *et al.* 2007). Various chemical and physical mutagens are used to produce mutant genotypes. Sometimes, due to the severity of breeding actions followed by severe erosions, a specific trait does not exist in plants. In such a case, the only possible way to achieve diversity and improve genetic materials, is employing a mutation induced by chemical or physical (nuclear energy) agents. Therefore, mutation is known as a process that leads to an increase in genetic diversity (Yilmaz and Boydak 2006). Most of the mutants are derived and introduced by using irradiation methods (Brown and Caligari 2008.). Accordingly, more than 2250 mutant varieties were introduced in the world (Ahloowalia *et al.* 2004). Among the radiation-induced mutant varieties, 64% were produced by applying gamma ray (Maluszynski *et al.* 2000). Using gamma rays, researchers have significantly reduced the height of rice seeds and increased their yield. The results of such studies have led to an introduction of two rice varieties (Arefii and Norozi 2008). Haris *et al.* (2013) treated two local rice cultivars with gamma rays at 200 and 300 Gray and produced some dwarf and early maturing mutants; however, the filled grain percentage was sharply dropped. On the other hand, while the number of tillers was increased, no significant difference was found in panicle length compared to the control group. In another study, different doses of gamma radiation were imposed on the rice cultivar Sakha-105. Studying morphological traits of the first and second generations showed that increasing the radiation dosage led to an increase in genetic diversity (El-Degwy 2013). Marn Oo *et al.* (2015) produced mutant genotypes by irradiating MK rice cultivars with gamma ray at 300 Gray. Among the mutants, the leaf relative water content of two mutant genotypes (i.e., MK-D-2 and MK-D-3) was higher than the control group. They concluded that the drought tolerance of these mutant genotypes was higher than the parent cultivar. In recent years, rice mutants have been produced and introduced to increase 1000-grain weight (Jeng *et al.* 2006), reducing plant height, creating early maturity and increasing yield (Domingo *et al.* 2007), improving grain quality (Luzi-kihupi *et al.* 2009) and promoting drought stress tolerance (Cha-um *et al.* 2012). Some efforts have also been made in Iran to breed plants with the use of gamma-ray-induced mutations. For example, Babaei *et al.* (2010) treated three rice varieties, Tarom-Hashemi, Sange-Tarom and Nemat, with gamma ray at 200, 250, 350 and 450 Gray. The 250 and 350 Gray dosages created a wide variation in several traits including panicle sterility, plant height, 1000-grain weight and fertile tillers.

Given the importance of producing drought-tolerant cultivars, this study aimed to evaluate and group rice mutants based on important agronomic traits to identify proper mutants under flooding and drought stress conditions using multivariate statistical methods.

Material and Methods

An initial cross between two rice cultivars of Ahlami Tarom and Sepidrood was conducted at Gonbad Kavous University in 2008 (Sabouri *et al.* 2008a; Sabouri *et al.* 2008b). Ahlami Tarom is a low yielding rice cultivar that is tolerant to drought stress and Sepidrood is a high yielding rice cultivar that is sensitive to drought stress at the reproductive stage (Sabouri *et al.* 2011; Kazerani *et al.* 2018). Segregating generations till the ninth generation were developed in the research field of the Faculty of Agriculture and Natural Resources of Gonbad Kavous University, Iran (54° 45' E longitude and 96° 36' N-W latitude, at an elevation of 52 m above sea level). Although several genotypes were found to be more tolerant to drought in the ninth generation, there was a need for maximizing genetic diversity for drought tolerance. Hence, gamma irradiation (at 250 Gray) was performed on 300 genotypes of the ninth generation in Karaj Nuclear Research Center for Agriculture and Medicine, Iran. The first and second mutant generations were screened to identify superior mutants. To perform initial screening, the parent genotypes and the mutant population (M_1) were planted in the research field of the Faculty of Agriculture and Natural Resources of Gonbad Kavous University, Iran. Transplanting of 300 mutant genotypes was carried out with a single seedling at a 25 × 25 cm spacing. Next to the M_1 field, the parent genotypes were planted as the control plants to provide a chance for making phenotypic comparisons and to estimate the percentage of mutation in the population. At flowering and maturity, mutant plants which looked better than the parents in terms of morphological and agronomical characteristics, and other important traits were selected and their traits were measured. After maturity, the seeds from the main tillers of 134 selected mutant plants were harvested to form the M_2 population. For performing a secondary screening, the mutant population (M_2) and their parent genotypes were plants in the five-liter pots in the greenhouse in May 2016. The seedlings were sprayed with the NPK solution (four kilograms of the NPK fertilizer solved in 1000 liters of water) three times, i.e., 10, 15 and 25 days after transplanting (Kazerani *et al.* 2019a; Kazerani *et al.* 2019b). Afterward, 134 mutant plants were evaluated in two separate experiments under the drought stress and flooding conditions using a randomized complete block design with three replications. Each experimental unit consisted of five rows of two-meters long with the planting pattern of 25 × 25 cm. The parent genotypes were again planted next to the M_2 generation as the control plants to provide a chance for performing the secondary screening. Using the results obtained from the soil analysis (Table 1), 150 kg/ha of urea was applied to supply the fertilizer need of the mutant genotypes. Half of this amount was applied at the planting time and the other half was used at the tillering stage. Additionally, 100 kg/ha of triple superphosphate fertilizer was applied at the planting time. During the growing season, 96 promising M_2 mutants were identified and their traits of interest were measured. During the growth period, weeding was done and the plants were sprayed with bentazon. Regarding the changes in temperature and rainfall in 2016 in Gonbad Kavous (Figure 1) and taking 150 to 200 dry days into consideration, the experimental site was considered as a region with the warm and dry Mediterranean climate. In this area, most celestial precipitations occurred during the cold season and summer was relatively warm and dry. Moreover, the surface water level and water depth in this region were 7.7 and 38.5 meters, respectively (Kazerani *et al.* 2018).

Table 1. Results of the soil analysis in the experimental site.

Soil texture			Absorbable K	Absorbable P	Total N	Organic carbon	Neutralizing materials	pH	Electrical conductance	Saturation of soil
Sand	Silt	Clay								
10 (%)	60 (%)	30 (%)	505 (ppm)	28 (ppm)	0.13 (%)	1.33 (%)	9 (%)	7.5	2.9 dsm^{-1}	45.9 (%)

Figure 1. The pattern of temperature and precipitation change in 2016 cropping season in Gonbad Kavous, Iran.

In the drought-stress experiment, irrigation was discontinued from 40 days after transplanting (the stage with maximum tillering) to the end of the growth period. Samples were taken on 50, 60, 70, 80 and 90 days after planting in the drought stress condition and the soil gravimetric wetness were estimated to be 0.32, 0.24, 0.18, 0.08 and 0.04 (kg/kg).

Since rice is sensitive to drought stress in its reproductive stage (Yoshida 1981), stress was applied after the vegetative stage (end of tillering), which occurred 40 days after transplanting. To prevent any water penetrations from the margins of the field, a distance of two meters was considered between the two experiments, and also, a plastic barrier with the depth of one meter was mounted around the drought- stress experiment. At the time of applying stress, in a period starting from July 10, 2015, to September 10, 2016, there was 33.6 and 36.1 mm rainfall, respectively.

To measure grain yield at maturity, 1.5 square meters was harvested and grain yield was calculated with a moisture content of 14% per hectare under both drought stress and flooding conditions. The moisture content of the grains was measured based on standard No. 2705 (ISIRI). Other traits were measured as follows, based on the Standard Evaluation System for Rice from International Rice Research Institute (IRRI 2013): leaf frying and leaf rolling (Table 2), plant height (the height of the highest tiller from the crown area on the soil surface to the tip of the panicle excluding the awns), number of tillers from 10 random plants from each plot, panicle length (the length of 10 main panicles in each plot from the nod below a panicle to the end of the panicle excluding the awns, panicle exsertion (the exsertion of panicles from the top of a flag leaf to the nod below the panicle at maturity), number of spikelets (number of spikelets from the main panicles of 10 plants per plot), 1000-grain weight (1000-grain weight from the main panicles of 10 plants per plot), the number of filled grains (number of filled grains in the main panicles of 10 random plants per plot, after the grains were completely filled) and number of days to flowering (days from planting to 50% of flowering). Fertility percentage was obtained by dividing the number of

Table 2. Codes related to the degree of leaf rolling and drying under drought stress at the vegetative stage of rice mutant lines.

Code	Leaf rolling	Leaf drying
0	Leaves healthy	No symptoms
1	Leaves start to fold (shallow)	Slight tip drying
3	Leaves folding (deep V-shape)	Tip drying extended up to 25% of the leaf length in most leaves
5	Leaves fully cupped (U-shape)	Between 25% to 50% of all leaves fully dried
7	Leaf margins touching (O-shape)	More than 67% of all leaves fully dried
9	Leaves tightly rolled	All plants dead

filled grains by the total number of grains (De Datta *et al.* 1988). Factor analysis was performed using the principal component analysis method and varimax factor rotation (Manly 2005). Bartlett's test of sphericity (Bartlett 1951) and KMO[1] statistic (Hair *et al.* 2014) were used to check the existence of correlation among variables and ensure that the data were fit for carrying out the factor analysis. To validate the data, they were first divided into two random groups. Then, factor analysis was separately performed for each group. The similar results obtained by both groups showed that changing people did not affect the results. Therefore, factor analysis was carried out for the whole data. After factor analysis, the selected factors were used for grouping the mutant genotypes by cluster analysis using Ward's algorithm and Euclidean distance at both moisture conditions (Romesburg 2004). To determine the best cut-off point of the dendrogram, a canonical discriminant function analysis (Manly 2005) was used. All multivariate analyses were performed by R 3.4.0 software.

Results and Discussion

Bartlett's test under both flooded and drought conditions was significant at the 1% significance level. Also, KMO values under the flooded and drought conditions were estimated as 0.76 and 0.79, respectively. Hence, the conduction of factor analysis was justified. Furthermore, dividing the data into two groups and running the factor analysis for each group showed almost similar results. Thus, all data within each environmental condition were included in the final factor analysis for data reduction. Considering the criterion of selecting the eigenvalues of greater than unity, three and four factors were chosen under the flooded and drought conditions, respectively (Tables 3 and 4). Most of the communalities were high and all of them were greater than 0.5, indicating that the selected factors were able to justify most of the variations within each trait.

In the flooding condition, the three selected factors explained 68.3% of the total variance (Table 3). The first factor covered 40.82% of the total variance. We considered the absolute values of 0.5 or greater for factor loadings to determine the important traits within each factor (Manly 2005). The first factor included grain yield, 1000-grain height, plant height, flag leaf frying, flag leaf rolling and the number of days to flowering. The

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coefficients for the grain yield, 1000-grain weight and the number of days to flowering were positive, and for plant height, flag leaf frying and flag leaf rolling were negative. Thus, the mutants with higher scores for the first factor had higher grain yield, 1000-grain weight and number of days to flowering, but were shorter and had smaller flag leaf frying and rolling. Under the flooding conditions, the late mutants had a longer vegetative growth period and greater opportunity to increase their canopy volume. Therefore, the late mutants produced more leaves and chlorophyll areas, enabling them to obtain higher grain yield. Furthermore, the presence of short stems aids the rice plant to be more resistant to lodging.

The eigenvalue of the second factor was 1.84 and it was able to explain 15.3% of the total variance (Table 3). Fertility percentage, number of filled grains and 1000-grain weight contributed to the second factor. The third factor with an eigenvalue of 1.46 was able to justify 12.2% of the total variance. In the third factor, panicle length and panicle exsertion had positive loads and the number of tillers had a negative load (Table 3). Thus, the individuals that had a higher score for the third factor, had a higher panicle length and panicle exsertion, and a lower number of tillers.

Results of the factor analysis under drought condition showed that four factors together explained 76.1% of the total variance (Table 4). The contribution of the first to fourth factors to the total variance, with eigen values of 4.99, 1.74, 1.32 and 1.08, was 41.61%, 14.5%, 10.97% and 8.97%, respectively. In the first factor, grain yield, 1000-grain weight and the number of tillers had positive loads and plant weight, flag leaf frying, flag leaf rolling and number of days to flowering had negative loads. Therefore, the mutants with the higher scores of the first factor, had greater 1000-grain weight, number of tillers and grain yield at drought stress conditions. On the other hand, the later and taller mutants had higher values of flag leaf frying and flag leaf rolling, and consequently lower grain yield. Under the drought stress conditions, the early mutants maintain their grain yield by escaping from the stress at the end of the growing season. The opposite signs for the factor loadings of grain yield and plant height can be explained by the lodging potential of tall mutants. Therefore, a considerable portion of their yields wasn't harvested. In this regard, the dwarf mutants under both moisture conditions had higher yields than the taller mutants. Blum and Sullivan (1986) stated that when responding to stress, plants reduce growth and leaf area. In the second factor, fertility percentage, number of filled grains and 1000-grain weight were important and had positive loads. Therefore, the individuals with higher factor loadings for the second factor had a higher fertility percentage, number of filled grains and 1000-grain weight. Number of spikelets was the only important trait that was included in the third factor, with the high and positive load. Thus, the individuals that had higher factor loadings for this factor had a higher number of spikelets. The fourth factor included panicle exsertion and panicle length with high and positive loads of 0.81 and 0.74, respectively (Table 4). Therefore, the individual mutants that had higher scores for the fourth factor had a higher panicle exsertion and panicle length. Ghorbani *et al.* (2011) identified three factors under the flooding condition and

Table 3. Results of factor analysis for morphological and agronomical traits of rice mutant lines under flooding conditions.

Traits	Factor 1	Factor 2	Factor 3	Communality
Grain yield	0.89	0.29	-0.11	0.90
1000-grain weight	0.67	0.58	-0.04	0.79
Number of filled grains	0.36	0.87	0.16	0.90
Number of tillers	0.22	-0.24	-0.67	0.55
Panicle length	0.19	-0.32	0.74	0.69
Number of spikelets	0.44	-0.04	0.47	0.51
Plant height	-0.90	-0.11	0.34	0.83
Panicle exsertion	-0.25	0.23	0.64	0.52
Flag leaf frying	-0.81	-0.15	0.06	0.68
Flag leaf rolling	-0.82	-0.18	0.01	0.71
Fertility percentage	0.17	0.93	0.04	0.90
Number of days to flowering	0.56	0.06	0.001	0.52
Eigen value	4.90	1.84	1.46	
Percent of variance	40.82	15.31	12.17	
Cumulative variance	40.82	56.13	68.3	

Table 4. Results of factor analysis for morphological and agronomical traits of rice mutant lines under drought conditions.

Traits	Factor 1	Factor 2	Factor 3	Factor 4	Communality
Grain yield	0.93	0.24	-0.001	-0.01	0.93
1000-grain weight	0.71	0.53	0.01	-0.11	0.79
Number of filled grains	0.17	0.90	0.12	0.05	0.86
Number of tillers	0.52	-0.20	-0.49	-0.05	0.56
Panicle length	0.19	-0.12	0.35	0.74	0.71
Number of spikelets	0.03	-0.003	0.91	0.03	0.82
Plant height	-0.82	-0.27	-0.13	0.02	0.76
Panicle exsertion	-0.21	0.14	-0.18	0.81	0.75
Flag leaf frying	-0.72	0.02	-0.02	-0.04	0.52
Flag leaf rolling	-0.82	-0.24	0.03	0.04	0.73
Fertility percentage	0.22	0.93	-0.06	0.02	0.92
Number of days to flowering	-0.88	-0.09	0.16	0.01	0.80
Eigen values	4.99	1.74	1.32	1.08	
Percent of variance	41.61	14.5	10.97	8.97	
Cumulative variance	41.61	56.11	67.08	76.05	

Sharifi *et al.* (2017) identified four principal factors under the drought stress conditions, which justified most of the total variance in the data.

The selected factors (three for flooding and four for drought stress conditions), were used for the cluster analysis of genotypes and the resulting dendrograms are shown in Figures 2 and 3. When the dendrograms were cut at distances of 190 and 130 under flooding and drought-stress conditions, respectively, the probability of being significant was minimized based on canonical discrimination function analysis (Table 5), and thus maximum differences were obtained among the clusters. Furthermore, the differences among clusters were significant (Table 5).

Under the flooding condition, the first to the fourth groups had 13, 20, 32 and 31 mutants, respectively (Figure 2). To show the value of each cluster in terms of the 12 measured traits, the deviations of the clusters from the grand mean were calculated. These deviations to some extent could indicate the diversity of the mutants under the study. Since the mutants of each cluster had greater genetic proximity compared to the mutants available in other clusters, hybridization, could be applied among the genotypes of different clusters to exploit transgressive segregation and heterosis, considering the mean values of the traits for each cluster. Results (Table 6) showed that the first and second clusters were higher than the grand mean in terms of grain yield, 1000-grain weight, number of filled grains, number of tillers, panicle length, number of spikelets, panicle exsertion, fertility percentage and number of days to flowering and were lower than the grand mean for plant height, flag leaf frying and flag leaf rolling. Accordingly, the mutants of the first and the second clusters have greater breeding importance in terms of grain yield and its components, shorter stature and fertility percentage under the flooding condition. The third and fourth clusters had the highest plant height, flag leaf frying and flag leaf rolling, but were lower than the grand mean in terms of other characteristics. Thus, the third and fourth clusters had the mutants with poor yield potentials under flooding conditions.

The cluster analysis of rice mutant lines under the drought condition resulted in four clusters (Figure 3). The first to the fourth groups consisted of 23, 41, 20 and 12 mutants, respectively. Examining means of the traits under the drought stress condition (Table 7) showed that the first and second clusters were higher than the grand mean in terms of plant height, panicle exsertion, flag leaf frying, flag leaf rolling and number of days to flowering, and were lower than the grand mean for grain yield, 1000-grain weight, number of filled grains, number of tillers, panicle length, number of spikelets and fertility percentage. The highest sensitivity to drought stress was observed in these clusters. Panicle exsertion and anther opening are indicators that make a plant sensitive to drought stress at the heading stage (O'Toole and Namuco 1983). The third and fourth clusters were higher than the grand mean in terms of the grain yield, 1000-grain weight, number of filled grains, number of tillers, panicle length, number of spikelets and fertility percentage, and were lower than the grand mean in terms of plant height, panicle exsertion, flag leaf frying, flag leaf rolling and number of days to flowering (Table 7). Hence, considering the better characteristics of these mutants, they could be exploited for increasing grain yield under drought stress conditions.

Under drought stress, the mutants of the first and second clusters, compared to the third and fourth clusters, had a lower yield. This may be because the fertility percentage of these clusters were low. In other words, a higher number of hallow grains in a panicle could be one of the effects of drought stress at the end of the growing season (Sharifi *et al.* 2017). Considering the weather and soil conditions of the experimental site, carbohydrate transfer under drought stress did not adequately address the demand for carbohydrate sinks (Guimaraes *et al.* 2016). Pollen abortion can also be considered as a reason for the decrease in the number of grains under the drought-stress conditions (Nguyen and Sutton 2009). In the drought-stress condition, the sterility of spikelets is an indication of the severity of water scarcity and regarded as the most important determinant of the grain yield (Guimaraes *et al.* 2010).

Panicle exsertion and anther dehiscence are

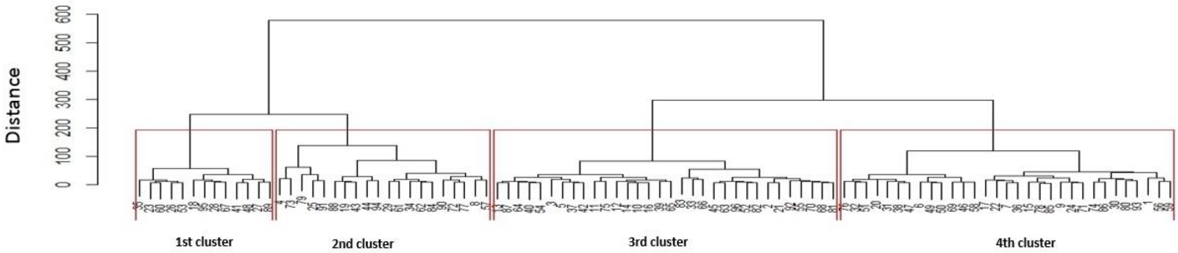

Figure 2. Grouping of the mutant rice genotypes based on the selected factors under the flooding condition.

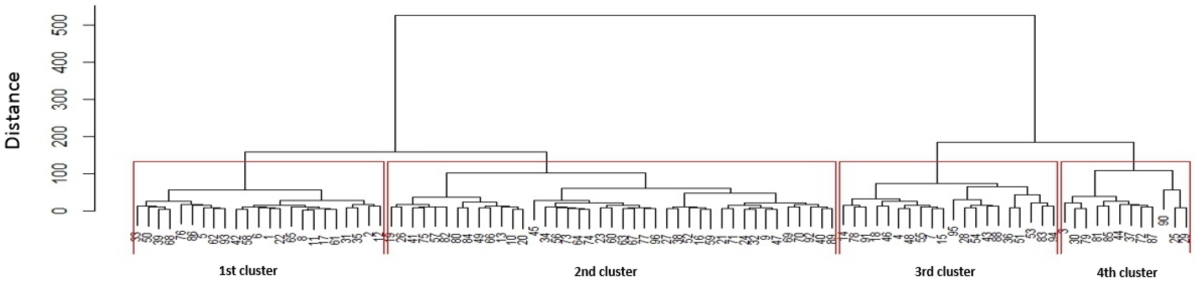

Figure 3. Grouping of the mutant rice genotypes based on the selected factors under the drought condition.

Table 5. Results of canonical discriminate analysis to determine the cut-off point in the dendrograms obtained from the cluster analysis of the rice mutant lines.

	Number of groups	Wilks' Lambda	Chi-square	Probability
Flooding condition	2	0.30^{**}	129.3^{**}	0.004
	3	0.18^{**}	296.9^{**}	0.002
	4	0.07^{**}	367.6^{**}	0.0003
Drought condition	2	0.31^{**}	191.1^{**}	0.002
	3	0.12^{**}	374.72^{**}	0.001
	4	0.07^{**}	519.75^{**}	0.0001

Table 6. Deviation of cluster means from the grand mean in the cluster analysis of rice mutant lines under flooding conditions.

Traits	1st cluster	2nd cluster	3rd cluster	4th cluster	Grand mean
Grain yield (t/ha)	0.56	0.4	-0.62	-0.34	4.28
1000-grain weight (gr)	2.98	2.63	-3.24	-2.37	26.1
Number of filled grains	20.65	16.7	-22.97	-14.38	709.6
Number of tillers	3.74	3.21	-4.06	-2.89	18.2
Panicle length (cm)	2.38	2.02	-2.57	-1.83	25.4
Number of spikelets	1.83	1.45	-2.06	-1.22	9.2
Plant height (cm)	-19.93	-11.04	17.06	13.91	104.42
Panicle exsertion (cm)	1.3	0.72	-1.43	-0.59	7.1
Flag leaf frying (range: 0-9)	-0.9	-0.45	0.81	0.54	2.8
Flag leaf rolling (range: 0-9)	-0.77	-0.58	0.74	0.61	3
Fertility percentage	16.74	13.52	-17.59	-12.67	68.3
Number of days to flowering	7.97	4.9	-9.19	-3.68	98.1

Table 7. Deviation of cluster means from the grand mean in the cluster analysis of rice mutant lines under drought conditions.

Traits	1st cluster	2nd cluster	3rd cluster	4th cluster	Grand mean
Grain yield (t/ha)	-0.63	-0.33	0.55	0.41	3.61
1000-grain weight (gr)	-2.72	-2.35	2.59	2.48	22.2
Number of filled grains	-28.74	-16.1	25.63	19.21	583.1
Number of tillers	-3.36	-2.16	3.01	2.51	15.17
Panicle length (cm)	-0.53	-0.21	0.45	0.29	22.7
Number of spikelets	-1.32	-0.87	1.17	1.02	8.7
Plant height (cm)	22.02	11.9	-19.62	-14.3	95.3
Panicle exsertion (cm)	1.24	0.81	-1.12	-0.93	6.7
Flag leaf frying (range: 0-9)	1.92	1.75	-2.25	-1.42	7.15
Flag leaf rolling (range: 0-9)	1.63	1.54	-1.89	-1.28	6.21
Fertility percentage	-21.1	-10.64	17.43	14.31	59.6
Number of days to flowering	14.1	5.85	-11.39	-8.56	91.93

sensitive to drought stress (O'Toole and Namuco 1983). Under drought stress, the sterility of spikelets is increased, because the unexserted spikelets are not able to complete anthesis and remain hallow (Guimaraes *et al.* 2016). In our experiment, however, the higher-yielding mutants (clusters 1 and 2) had only higher panicle exsertion values in the flooding conditions. In the contrary, under drought stress, the higher-yielding mutants of the third and fourth clusters had a higher number of filled grains and the number of spikelets, even though they had lower than average panicle exsertion. It seems that under drought stress conditions, the mutants with higher yield have used other mechanisms to compensate for the adverse effects of the reduction in the panicle exsertion.

The mutants No. 4, 18, 25, 28, 29, 43, 44, 48, 53, 72, 79, 88, 90, 91, 94, and 95, which were present in the first and second clusters under flooding condition, and in the third and fourth clusters under drought-stress condition, can be regarded as the promising mutants in terms of yield potential and drought tolerance.

Under the flooding and drought stress conditions, the late and early mutants, respectively, had more optimal characteristics in terms of grain yield; therefore, in the conditions of our experiments, late mutants were favored in the flooding conditions and early mutants in the drought-stress environments.

Conclusions

The results of the present study showed that under the flooding condition, the mutants of the first and second clusters had greater breeding importance. Under the drought stress condition, the mutants of the third and fourth groups, due to having most of the optimal values of the traits (e.g. short stature, early maturity, higher yield and yield components), can be regarded as the mutants with higher drought-stress tolerance. Mutants No. 4, 18, 25, 28, 29, 43, 44, 48, 53, 72, 79, 88, 90, 91, 94, and 95 were located in higher-yielding clusters under both normal and the drought stress conditions; therefore, they are promising mutants in terms of yield potential and drought tolerance. The results obtained from the canonical discriminant function analysis confirmed the existence of genetic differences among the four clusters of mutants under different moisture conditions. The mutants of the different groups can be used in the breeding programs aimed at developing new cultivars by hybridization among different clusters.

Given the existence of genetic diversity among the mutants under study and the presence of a significant number of high-yielding and drought-stress tolerant mutants, this population can be exploited for carrying out supplementary studies (e.g. genomics, proteomics, interactomics, transcriptomics, metabolomics) for increasing drought-stress tolerance and producing high-yielding rice cultivars.

References

Ahloowalia BS, Maluszynski M and Nichterlein K, 2004. Global impact of mutation-derived varieties. Euphytica 135(2): 187-204.

Arefi H and Norozi M, 2008. Introduced two new rice varieties through mutation (gamma rays). Rice Research Institute of Iran, Deputy of Mazandaran. 1: 2-10 (In Persian).

Babaei A, Nematzadeh GA, Avagyan V and Hashemi-Petrodi SH, 2010. Radio sensitivity studies of morpho-physiological characteristics in some Iranian rice varieties (*Oryza sativa* L.) in M_1 generation. African Journal of Agricultural Research 5(16): 2124-2130.

Barnabas B, Jager K and Feher A, 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell & Environment 31: 11-38.

Bartlett MS, 1951. The effect of standardization on a chi-square approximation in factor analysis. Biometrika 38(3-4): 337-344.

Blum A and Sullivan CY, 1986. The comparative drought resistance of landraces of sorghum and millet from dry and humid regions. Annals of Botany 57: 835-846.

Brown J and Caligari P, 2008. An Introduction to Plant Breeding. Blackwell Publishing, USA.

Cha-um S, Yooyongwech S and Supaibulwatana K, 2012. Water-deficit tolerant classification in mutant lines of Indica rice. Scientia Agricola 69(2): 135-141.

de Datta SK, Malabuyoc JA and Aragon EL, 1988. A field screening technique for evaluating rice germplasm for drought tolerance during vegetative stage. Field Crops Research 19(2): 123-124.

Domingo C, Andres F and Talon M, 2007. Rice cv. Bahia mutagenized population: a new resource for rice breeding in the Mediterranean Basin. Spanish Journal of Agricultural Research 5(3): 341-347.

El-Degwy IS, 2013. Mutation induced genetic variability in rice (*Oriza sativa* L.). International Journal of Agriculture and Crop Sciences 5(23): 2789-2794.

FAO, 2018. FAOSTAT. Food and Agriculture Organization of the United Nations. http://fao.org/crop/statistics.

Ghorbani H, Samizadeh Lahiji HA, Rabiei B and Allahgholipour M. 2011. Grouping different rice genotypes using factor and cluster analyses. Journal of Agricultural Science and Sustainable Production 21(3): 89-104 (In Persian with English abstract).

Guimaraes CM, de Castro AP, Stone LF and de Oliveira JP, 2016. Drought tolerance in upland rice: identification of genotypes and agronomic characteristics. Acta Scientiarum 38(2): 201-206.

Hair Jr JF, Black WC, Babin BJ, Anderson RE and Tatham RL, 2014. Multivariate Data Analysis. Seventh edition. Pearson Education Limited, UK, 729 p.

Haris A, Abdullah, Bakhtiar, Subaedah, Aminah and Jusoff K, 2013. Gamma ray radiation mutant rice on local aged dwarf. Middle-East Journal of Scientific Research 15(8): 1160-1164.

ISIRI, 1987. Determination of moisture content in cereals. Institute of Standards and Industrial Research of Iran. Retrieved October 14, 2017, from http://www.isiri.gov.ir.

IRRI, 2013. Standard evaluation system (SES) for rice. 5th ed. International Rice Research Institute, Manila, Philippines. 55pp.

Jeng TL, Tseng TH, Wang CS, Chen CL and Sung JM, 2006. Yield and grain uniformity in contrasting rice genotypes suitable for different growth environments. Field Crops Research 99(1): 59-66.

Kazerani B, Navabpour S, Sabouri H, Ramezanpour SS, Zaynali Nezhad K and Eskandari A, 2018. Determination of the best selection indices in mutant lines of rice at different moisture conditions. Journal of Crop Improvement (Journal of Agricultural) 20(1): 173-189 (In Persian with English abstract).

Kazerani B, Navabpour S, Sabouri H, Ramezanpour SS, Zaynali Nezhad Kh and Eskandari A, 2019a. Evaluation and selection of rice mutant lines based on drought tolerance indices. Journal of Plant Production Research 25(4): 15-31 (In Persian with English abstract).

Kazerani B, Navabpour S, Sabouri H, Ramezanpour SS, Zaynali Nezhad Kh and Eskandari A, 2019b. Evaluation of drought tolerance in rice recombinant inbred lines using stress tolerance indices. Seed and Plant Production 35(1): 59-81 (In Persian with English abstract).

Luzi-Kihupi A, Zakayo JA, Tusekelege H, Mkuya M, Kibanda NJM, Khatib KJ and Maerere A, 2008. Mutation breeding for rice improvement in Tanzania. International Symposium on Induced Mutations in Plants, 12-15 August, Vienna, Austria, p. 177.

Maluszynski M, Nichterlein K, van Zanten L and Ahloowalia BS, 2000. Officially released mutant varieties – the FAO/IAEA Database. Mutation Breeding Review 12: 1-84.

Manly BFJ, 2005. Multivariate Statistical Methods, A Primer. Third edition. Chapman and Hall/CRC, USA.

Marn Oo SH, Minn M, Linn KZ, Mar NN and Thin PP, 2015. Development of drought tolerant mutant from rice var. Manawthukha through mutation breeding technique using ^{60}Co gamma source. International Journal of Innovative Research in Science, Engineering and Technology 4(11): 11205-11212.

Nguyen GN and Sutton BG, 2009. Water deficit reduced fertility of young microspores resulting in a decline of viable mature pollen and grain set in rice. Journal of Agronomy and Crop Science 195(1): 11-18.

O'Toole JC and Namuco OS, 1983. Role of panicle exsertion in water-stress induced sterility. Crop Science 23: 1093-1097.

Pandey V and Shukla A, 2015. Acclimation and tolerance strategies of rice under drought stress. Rice Science 22(4): 147-161.

Romesburg C, 2004. Cluster Analysis for Researchers. Lulu Press, USA, 332 p.

Sabouri H, Biabani A, Fakhzri Moghaddam A, Katouzi M and Ebadi AA, 2008a. Genetic analysis of agronomic and qualitative traits in Iranian rice. Final Report of Project. Gonbad Kavous University, Gonbad Kavous, Iran. 48p. (In Persian with English abstract).

Sabouri H, Biabani A, Fakhzri Moghaddam A, Mollashahi M, Sabouri A and Katouzi M, 2008b. Genetic analysis of agronomic traits in Iranian rice using diallel method. Final Report of Project. Gonbad Kavous University, Gonbad Kavous, Iran. 49p. (In Persian with English abstract).

Sabouri H, Sabouri A, Jafarzadeh MR, Sajadi SJ, Mollashahi M and Jafarian HA, 2011. Introduction of tolerant rice cultivars for Gonbad Kavous region. Final Report of Project. Gonbad Kavous University, Gonbad Kavous, Iran. 46p. (In Persian with English abstract).

Senthamizh Selvi B, Ponnuswami V and Sumathi T, 2007. Identification of DNA polymorphism induced by gamma ray irradiation in Amla (*Emblica officinalis* Gaertn.) grafts of V_1M_1 and V_2M_1 generation. Journal of Applied Sciences and Research 3(12): 1933-1935.

Sharifi P, Aminpanah H and Ebadi AA, 2017. Classification of mutant rice (*Oryza sativa* L.) genotypes under drought stress conditions. Iranian Journal of Crop Sciences 19(2): 148-164 (In Persian with English abstract).

Yilmaz A and Boydak E, 2006. The effects of cobalt-60 applications on yield and yield components of cotton (*Gossypium barbadense* L.). Pakistan Journal of Biological Sciences 9(15): 2761-2769.

Yoshida S, 1981. Fundamentals of rice crop science. International Rice Research Institute, Los Banos, Laguna, Philippines. 269 p.

گروه‌بندی لاین‌های موتانت برنج از نظر صفات مهم مورفولوژیک و زراعی در شرایط متفاوت رطوبتی با استفاده از روش‌های آماری چند متغیره

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چکیده

به‌منظور مطالعه صفات مورفولوژیک و زراعی و تعیین الگوی هتروتیک در مرحله زایشی ۹۶ ژنوتیپ موتانت برنج (M_2)، دو آزمایش جداگانه تحت شرایط تنش خشکی و غرقاب در قالب طرح بلوک‌های کامل تصادفی با سه تکرار در دانشگاه گنبد کاووس در سال ۱۳۹۵ اجرا گردید. تجزیه به عامل‌ها به منظور کاهش تعداد متغیرها به تعداد کمتری از عامل‌های مستقل از هم منجر به شناسایی سه و چهار عامل، به ترتیب در شرایط غرقاب و خشکی، شد که ۶۸/۳ و ۷۶/۰۵ درصد واریانس کل را توجیه کردند. تجزیه خوشه‌ای با استفاده از عامل‌های انتخابی به روش Ward و فاصله اقلیدوسی منجر به گروه‌بندی ژنوتیپ‌های موتانت برنج شد. به منظور تعیین بهترین نقطه برش دندروگرام‌ها از تجزیه تابع تشخیص استفاده شد که نتیجه آن تولید چهار خوشه با تفاوت معنی‌دار ازهم در هر دو شرایط آبیاری شد. در شرایط غرقاب ژنوتیپ‌های خوشه‌های اول و دوم بیشترین عملکرد و اجزای عملکرد را داشتند. از طرفی، در شرایط خشکی ژنوتیپ‌های خوشه‌های سوم و چهارم پرمحصول‌تر و متحمل‌تر به تنش خشکی بودند. موتانت‌های شماره ۴، ۱۸، ۲۵، ۲۸، ۲۹، ۴۳، ۴۴، ۴۸، ۵۳، ۷۲، ۷۹، ۸۸، ۹۰، ۹۱، ۹۴ و ۹۵ در شرایط غرقاب در خوشه‌های اول و دوم و در شرایط تنش خشکی در خوشه‌های متحمل سوم و چهارم قرار گرفتند و بنابراین از نظر عملکرد دانه و تحمل به تنش خشکی به سایر موتانت‌ها برتری داشتند. از نتایج حاصل از این پژوهش می‌توان برای انتخاب هدفمند والدین مناسب از خوشه‌های متفاوت به‌منظور دورگ‌گیری و تولید رقم‌های جدید برنج استفاده نمود.

واژه‌های کلیدی: برنج؛ تجزیه به عامل‌ها؛ تجزیه تابع تشخیص؛ تجزیه خوشه‌ای؛ خشکی.