

Study of genetic variation of some Iranian rice (*Oryza sativa*) genotypes based on morphological traits, physicochemical properties and molecular markers

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ABSTRACT: Assessment of genetic diversity and individual relationships in rice (*Oryza sativa*) germplasm collections seems to be necessary for future rice breeding program. In order to understand genetic relationships of 30 rice genotypes, nine morphological traits, seven physicochemical properties and twelve RAPD primers were used for study of 30 rice genotypes. Among morphological traits, number of unfilled grain, number of tiller, number of filled grain and plant height had the highest CV value that indicated the high range of genetic diversity for studied genotypes. Pairwise correlation of morphological traits and physicochemical properties showed plant height had a strong positive correlation with panicle length ($r = 0.721$, $P < 0.0001$). Also, ratio of white rice to paddy rice and milling ratio had a negative correlation with plant height and 1000-grain weight, respectively. Cluster analysis of physicochemical properties and morphological traits grouped all genotypes into three main clusters. A total of 105 obtained RAPD bands, a number of 35 bands were polymorphs which range 7 to 19 bands per primer. OPB-14 and OPH-12 primers shown that lowest and the highest number of bands per primers, respectively. Cluster analysis of molecular data based on UPGMA algorithm and Jaccard's similarity coefficient grouped 30 rice genotypes into three clusters. The findings of this study might provide valuable information about local rice cultivar relationships in terms of their genetic distance, and can be useful in rice breeding program.

KEYWORDS: *Oryza sativa*, Genetic variation, Morphological trait, Physicochemical property, RAPD

INTRODUCTION

Various estimates show that the world food production must be doubled by 2025 to meet the needs of 11 billion people (25). Considering the global climatic changes, this must be obtained on the smaller land area and with the less water resources (19). Rice (*Oryza sativa*) belongs to Poaceae family, and is one of the most important food sources worldwide (3). After bread wheat, rice has the second ranks of calories consumed in the human diet, and is served for over fifty percent of world's population especially in Asia (3, 16).

Improvement of plant agronomic parameters, such as physiological, physicochemical and morphological traits is the final aim of rice breeding program (15), and genetic diversity as the basis of plant breeding program has a key role in this regard (5, 12). On the one hand, assessment of genetic diversity and relationships among different cultivars and related accessions is very important for conservation of genetic resources (21). On the other hand, knowledge of genetic diversity in cultivated species is essential for choosing appropriate parental lines in order

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to exploit the heterosis (hybrid vigor), especially in commercial crops (4, 11). Generally, the first step towards crop improvement is rapid identification of germplasm, maintain and expand germplasm diversity in a short time (13, 14). In this view, the role of different morphological, physicochemical and molecular markers for genetic diversity assessment is undeniable. Genetic variation of different Iranian rice landraces has been studied using different morphological traits (6, 36, 37), physicochemical properties (22, 34) and molecular markers including isozymes (26), PCR-based RFLP (restriction fragment length polymorphism) (9), RAPD (random-amplified polymorphic DNA) (35), AFLP (amplified fragment length polymorphism) (1, 32), SSR (Simple sequence repeats) (2, 30, 32), and so on.

However, the characterization of germplasm based on morphological and physicochemical traits is influenced by environmental conditions, but they are still considered as the initial prerequisites of many population genetic analyses such as mapping studies. In this view, the evaluation of genetic variation will be more appreciated if both molecular and non-molecular markers are utilized together (29). This research was conducted to study the genetic variation among different genotypes of Iranian rice genotypes using morphological traits, physicochemical properties and RAPD markers.

MATERIALS AND METHODS

Plant materials, field experiments, and traits evaluation

This study was conducted at the Genetic and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari Agricultural Sciences and Natural Resources University (SANRU), Sari, Iran ($36^{\circ}39'36''N$ latitude, $54^{\circ}04'05''E$ longitude, -26 m altitude). The plant materials (30 cultivars) were obtained from Rice Research, Amol, Iran (Table 1). The seeds of collected samples were sown in an incomplete block design, lattice, with three replications. Each plot consisted of five rows of 2.0 m length with a plant spacing of 0.25 m in either direction. Standard agronomic practices were followed during crop growth period to minimize weed and insect damage in order to reach maximum grain yield. Any possible border effect was controlled by measuring the central three rows in each plot. The morphological traits including plant height, tiller number, panicle length, 1000-grain weight,

Table 1. Different rice genotypes used in this study.

No.	Genotype name	No.	Genotype name
1	Sangejoe	16	Spidrod
2	Shahpasand	17	Gerderashti
3	IR58	18	Sangetarom
4	Hassansarai	19	Domsiah
5	Zirehbandpai	20	Binam
6	Salari	21	Amol 2
7	Rashtisard	22	Haraz
8	IR56	23	Dadras
9	Taromamiri	24	Garmetaram
10	Rashti	25	Sadrimolaei
11	Bijar	26	Akhondmolaei
12	Gardeh	27	Sadri
13	Mosatarom	28	Sadriatri
14	Mirtarom	29	Neda
15	Anbarbo	30	Nemat

grain length, grain width, grain length/width ratio, number of filled grain, number of unfilled grain were measured based on standard evaluation system (SES) (31). Physicochemical properties such as the milling ratio, ratio of white rice to paddy rice, head rice grain percentage, grain length after cook, elongation were measured based on SES. Gelatinization temperature (20) and amylose content (17) also were evaluated with minor modifications. All traits that distributed normally at least with one of normality tests (Shapiro-Wilk test, Anderson-Darling test, Lilliefors test and Jarque-Bera test) were considered as normal data. Cluster analysis for all eight agronomical traits and seven physiochemical properties was performed using the Infostat software (Version 2012) based on Euclidean distance and Ward cluster algorithm. Coefficient of variation (CV) for morphological traits was calculated by using EXEEL software and factor analysis of morphological traits was computed by using SPSS15 software.

RAPD analysis

Fresh leaves were collected at tiller stage and immediately were transferred to $-20^{\circ}C$. DNA was extracted according to a Dellaporta *et al.* procedure with minor modifications. The quality and quantity of extracted DNA were determined using agarose gel electrophoresis and spectrophotometry, respectively. Twenty-four RAPD primers were used in this study. The list of primers is given in Table 2. RAPD analysis was performed in 12.5 l reaction volume which contained 15 ng DNA, 3 mM MgCl₂, 2 mM of each dNTPs, 1 μ M primer, 1 U *Taq* DNA polymerase. PCR (polymerase chain reaction) was done in the MJ-Mini™ thermal cycler (Bio-Rad, USA). The PCR program used for ampl-

Table 2. RAPD primers and their features.

Primer	Sequence	Number of band
OPH-10	CCTACGTCAG	13
OPH-12	ACGCCATGT	19
OPH-15	AATGGCGCAG	12
OPH-13	GACGCCACAC	9
OPH-18	GAATCGGCCA	12
OPH-05	AGTCGTCCCC	8
OPH-19	GGGAGACATC	8
OPH-01	GGTCGGAGAA	12
OPH-20	AACGGTGACC	11
OPC-08	TGGACCGGTT	12
OPH-14	ACCAGGTTGG	8
OPB-14	TCCGCTCTGG	7

ification was as follow: 4 min at 94°C, followed by 35 cycles of 94°C for 1 min, 38°C for 1 min and 72°C for 10 min. The final extension was 7 min at 72°C. The amplified products were resolved in 1.5% agarose gel (0.5 X TBE), stained with ethidium bromide (10 µg/ml) and photographed under UV light. In order to generate a binary matrix, each locus base on presence or absence a band across all the genotypes was scored as 1 and 0, respectively. It should be noted that, Co-migrating bands were assumed to be originating from the same genetic locus. The obtained binary matrix was analyzed using the NTSYS-PC version 2.02 software in order to calculate the similarity matrices, construct the dendograms, and compare clusters base on Mantel test (11). Dendograms were regenerated by dendroscope software Ver 3.

RESULTS and DISCUSSION

On the contrary of other cereal grains such as wheat, barley and sorghum, quality characteristics of rice grains play a key role for the choice and demand of rice consumers (27, 28). These characteristics largely depend on the physicochemical properties, which are greatly influenced by the genotype (18). For this base, the genetic potential of a variety/cultivar determines the diversity of future use (33). In this study, genetic variation of some Iranian rice was investigated base on physicochemical properties and morphological traits in order to identify characteristics that can be useful for rice breeding programs. Physicochemical properties were milling ratio, ratio of white rice to paddy rice, head rice grain percentage, grain length after cook, elongation,

gelatinization temperature and amylose content. Morphological traits were plant height, tiller number, panicle length, 1000-grain weight, grain length, grain width, grain length/width ratio, number of filled grain, number of unfilled grain. Our results showed extensive variations in quality traits among studied genotypes. Among morphological traits, number of unfilled grain, number of tiller, number of filled grain and plant height had the most variation with the CV value of 76.7%, 37.66%, 24.73% and 21.55%, respectively. These values reflect the high range of genetic diversity between studied genotypes for given traits. Our results are also in agreement with Bagheri *et al.* findings (2008). The box-plot graph of the rice physicochemical properties and morphological traits has depicted in Figure 1.

Results of pairwise correlation of morphological traits and physicochemical properties are given in Table 3. Plant height showed a strong positive correlation with panicle length ($r = 0.721$, $P < 0.0001$). Grain length/width ratio was found to have a strong positive correlation ($r = 0.747$, $R^2 = 0.557$, $P < 0.0001$) with grain length, while had strong negative correlation ($r = -0.885$, $R^2 = 0.782$, $P < 0.0001$) with grain width. As it is shown in Table 3, ratio of white rice to paddy rice and milling ratio had a negative correlation with plant height and 1000-grain weight, respectively. Number of unfilled grain also had negative correlation with tiller number and 1000-grain weight. A significant positive correlation was observed for milling ratio with ratio of white rice to paddy rice and head rice grain percentage. Our results are in agreement with Haque *et al.* study, that reported there was a strong positive correlation between milling ratio and head rice grain (10). The distance matrix based on the Euclidean coefficient among genotypes ranged from 13.97 to 183.99 with an average distance index of 98.98. The lowest genetic distance was observed between local Binam and Domsiah and the highest one belongs to Mosataom and Salari. Results obtained from cluster analysis of physicochemical properties and morphological traits grouped all genotypes into three main clusters (Figure 2). Cluster I contains genotypes of

Shahpasand, Garmetarom, Akhonomolaei, Hasansaraei, Sadrimolaei, Domsiah, Binam, Gerdeh, Zirehbandepai, Anbarbo, Sadriatri, Rashtisard, Taromamiri, Rashti, Mirtarom, sangehjoe, Dadras, Bejar, and Mosatarom. The most Iranian local cultivar placed in this cluster. Two genotypes of Salari and Sangtarom were located in a distinct group (cluster II). High-yielding rice varieties and

Table 3. Pairwise correlation of morphological traits and physicochemical properties.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16
X1	1															
X2	-0.063	1														
X3	0.721**	-0.295	1													
X4	-0.069	0.044	-0.126	1												
X5	0.038	-0.187	0.123	0.156	1											
X6	0.129	-0.093	0.136	-0.108	-0.402*	1										
X7	-0.112	-0.003	-0.063	0.260	0.747**	-0.885**	1									
X8	-0.134	-0.321	-0.068	0.345	0.139	0.015	0.068	1								
X9	0.003	-0.450*	0.254	-0.492**	0.121	0.109	-0.056	0.063	1							
X10	0.112	0.095	0.109	-0.474**	-0.069	0.309	-0.289	0.026	0.192	1						
X11	-0.441*	0.002	-0.252	0.034	-0.055	0.230	-0.170	0.211	-0.041	0.447*	1					
X12	0.012	-0.080	0.013	-0.339	-0.259	0.202	-0.274	0.099	0.210	0.678**	0.408*	1				
X13	-0.153	-0.040	-0.028	0.205	0.268	0.135	0.075	0.177	0.156	0.175	0.453	-0.076	1			
X14	0.215	-0.098	0.161	-0.032	0.156	0.112	0.002	0.144	0.137	0.332	0.249	0.356	0.334	1		
X15	0.091	0.160	0.073	0.232	-0.032	-0.158	0.127	0.206	-0.337	-0.033	0.002	-0.037	-0.149	0.076	1	
X16	0.191	-0.127	0.225	-0.016	0.172	0.129	-0.023	-0.103	-0.022	-0.073	-0.198	-0.329	-0.076	-0.036	0.286	1

**Indicates a significant difference at $p < 0.01$, * significant difference at $p < 0.5$

Plant height (X1), Tiller number (X2), Panicle length (X3), 1000-grain weight (X4), Grain length (X5), Grain width (X6), Grain length/width ratio (X7), Number of filled grain (X8), Number of unfilled grain (X9), Milling ratio (X10), Ratio of white rice to paddy rice (X11), Head rice grain percentage (X12), Grain length after cook (X13), Elongation (X14), Gelatinization (X15), temperature (X16) Amylose content

foreign lines including Amol 2, IR58, IR56, Sepidrod, Neda, Nemat, and Haraz as well as Sadriatri and Gerdehrashti allocated in cluster III. From 24 RAPD primers used in this study, only 12 primers produced clear and scorable bands (Table 2). A total of 105 obtained bands, a number of 35 bands were polymorphs which range 7 to 19 bands per primer. OPB-14 and OPH-12 primers shown that lowest and the highest number of bands per primers, respectively. Average number of bands was 10. The data generated from 12 RAPD markers for 30 rice genotypes were applied for calculating similarity coefficient and constructing dendrogram. The Mantel test was used to determine the correlation coefficient between similarity matrices and cophenetic correlation values (21). Similarity coefficient based on RAPD molecular data were ranged from 0.31 to 0.88. The lowest genetic

similarity was observed between Salari and Haraz with a value of 0.31. The highest genetic similarity value was 0.88 that were observed between Sadri and Sadrimolaei; Sadri and Akhondmolaei; Rashtisard and Akhondmolaei. Cluster analysis based on UPGMA algorithm and Jaccard's similarity coefficient grouped 30 rice genotypes into three main clusters (Figure 3).

Cluster 1 includes 12 genotypes, namely Rashtisard, Dadras, Domsiah, Sadri, Sadriatri, Shahpasand, Sangejoe, Haraz, Binam, Sangetarom, Anbarbo, Rashti and Taromamiri. This cluster is further divided into three subgroups. Cluster-II includes five genotypes (Nemat, Gerderashti, Amol 2, Neda and Sadrimolaei) which except Gradeh-rashti all others are cultivated in Mazandaran province. The remaining genotypes namely Sadriatri, Garmetarom, Zirehbandepai, Akhondmolaei,

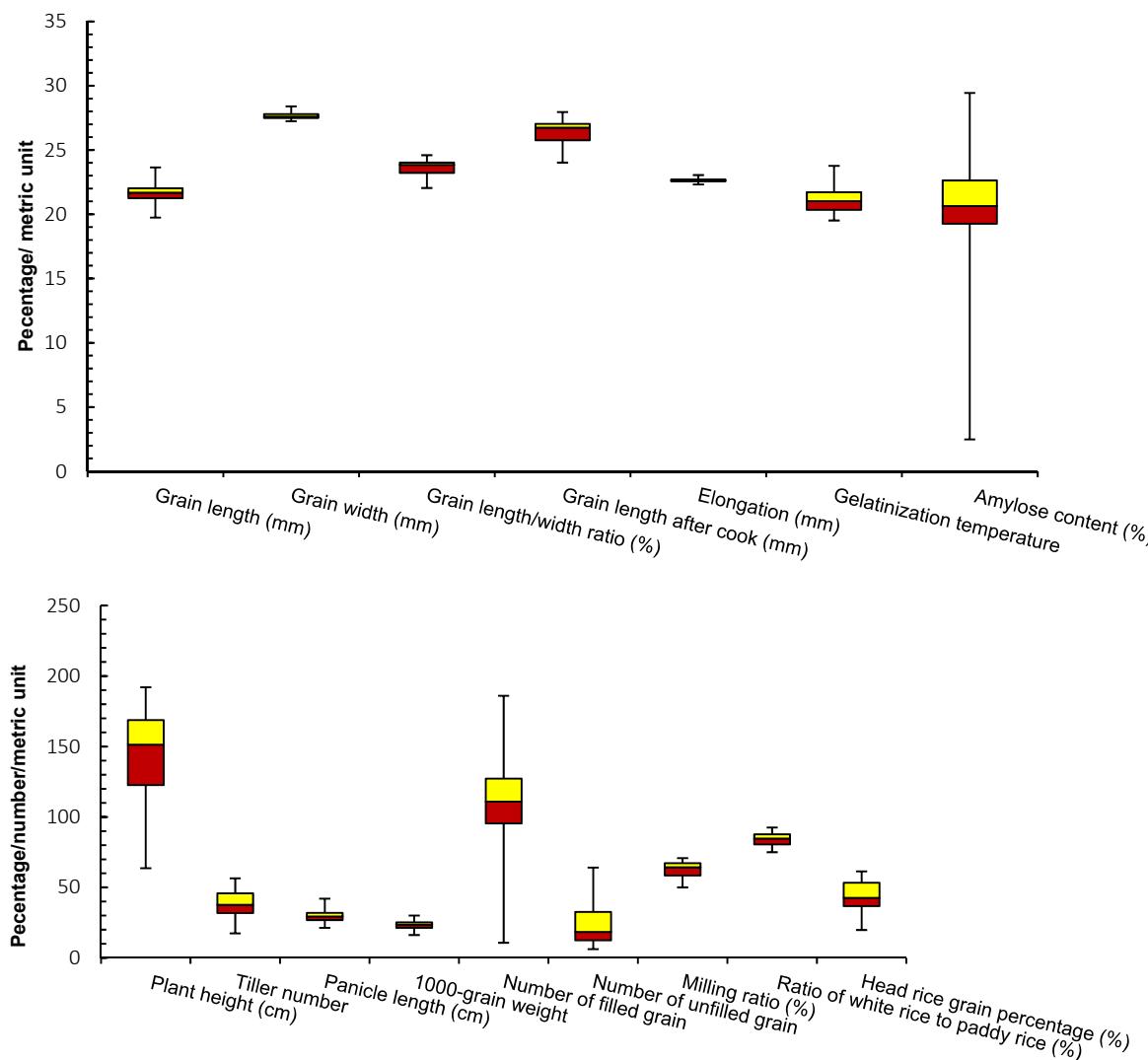


Figure 1. The box-plot graph of the rice morphological traits and physicochemical properties of 30 rice genotypes.

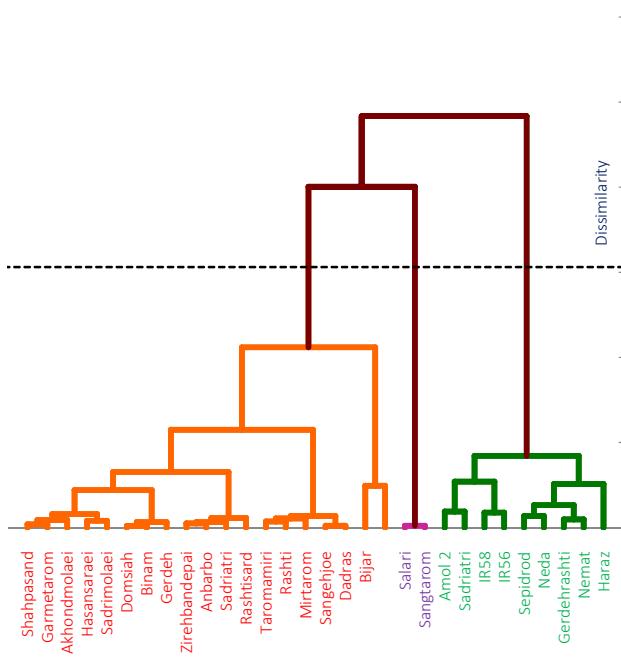


Figure 2. Cluster analysis of different rice genotypes based on physicochemical and morphological traits.

Spidrod, IR56, IR58, Salari, Hassansaraei, Mirtarom, Mosatarom, Bijar and Gardeh were allocated in cluster III.

Generally, the primary purpose of cluster analysis is classification of genotypes based on their similarities or dissimilarities (24). Individuals which are grouped in one cluster are closer genetically to each other. On the other side, individuals which are grouped on different clusters show more genetic variance. Theoretically, hybridization between varieties with high genetic distance, can produce more heterosis (23).

Relatively large amounts of physicochemical, morphological and molecular variability were measured for all given genotypes. The high level of genetic variation suggests that this collection of local and foreign lines would enhance the selection efficiency of desirable varieties, and also is appropriate for future rice breeding program.

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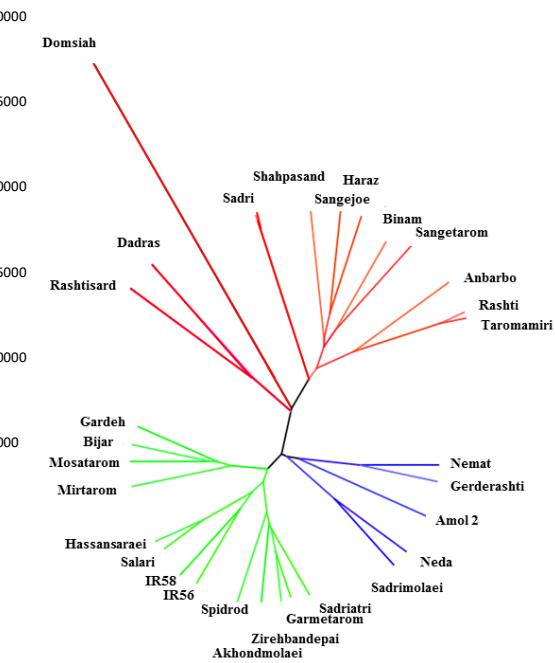


Figure 3. Dendrogram of rice genotypes based on RAPD random markers.

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بررسی تنوع ژنتیکی برخی از ژنوتیپ‌های برنج (*Oryza sativa*) ایرانی بر اساس صفات مورفولوژیک، فیزیکوشیمیایی و نشانگرهای مولکولی

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چکیده

بررسی تنوع ژنتیکی و روابط افراد در کلکسیون‌های ژرم‌پلاسم| برنج (*Oryza sativa*) برای توفیق برنامه‌های بهنژادی این گیاه ضروری می‌باشد. به منظور بررسی روابط ژنتیکی ۳۰ ژنوتیپ برنج، از نه صفت مورفولوژیک، هفت صفت فیکوشیمیایی و ۱۲ آغازگر RAPD استفاده شد. بیشترین میزان ضریب تغییرات در صفات تعداد دانه پوک، تعداد پنجه، تعداد دانه پر و ارتفاع بوته برآورد شد که نشان دهنده دانه گستردگی از تنوع ژنتیکی در میان ژنوتیپ‌های مورد بررسی بود. تجزیه همبستگی صفات مورفولوژیکی و فیکوشیمیایی نشان داد که ارتفاع بوته به شکل معنی‌داری دارای همبستگی منفی با طول شلتونگ می‌باشد ($r = -0.721$, $P < 0.0001$). همچنین صفات راندمان تیدیل و درجه تبدیل همبستگی منفی به ترتیب با ارتفاع گیاه و وزن هزار دانه نشان دادند. تجزیه کلاستر براساس صفات مورفولوژیک و فیکوشیمیایی، ژنوتیپ‌های مورد بررسی را در سه گروه مجزا دسته‌بندی نمود. از مجموع ۱۰۵ باند RAPD حاصله، تعداد ۳۵ باند چندشکل بودند که تعداد آنها بین ۷ تا ۱۹ باند در هر آگاگر محاسبه گردید. بیشترین و کمترین تعداد باند در هر پرایمر به ترتیب در پرایمرهای OPB-14 و OPH-12 مشاهده شد. بر مبنای تجزیه کلاستر بر اساس الگوریتم بندي UPGMA و ضریب تشابه Jaccard ژنوتیپ‌های در سه گروه قرار گرفتند. یافته‌های این تحقیق می‌تواند اطلاعات ارزشمندی در رابطه با روابط ارقام برنج با توجه به فاصله ژنتیکی آنها داشته و در برنامه‌های بهنژادی برنجی مثمر ثمر باشد.

کلمات کلیدی: *Oryza sativa*: تنوع ژنتیکی، صفت مورفولوژیکی، خصوصیات فیکوشیمیایی، نشانگر مولکولی RAPD