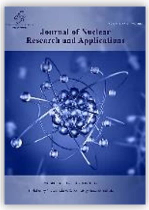
Nuclear Science & Technology Research Institute

Journal of Nuclear Research and Applications

Research Paper

Journal homepage: https://jonra.nstri.ir



Kian is the First Drought-Tolerant Rice Cultivar in Iran Developed Through Mutation of Local Varieties

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(Received: 4 August 2024, Revised: 11 November 2024, Accepted: 12 November 2024)

ABSTRACT

Iranian local rice cultivars have excellent cooking quality but unfortunately, are sensitive to water stress. In the present study, we tried to introduce a drought-tolerant variety using mutation by radiation. The first step was the determining the optimal dose of gamma radiation for the Tarom variety (230 Gy) to obtain a mutant population. Selection was based on drought stress tolerance and other agronomic traits, including plant height, panicle length, number of filled grains per panicle, percentage of fertility, 1000-grain weight, and grain yield from 2009 to 2012. This process led to the identification of 56 promising drought-tolerant mutant lines. Based on preliminary evaluations conducted over two years (2012 and 2013) under both normal and drought stress conditions, 14 lines were selected. The stability of these 14 mutant lines was further assessed in three regions-Rasht, Chaparsar, and Fars-from 2014 to 2015. After the evaluation, the Kian variety was chosen as the superior line for yield, drought stress tolerance, early maturity, and good cooking quality. Agronomic tests determined that applying 90 kg of nitrogen per hectare with a planting distance of 20×20 cm was optimal for this variety. In 2017, Kian was cultivated alongside Hashemi, the check cultivar, in three cities of Guilan province. Results showed that the average grain yield of the Kian variety was 5383 kg/ha under normal conditions and 4093 kg/ha under drought stress, compared to an average yield of 3900 kg/ha for the Hashemi cultivar under normal conditions. The growth period from sowing to maturity ranged from 99-109 days for Kian, while it was 112-114 days for Hashemi.

Keywords: Water stress; Rice; Mutant variety.

1. Introductions

Rice (*Oryza sativa*) a member of the cereal family (Poaceae) and is one of the world's most important grains, cultivated in one-third of the world's cereal-growing areas. It provides 21% of global energy and 15% of global protein. Among cereals, rice requires the most water,

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DOI: https://doi.org/10.24200/jonra.2024.1633.1137.

needing approximately 8,000 to 20,000 cubic meters and 700 liters of water to produce one kilogram of dry matter until the physiological maturation of seeds [1]. However, water deficit stress is a significant factor that reduces rice yield [2].

In Iran, of the 11.38 million hectares of cultivated land, 52.9% is irrigated while 47.1% relies on rainfed. The highest harvest level is observed in the cereals group, with a total of 71.86%, of which 44.52% comes from irrigated cereals. Rice cultivation, which is irrigated across all regions of the country, plays a vital role in this context [3]. To minimize the impact of water deficit and ensure the sustainability of rice production, it is necessary to identify or develop drought-tolerant genotypes with high yield potential, especially for areas prone to drought stress. Considering the limitation of available water for most crops, including rice, optimizing water use is critical for addressing the ongoing water scarcity crisis. Therefore, identifying varieties with higher water use efficiency is crucial for future breeding programs aimed at producing drought-tolerant rice varieties.

Rice is particularly sensitive to drought stress during the reproductive stage [4]. It is estimated thatthat 50% of global rice production is affected by the drought [5]. The reproductive stage is more susceptible to drought stress than the vegetative stage, with a significant impact on grain yield and its components. Accordingly, in breeding projects, late-season drought stress is commonly applied in the evaluation of rice genotypes [6-8]. In Guilan province, approximately 70% of paddy fields are irrigated by the Sepidroud dam. Typically, the level of water behind the dam decreases significantly towards the end of the growing season. During periods of water scarcity, the release of water is often reduced at the end of the growing season, at critical stages, such as flowering and grain filling. This shortage affects downstream areas, leading to drought stress in the paddy fields and contributing to the challenge of cultivating late high-yielding cultivars in Guilan.

Various research has been done by different scientists to determine the most effective mutagenic treatment for the induction of desirable traits in rice [9,10]. Mutation breeding methods have played significant methods in the improvement of rice by developing a large number of semi-dwarf and high-yielding varieties in many countries [11,12]. Bughio et al. developed a high-yielding rice mutant variety; Mehak (fragrance) from a fine aromatic variety of Basmati-370, that is significantly better than its parent with respect to yield and yield contributing traits [13]. IAEA/FAO reported that 873 rice varieties have been developed through induced mutations from 1966 to 2022 [14].

Local cultivars are highly valued for their exceptional taste and increased grain length after cooking. Despite their many disadvantages, these varieties remain widely cultivated, and most paddy fields are dedicated to them. Thus, enhancing local cultivars through various breeding methods can improve average yield and overall rice production.

One promising breeding approach to address the limitations of local cultivars is mutation breeding, which utilizes physical mutagens to introduce the necessary genetic diversity. This method has been successfully employed worldwide to develop new varieties with superior qualitative and quantitative traits. However, due to the quantitative nature of the traits studied and the challenges associated with producing drought-tolerant cultivars using gamma radiation, research in this area remains limited.

2. Materials and Methods

In this research, the improved cultivars "Fajr" and "Khazar" along with the local cultivars "Tarom Mahali" and "Hashemi" were utilized as plant materials. To determine the optimum dose, the Survival LD50 of seedlings was calculated in different doses, three weeks after sowing the seeds. To obtain the optimum dose, seeds were irradiated with various doses of (0, 150, 180, 200, 230, 250, and 300 Gy) of gamma rays from a Co60 source in a gamma cell (dose rate, 0.2 Gy/sec and special activity, 2000 Ci). Following this, based on the irradiation results and the established optimal dose, seeds of the main cultivars were irradiated with both the optimal and a higher dose to create the M1 generation plant population. The lines evaluated in this research were derived from induced mutations of the local Tarom variety, resulting from several collaborative projects between the Rice Research Institute of Iran (RRII) and the Nuclear Science and Technology Research Institute (NSTRI) [15,16].

The seeds of all plants from the M1 generation were harvested. For the M2 generation, plants were subjected to drought stress to identify drought-tolerant individuals. Soil moisture levels in drought-stressed plots were periodically monitored using a tensiometer. Water stress was applied in the field approximately 10 days before flowering and continued for 4 days after flowering, spanning two weeks [17].

Phenotypic analysis of the rice plants under drought stress was conducted based on the International Rice Research Institute (IRRI) standard evaluation systems [18]. All phenotypic evaluations during the 2009-2012 growing seasons were carried out of rice plants under drought stress across three generations of mutations, involving the Tarom, Fajr, Hashemi and Khazar genotypes.

In 2013, 56 mutant lines from the M5 and M6 generations were develped through the mutation of two local cultivars (Hashemi and Tarom Mahali) and two improved cultivars (Khazar and Fajr). These lines were evaluated alongside the four parents cultivars and four control cultivars (Sengjo, Baynam, Ali Kazemi and Sepidroud), which are known for their drought stress tolerance. The evaluation was conducted using two separate square lattice designs (8×8) with two replications in the experimental field of the Rice Research Institute of Iran, under both normal and drought stress conditions. For the drought stress treatment, irrigation was completely ceased from the panicle initiation stage until harvest.

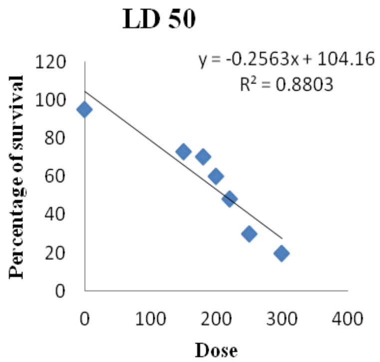
Fourteen selected mutant lines (seven Hashemi mutant lines, six Tarom mutant lines and one Khazar mutant line), along with four cultivars (Hashemi, Tarom, Khazar and Gilaneh), were evaluated in three locations (Rasht, Chaparsar and Fars) over two consecutive years (2014-2015). In Rasht, the evaluations were conducted under both normal and drought stress conditions. For the drought stress experiment, irrigation was completely withheld from panicle initiation until harvest. Throughout the growth period and and post-harvest, various traits were measured, including grain yield (kg/ha), number of panicles per plant, 100-grain weight (g), number of filled grains per panicle, plant height (cm), panicle length (cm) and days to 50% flowering. Furthermore, grain quality traits such as amylose content, gelatinization temperature and a gel consistency, along withphysical characteristics like grain length, width, elongation after cooking and head rice yield were thoroughly analyzed.

The optimal planting distance and an appropriate nitrogen fertilizer amount for the Kian variety (promising line TM8-B-7-1 or MN5) were determined in a research study conducted in 2017. The study employed a factorial experiment based on a randomized complete block design (RCBD) with three replications. Five levels of pure nitrogen fertilizer (0, 60, 75, 90 and 105 kg/ha) and two planting distances (20×15 cm and 20×20 cm) were evaluated. In 2016-2017, Kian was cultivated alongside Hashemi in 500-square-meter plots in three cities in Guilan province (Lashtenesha, Soumesara and Langrod) on the fields of leading farmers. During the growing period, key morphological traits, including the number of panicles, plant height, growth period length and grain yield, were measured by sampling the required number of plants. Statistical analysis was performed by SAS version 9.1 software.

3. Results and discussion

The results of the dose tests indicated that the optimal gamma radiation dose for the local Tarom genotype is 230 Gy (Fig. 1), while for the Fajr, it ranges between 200 and 230 Gy. An optimal dose is defined as the radiation level that induces a high frequency of favorable mutations with minimal damage to the plant. For Hashmi and Khazar, the optimal doses were determined to be 250 Gy and 200 Gy, respectively. Sensitivity to gamma rays varied between local and improved cultivars, with the native cultivars (Tarom Mahali and Hashemi) exhibiting distinct patterns and levels of sensitivity compared to the improved cultivars (Fajr and Khazar).

Two weeks after applying drought stress to the M2 generation of Tarom mutant lines, 64 mutant plants exhibiting drought tolerance (based on leaf rolling scale) and 81 mutants early- and very-early flowering mutant plants were selected resulting in a total of 145 plants. After an additional 2-3 weeks of drought stress, 49 drought-tolerant lines (based on the leaf rolling scale) and 5 early flowering lines were identified in the M3 generation totaling 54 plants. In the M4 generation, approximately one month of drought stress was applied to the Tarom mutant plants in the experimental field. From this population, 10 drought-tolerant lines (based on the leaf rolling scale) and 3 early flowering lines were selected, amounting to a total of 13 lines.



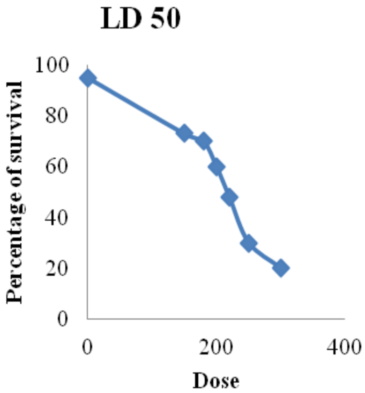


Fig. 1. LD 50 of seedling survival in the "Tarom Mahali" genotype.

A total of 56 promising mutant lines tolerant to drought stress were selected from the M4 generation of local Tarom and Fajr cultivars, as well as the M3 generation of Hashemi and Khazar cultivars. These lines were compared with normal controls and demonstrated superior performance. Preliminary yield evaluations under both normal and drought stress conditions revealed that Kian (promising line TM6-B-7-1) achieved the highest grain yield among all the examined lines, with an average yield of 5861 kg/ha under normal conditions. Under drought stress conditions, Kian produced a yield of 2217 kg/ha, which was statistically lower than only two lines (numbers 12 and 43), placing it in the second-highest yield group. After conducting a simple analysis of the traits investigated across different locations and years in 14 mutant lines, along with 4 control cultivars, and verifying the uniformity of experimental error variances for common traits measured in all three locations and two years, a combined variance analysis was performed. The main effect of genotype was significant at the 1% level for all studied traits. The interaction effect of genotype by year by location was also significant at the 1% level for all studied traits. In Kian, the plant height averaged 119 cm, which was significantly different from the local Tarom and Hashemi cultivars. The average panicle length in Kian was 24.86 cm, placing it in the second-highest group for this trait. The number of filled grains per panicle in Kian was 89.05, which was not significantly different from the highest value observed among the studied varieties and lines. However, the average number of filled grains per panicle in Kian was lower than that of the control cultivars. The fertility percentage for Kian was 91.35%, exceeding that of the control cultivars and not significantly different from the highest value observed in line number 7. Kian had an average grain weight of 2.72 grams, placing it in the top group for this trait with significant differences compared to the control cultivars. Additionally, Kian had the highest average grain yield of 5684 kg/ha among the investigated genotypes across the three locations and two years.

To identify the most stable genotypes, stability analysis was conducted using various statistical methods. The stability parameters, including intra-spatial variance and the environmental variation coefficient, indicated that Kian (line no. 5) and lines no. 7, 8 and 12, which had the lowest values for these indicators, are the most stable genotypes in terms of grain yield. Three indices were used for genotype stability classification: the regression coefficient (bi), the average yield of genotypes ($\bar{g}$) and the average yield of all genotypes ($\bar{X}$).

Kian, along with genotypes 1, 2, 7, 8, 12 and 18, exhibited a regression coefficient of less than one and an average yield higher than the overall average of all genotypes. Among these, the deviation from regression was minimal in Kian and genotype 12 compared to others. Overall, Kian and genotype 12 demonstrated high yield stability, characterized by an average grain yield above the overall average, a regression coefficient of less than one, minimal deviation from regression, a low coefficient of environmental variation, and low intra-spatial variance (Table 1). Supporting this finding, previous research has also used similar parametric methods to identify stable rice genotypes. For instance, Nahvi et al. [19] identified line 424 (Dorfak) as the most stable and productive due to its low mean square and coefficient of variation. Additionally, Sharifi et al. [20] employed various stability analysis methods, including environmental variance, coefficient of variation, Shukla's variance, Rick's equivalence, regression coefficient, detection coefficient, Eberhart-Russell variance analysis, and yield stability statistics (YSi), to identify stable genotypes. Sharifi and AminPanah [21] similarly identified the most stable genotype based on low intra-spatial variance and coefficient of variation.

As shown in the diagram, genotypes 5 (Kian), 7 (promising line HM8-250-5E-1-1 or MN7), and 12, which had higher average yields compared
to other genotypes, were well-suited to all six investigated environments. Specifically, genotypes 7 and 12 were highly compatible with environments 2, 3 and 6, while genotype 5 (Kian) was better suited to environments 1, 4 and 5 (Fig. 2).

The evaluation of key agronomic characteristics of Kian and Hashemi across three cities in Guilan province revealed that under normal conditions, Kian achieved the highest grain yield in Soumesara (6400 kg/ha) and the lowest in Lashtnesha (4500 kg/ha) (Fig. 3). This variation in yield between the two regions is primarilyattributed to deviations from agricultural guidelines and poor nutrition management in Lashtnesha. Adhering to agricultural best practices could significantly enhance Kian's yield, which already demonstrates a higher yield potential compared to the local Hashemi variety. Moreover, under drought stress, the yield reduction in Kian was minimal, matchingthe yield of the Hashemi variety under normal conditions.

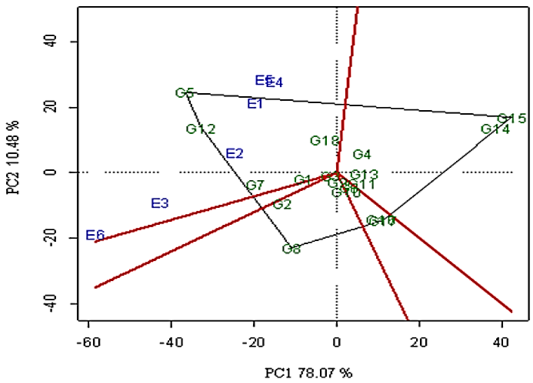


Fig. 2. Polygon view of GGE biplot based on grain yield data for 18 genotypes across three locations and two years (6 environments).

Table 1. Parameters of grain yield stability for rice genotypes across three locations and three years.

Genotype	Grain Yield (kg/ha)	Regression Coefficient (bi)	Deviation from regression	Intra-spatial variance (Msy/l)	Environmental Coefficient Variation (CVy/i)
G1	4607	0.996	111284	412652	13.94
G2	4699	0.711	279690	391490	13.31
G3	4418	1.08	96356	453456	15.24
G4	4227	1.25	122539	608946	18.46
Kian	5684	0.69	72194	215927	8.18
G6	4237	0.795	47371	246212	11.71
G7	5009	0.265	113668	118698	7.02
G8	4466	0.0863	248161	206154	10.17
G9	4323	0.828	21113	227225	11.03
G10	4240	0.977	103534	394590	14.81
G11	4175	1.34	130530	686332	19.93
G12	5470	0.526	69483	149672	7.02
G13	4148	0.989	78619	382271	14.90
G14	3353	2.351	241389	2216414	44.40
Khazar	3252	2.72	283012	2603394	49.61
Hashemi	3908	0.774	97766	261180	13.08
Tarommohali	3883	0.789	34495	232571	12.42
Gilaneh	4504	0.71	260708	375491	13.60

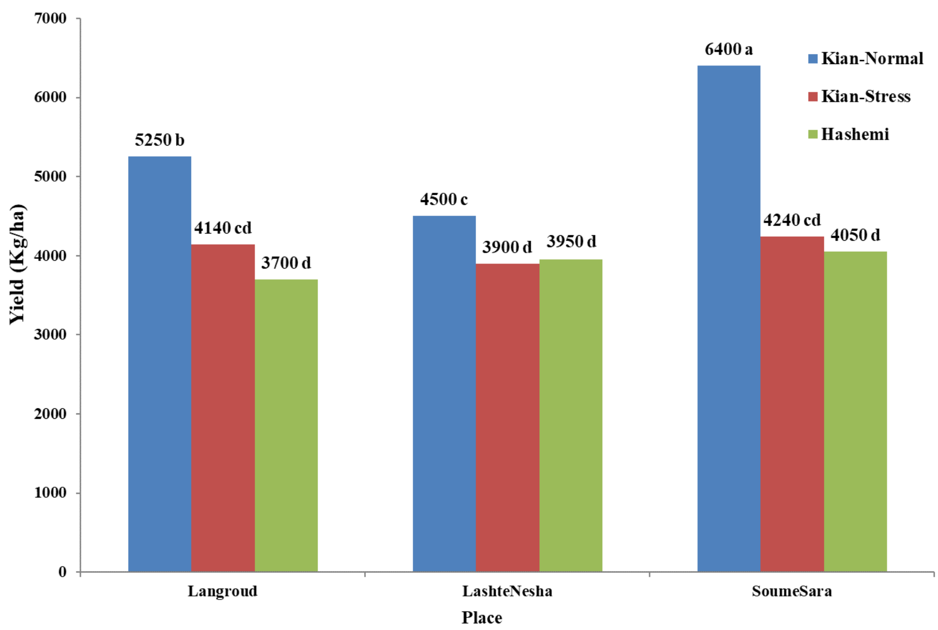


Fig. 3. Average grain yield (kg/ha) of Kian (under normal conditions and drought stress) and Hashemi across three cities in Guilan province.

Based on a T-test, the yield of the Kian rice variety was significantly higher with an average of 5383 kg/ha,compared to the Hashemi variety which averaged 3900 kg/ha, across three regions of Gilan Province. The cultivar Kian had an average plant height of 134 cm, which was significantly shorter than the Hashemi cultivar with an average height of 152 cm, across the three test regions. The Kian variety had an average growth period of 109 days, which was significantly earlier than the Hashemi variety with an average growth period of 116 days. The head rice yield in the Kian variety with an average of 60% was significantly higher than the Hashemi variety with an average of 56%. Additionally, the number of filled grains per panicle was significantly higher in Kian variety with an average of 113 grains per panicle than Hashemi variety with an average of 90 grains per panicle (Table 2).

Table 2. Comparison of the means of different traits between two cultivars of Kian under normal conditions and the Hashemi cultivar in three different counties using a two-tailed t-test.

Treat	Mean ± Standard Error in Kian-N	Mean ± Standard Error in Hashemi	T value	P value
Yield (Kg/ha)	5383 ± 957	3900 ± 104	-3.64	0.047
Plant Height (cm)	134 ± 2.3	152 ± 1.4	6.55	0.003
Growth duration (days)	109 ± 0.6	116 ± 0.9	7.27	0.002
Head Rice Yield (percentage)	60 ± 0.58	56 ± 0.58	-4.90	0.008
Filled grains number per panicle (Number)	113 ± 1.2	90 ± 0.58	-17.0	<.0001

An evaluation of planting distances and nitrogen application rates for Kian revealed that the average grain yield was significantly higher with a planting distance of 20×20 cm compared to 15×20 cm. Additionally, the harvest index, nitrogen recycling efficiency and agronomic efficiency of nitrogen application were significantly better at the 20×20 cm distance. However, the amount of nitrogen absorbed by the straw was significantly higher with the 20×15 cm planting distance compared to the 20×20 cm distance. The highest grain yield, 4492 kg per hectare, was achieved with the application of 90 kg of nitrogen per hectare at a planting distance of 20×20 cm. Based on thesignificant yield difference, it is recommended to apply 90 kg of nitrogen per hectare with a planting distance of 20×20 cm for Kian.

Kian, developed through mutation induced by gamma radiation in the local cultivar, is drought-tolerant. Under normal conditions, Kian yields between 5.5 to 6 tons per hectare, with a 100-grain weight of 3.2 to 3.8 grams, an average of 111 to 112 filled grains per panicle and a plant height of 135 to 147 cm. Under drought stress, the yield drops about 3.5 tons per hectare, with a 100-grain weight of 2.2 to 2.7 grams, 92 to 97 filled grains per panicle and a height of 128 to 132 cm. Kian not only outperforms its parent varieties but also is an early, semi-dwarf variety with cooking quality comparable to local Iranian varieties. Its wide leaves enhance photosynthesis and overall yield, while rapid seed germination ensures effective growth. Once planted in the main field, the long leaves provide effective shading between plants, which helps reduce weed growth. Substituting the Kian for 10% of the paddy fields in Guilan and Mazandaran provinces (approximately 40,000 hectares) could increase production by 60,000 to 80,000 tons of paddy. In other words, replacing local varieties such as Hashemi with Kian could generate an additional income of 750 to 1,000 million Rials for rice farmers, based on an increased yield of 1,500 to 2,000 kg of paddy per hectare at a price of 500,000 Rials per kg. Moreover, this variety could enhance production stability in areas facing water scarcity, reducing potential losses for rice farmers. Kian, developed through these efforts, is characterized for its drought stress tolerance, shorter plant height, and higher yield under both normal and drought stress conditions. Additionally, Kian possesses desirable traits similar to local varieties, including an amylose content of 21%, a medium gelatinization temperature, a pleasant aroma, a head rice percentage of 62% and cooking quality comparable to that of local varieties.

Conflict of interest

The authors declare no potential conflict of interest regarding the publication of this work.

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How to cite this article
A. Fakhraei, F. Faghihi, *Development of Transient Dynamics Code for a Helically-Coiled Steam Generator Analysis Using Multi-Node Moving Boundary Model*, Journal of Nuclear Research and Applications (JONRA), Volume 5 Number 1 Winter (2025) 56-65, **URL:** https://jonra.nstri.ir/article_1693.html, **DOI:** https://doi.org/10.24200/jonra.2024.1633.1137.