



## BREEDING FOR DROUGHT AND SALINITY STRESS RESISTANT RICE (*ORYZA SATIVA* L.) WITH THE INFLUENCE OF IONIZING RADIATIONS

**K. BAKIRULY<sup>1</sup>, A. ZHALBYROV<sup>1</sup>, Yu. ALEKSIAYENAK<sup>2</sup>, A. KRUGLYAK<sup>2</sup>,  
 L. TOKHETOVA<sup>3\*</sup>, G. BAIMBETOVA<sup>1</sup>, Yu. GLEDENOV<sup>2</sup>, N. APPAZOV<sup>3</sup>, and  
 A. DOROSHKEVICH<sup>2</sup>**

<sup>1</sup>Kazakh Research Institute of Rice named after I. Zhakhaev, Kyzylorda, Kazakhstan

<sup>2</sup>Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Moscow Region, Russia

<sup>3</sup>Korkyt Ata Kyzylorda University, Kyzylorda, Kazakhstan

\*Corresponding author's email: lauramarat\_777@mail.ru

Email addresses of co-authors: kurmanbekbakiruly@mail.ru, aidos090@mail.ru, beataa@gmail.com, anastasiya.Kruglyak@nf.jinr.ru, baimbetova.g@bk.ru, gledenov@nf.jinr.ru, nurasar.82@mail.ru, doroh@jinr.ru

### SUMMARY

The present research aimed to assess the pivotal role of gamma (γ-ray) and fast neutron (FN) radiations in developing rice (*Oryza sativa* L.) mutant types resistant to salinity (NaCl) and drought stress conditions. Local rice cultivars irradiated with different levels of ionizing radiation had their seeds subjected to salinity and drought stress conditions. Rice cultivars showed varied responses to ionizing radiation and stress factors for morphological and yield-related traits. The highest number of mutant forms resulted from the local rice cultivar Syr Suluy, followed by two other cultivars, viz., Leader and Aikerim. The resulting M<sub>1</sub> genotypes significantly differed from the parental forms for morphological and yield-related parameters, i.e., plant height, panicle length, grain size, and grain weight. This genetic material can be effective in the development of synthetic cultivars adapted to the soil and climate stress conditions of the Aral Sea area in Kazakhstan.

**Keywords:** Rice (*O. sativa* L.), cultivars, mutation, gamma rays, fast neutrons, salinity and drought stress conditions, morphological and yield-related traits

**Key findings:** Determining the average lethal irradiation doses was successful for the local rice (*O. sativa* L.) cultivars that had been bred for the specific environmental conditions of Kazakhstan, as well as establishing their salinity and drought treatment levels. Rice cultivar Aikerim showed a higher productivity than the control. The fast neutron-irradiated seeds produced more surviving rice plants with higher productivity than γ-rays.

Communicating Editor: Prof. Naqib Ullah Khan

Manuscript received: September 13, 2024; Accepted: November 14, 2024.

© Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) 2025

**Citation:** Bakiruly K, Zhalbyrov A, Aleksiayenak Yu, Kruglyak A, Tokhetova L, Baimbetova G, Gledenov Yu, Appazov N, Doroshkevich A (2025). Breeding for drought and salinity stress resistant rice (*Oryza sativa* L.) with the influence of ionizing radiations. *SABRAO J. Breed. Genet.* 57(6): 2334-2344. <http://doi.org/10.54910/sabrao2025.57.6.8>.

## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major staple food crops worldwide, especially in Asia. According to 2025 statistics, 89.9% of global rice production was central in Asia, with China and India being the leading producers. In 2025, China produced 214.4 million tons of rice, and India produced 195.4 million tons (FAOSTAT, 2025). The total rice production in Asia amounted to approximately 720.0 million tons, confirming the region's dominant role in global output (World O Stats, 2025). Together, China and India account for nearly 50% of the world's rice production, which has an estimate of around 800 million tons. According to the latest Food and Agriculture Organization (FAO) report, in 2025, the global milled rice production reached a record level of 551.5 million tons, with the growth mainly driven by Asian countries (FAO, 2025). Meanwhile, in 2024, Kazakhstan produced approximately 315,000 tons of milled rice (about 485,000 tons in rough rice), with projections of 335,000 tons for 2025 (USDA/FAS IPAD, 2025).

The Kyzylorda Region is the main rice-growing region in Kazakhstan. The Aral Sea Region in the Kyzylorda territory also experienced intensive desertification, salinization, and soil deflation (Karlykhanov and Toktaganova, 2016; Suska-Malawska *et al.*, 2022). Therefore, these stress conditions necessitate the development of salt- and drought-resistant rice cultivars characterized by high productivity, vigorous initial growth, resistance to diseases and pests, and desirable grain quality (Abikenova *et al.*, 2014; Olzhabayeva *et al.*, 2016; Haque *et al.*, 2021).

However, there is a tendency to reduce the rice varietal diversity, which considerably enhances its genetic vulnerability, and this occurrence depends on the genetic uniformity in rice cultivars. Therefore, the source material is of decisive importance and requires constant resumption, with the introduction of new, economically valuable genes into it. In achieving such goals, one of the effective breeding methods is induced mutagenesis, considered as a source of creating fundamentally new forms worldwide (Sikora *et al.*, 2011; Oladosu *et al.*, 2016). This would

also facilitate the expansion of the synthetic breeding possibilities through the use of mutant forms in the hybridization with unique breeding and valuable traits.

According to the FAO-UN/International Atomic Energy Agency–Mutant Variety Database (MVD) data reports in 2022 on the developed and officially released mutants, 40% of the cereal mutant cultivars consisted of rice (FAO/IAEA-MVD, 2023). In this regard, when breeding new rice cultivars adapted to the stressful soil and climatic conditions of the Kazakhstan Aral Sea Region, radiation breeding plays a significant role. This technique makes it possible to obtain the mutant lines resistant to abiotic stress factors, as well as lines with individual or complex positive traits (Kharitonov *et al.*, 2017; Bakiruly *et al.*, 2022).

In previous years, scientists have begun to perform intensively induced mutagenesis (Viana *et al.*, 2019), using gamma rays (Schiocchet *et al.*, 2014), heavy ions (Ichida *et al.*, 2019; Li *et al.*, 2019), salinity, and drought factors (Abdelnour-Esquivel *et al.*, 2020) to develop new forms of mutant lines resistant to various stress conditions. The gamma rays are the most used physical mutagens (Viana *et al.*, 2019); rice neutron irradiation also showed better results in developing mutants with various desirable traits (Ruengphayak *et al.*, 2015; Li *et al.*, 2016, 2017). Therefore, the following research aimed to assess the effect of gamma rays and fast neutrons on three rice cultivars under salinity and drought stress conditions (Bakiruly *et al.*, 2022; Kruglyak *et al.*, 2022).

## MATERIALS AND METHODS

### Breeding material and procedure

For the presented study, the selected three rice (*Oryza sativa* L.) cultivars were Aikerim, Leader, and Syr Suluy. These rice cultivars already attained approval for widespread cultivation in the Kyzylorda Region, Kazakhstan. The cultivars Aikerim and Syr Suluy entailed specific breeding for the local climate and soil conditions. Therefore, these cultivars were visible with higher grain yields

and rapid adaptation. In managing the field experiment, the following certified guidelines and methodologies proceeded: a) Guidelines for the study of the world collection of rice and the classifier of the genus *Oryza sativa* L. (Rice Growing Guide, 2021), b) Methodology of the field experiment (Rice Growing Guide, 2021), and c) Methodology for performing a cultivar testing (USDA-The Grower's Guide, 2018).

### Experimental design

Seeds of these three rice cultivars experienced two different ionizing irradiations (gamma and fast neutron-FN). One of the goals was to determine the median irradiation lethal dose (LD<sub>50</sub>) for these rice cultivars since literature does not provide unambiguous data on the matter, as well as to determine optimal salinity and drought levels. The considered LD<sub>50</sub> is favorable for induced mutation in breeding, as it provides enough genetic material for selection (Bado *et al.*, 2015).

The relevant study progressed in three stages. The first stage determined the LD<sub>50</sub> for different applied factors, i.e.,  $\gamma$ -rays, FN, NaCl, and sorbitol. For this purpose, using the rice cultivar Syr Suluy seeds was appropriate. The second stage sought to irradiate seeds of the three rice cultivars with the established LD<sub>50</sub> and treat them with drought and salinity agents, enabling plant growth from these seeds. The third stage was the selection of promising mutant lines resistant to salinity and drought, as well as their further study in laboratory and field conditions for the heritability of acquired traits and their breeding values.

### Radiosensitivity determination

The determination of LD<sub>50</sub> for  $\gamma$  irradiation had the cultivar Syr Suluy seeds irradiated with five different doses of  $\gamma$ -rays (50, 100, 150, 200, and 250 Gy). The irradiation was conducted at the ILU-10 Electron Linear Accelerator in the JSC 'Park of Nuclear Technologies' (Kurchatov, Kazakhstan) with the following parameters:  $E = 4$  MeV; the average current value,  $I_{av}$ , was 6.84 mA.

In determining the fast neutron's LD<sub>50</sub>, the rice seeds sustained irradiation with five different doses of FN (5, 25, 50, 75, and 100 Gy) using the EG-5 electrostatic generator in the Joint Institute for Nuclear Research (Dubna, Russian Federation) with the following parameters  $E_n = 4.1$  MeV; deuteron beam current (reaction  $D(d,n)^3\text{He}$ ) was 1.7–2.0  $\mu\text{A}$  with an energy of 2.5 MeV; and neutron flux intensity =  $3 \times 10^7$  particles/cm<sup>2</sup>.

The rice seeds' radiosensitivity determination focused on growth indicators at the initial stage of ontogeny—the germination energy and seeds' germination percentage and the height and weight of 15-day-old seedlings.

### Sensitivity to the stress factors

To establish the sensitivity to salinity and drought factors, unirradiated seeds of the rice cultivar Syr Suluy were left to germinate in thermostat. For the NaCl aqueous solution, the concentrations were 0%, 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% (w/v). For sorbitol, the used concentrations were 5%, 7.5%, 10.0%, and 12.5% (w/v). Defining the rice cultivar sensitivity to salinity and drought and their median lethal concentrations ensued through the energy of growth and seed germination percentage, as well as the height and weight of the 15-day-old seedlings.

### Producing stress-resistant rice lines

To screen for stress-tolerant rice lines, irradiated seeds were treated with salinity and drought stress agents. Seeds from three rice cultivars were used: 1500 seeds per cultivar for  $\gamma$ -irradiation, and 900 seeds per cultivar for FN irradiation at the LD<sub>50</sub> dose. The irradiated seeds totaled 7200, where 4500 seeds were subjected to  $\gamma$ -rays and 2700 to neutrons. The irradiated rice seeds were then divided into three equal groups and treated as follows: two groups with the LD<sub>50</sub> concentration of NaCl and sorbitol, and one group with a combined solution of NaCl and sorbitol, each at half of its respective LD<sub>50</sub> concentration.

For every rice cultivar after the gamma irradiation, 500 seeds entailed NaCl treatment, 500 seeds with sorbitol, with the remaining processed with a combination of NaCl and sorbitol. For neutron irradiation, the scheme was the same as with the 300 seeds for every salinity and drought agent. To see the effect of the abiotic stress and irradiation, the 500 seeds in control were grown under the same conditions to compare with the gamma irradiated plants, as for FN number for control was 300 seeds.

### Field work

Rice plants that survived after irradiation, as well as salinization (NaCl) and drought (sorbitol) factors, experienced placement in the special paddy-field containers during their tillering phase. Containers were 35 cm × 15 cm × 15 cm in size and filled with soil taken from the original paddy fields. Furthermore, the transplanting of plants continued directly into the soil.

The experimental site location was the Scientific Production Station of the Kazakh Rice Research Institute named after I. Zhakhayev (Field No. 4, Check No. 3). The soils' classification was meadow-boggy, typical of rice-growing areas in the region. Soil analysis revealed a slightly alkaline pH of 8.1–8.3 in the 0–40 cm layer. The residual density was 1.12% in the topsoil (0–20 cm) and 1.15% at 20–40 cm depth. The dominant anions were sulfates, with concentrations reaching 0.857 and 0.837 meq/100 g, respectively, while bicarbonate ( $\text{HCO}_3^-$ ) and chloride ( $\text{Cl}^-$ ) levels remained low. Among the cations, calcium (0.19–0.21 meq/100 g) and magnesium (0.026–0.032 meq/100 g) were prevalent, with moderate sodium content (0.023–0.026 meq/100 g). The total salt content exceeded the critical threshold for rice, measuring 0.758% in the upper layer and 0.795% at deeper levels. Based on ion composition and concentration, the soil attained a strongly saline classification of the sulfate type.

During the growing season, phenological observations of plant growth and development took place, including two-time

fertilization with nitrogen. Before harvest, the remaining plants were counted with all the surviving rice plants removed by the roots for agronomic and morphological analysis and photographs.

### Data analysis

The analysis of the obtained data continued in Statistica 8.0. Samples with normal distribution were analysed by parametric statistics, while the non-normal distribution sample methods acquired non-parametric statistics, i.e., the Kruskal-Wallis Test. Survival of the emerged plants' percentage (plant survival rate – PSR) and general survival (1, 2) entailed calculation according to the guidance of the United States Environmental Protection Agency (USEPA, 2012, 2018), as well as the percentage of seed germination and the lethality (3, 4) (Krebs, 2014; Mondal *et al.*, 2021).

$$\text{Survival of emerged plants(\%)} = \frac{\text{Number of plants at maturity}}{\text{Number of seeds germinated}} \times 100\%$$

$$\text{Survival(\%)} = \frac{\text{Number of surviving plants at the end of the study}}{\text{Number of planted seeds}} \times 100\%$$

$$\text{Survival of emerged plants(\%)} = \frac{\text{Number of plants at maturity}}{\text{Number of seeds germinated}} \times 100\%$$

$$\text{Lethality(\%)} = \frac{\text{Contol} - \text{Treated}}{\text{Control}} \times 100\%$$

### RESULTS

The results showed the rice with LD<sub>50</sub> of γ-rays and FN were 100 and 50 Gy, respectively. The median lethal concentration of NaCl was 1.0% aqueous solution and 7.5% for sorbitol. The analysis revealed significant differences existed among the rice seedlings grown from unirradiated seeds (control) and the seedlings grown from γ-rays and FN-irradiated seeds (Table 1). These differences were statistically

**Table 1.** Mean values of rice seedlings grown from irradiated and control seeds and T-test results.

Rice seedlings	Gamma irradiation			FN irradiation		
	Mean	Mean Control	t-value, <i>P</i> < 0.05	Mean	Mean Control	t-value, <i>P</i> < 0.05
Seedling number	91.3±36.3	484.0±3.1	6.05	111.3±23.0	292.0±1.0	4.4

**Table 2.** Mean values of rice cultivars with irradiation and control under salinity and drought stress conditions in terms of germination, survival, and lethality percentage.

Cultivars	Physical and chemical impacts	Germination (%)	Survival of the emerged plants (PSR) (%)	Survival (%)	Lethality (%)
Aikerim	gamma + NaCl	45.2	1.3	0.6	53.9
	gamma + sorbitol	3.0	6.7	0.2	96.9
	gamma + NaCl + sorbitol	3.6	5.6	0.2	96.3
	Control	98.0	71.4	70.0	0.0
	FN + NaCl	56.0	4.2	2.3	42.3
	FN + sorbitol	4.3	15.4	0.7	95.5
	FN + NaCl + sorbitol	5.3	12.5	0.7	94.5
	Control	97.0	74.2	72.0	0.0
Leader	gamma + NaCl	63.8	0.0	0.0	33.8
	gamma + sorbitol	4.2	28.6	1.2	95.6
	gamma + NaCl + sorbitol	6.2	25.8	1.6	93.6
	Control	96.4	71.6	69.0	0.0
	FN + NaCl	47.0	0.7	0.3	52.0
	FN + sorbitol	35.0	12.4	4.3	64.3
	FN + NaCl + sorbitol	45.0	11.8	5.3	54.1
	Control	98.0	66.3	65.0	0.0
Syr Suluy	gamma + NaCl	21.0	8.6	1.8	78.1
	gamma + sorbitol	7.2	8.3	0.6	92.5
	gamma + NaCl + sorbitol	10.2	23.5	2.4	89.4
	Control	96.0	69.8	67.0	0.0
	FN + NaCl	72.0	13.9	10.0	25.8
	FN + sorbitol	20.3	23.0	4.7	79.0
	FN + NaCl + sorbitol	49.0	20.4	10.0	49.5
	Control	97.0	73.2	71.0	0.0

significant, with t-values of 6.05 and 4.4, respectively, indicating a strong negative impact of irradiation on seedling survival.

For the seed germination, the highest seed survival rate—germination (Krebs, 2014)—in all the rice cultivars emerged with the NaCl treatment and irradiation with  $\gamma$ -rays (21.0%–64.0%) and FN (47.0%–72.0%). The control's survival rate was in the range of 96% to 98% for all the rice cultivars (Table 2). The least number of germinating grains appeared after irradiating rice seeds with the sorbitol treatment. In general, out of 7200 treated seeds, only 1824 seeds provided sprouts.

However, after 10 days of the seedlings' emergence, most seedlings began to die, especially those in the gamma-ray-irradiated rice cultivars. As a result, 20 days after the seed's germination of all the variants' versions, only one to 30 plants remained alive. Hence, out of 1824 sprouted rice plants, only 197 stayed alive and succeeded in transplanting to paddy fields in special containers. It should be noteworthy that all transplanted plants survived until the end of the study. Median plant survival rate (PSR) was 10.8%, while for some rice cultivars and treatments, it was up to 28.6% (Table 2).

**Table 3.** Duration of interphase and vegetation periods of M<sub>1</sub> plants obtained after treating the rice cultivar seeds with gamma rays and fast neutrons under salinity and drought stress conditions.

Cultivar	Physical and chemical impacts	Duration of the interphase period (days)				Growing season (days)
		Flooding of seedlings	Shoots – tillering	Tillering – earing	Earing – full ripeness	
Aikerim	gamma + NaCl	13-14	33-35	31-32	43-45	119-125
	gamma + sorbitol	13	33	32	43	121
	gamma + NaCl + sorbitol	13	33	32	44	122
	FN + NaCl	12-13	32-34	33-35	43-46	120-128
	FN + sorbitol	13	32	35-36	44	124-125
	FN + NaCl + sorbitol	12-13	31-32	36-37	43-45	122-127
	Control	12	32	30	42	116
Leader	gamma + NaCl	No shoots				
	gamma + sorbitol	12-13	33-35	31-33	44-46	120-127
	gamma + NaCl + sorbitol	12-13	32-35	31-33	44-45	119-126
	FN + NaCl	12	34	33	45	124
	FN + sorbitol	12-13	32-33	31-33	44-46	119-125
	FN + NaCl + sorbitol	12-13	33-34	31-33	44-46	120-126
	Control	12	32	31	43	118
Syr Suluy	gamma + NaCl	11	32	30	41-42	114-115
	gamma + sorbitol	11	33	30-31	42-43	116-118
	gamma + NaCl + sorbitol	12	33	31-32	42-45	117-122
	FN + NaCl	10-12	31-33	31-34	41-44	113-123
	FN + sorbitol	10-11	32-33	32-34	42-45	116-123
	FN + NaCl + sorbitol	11-12	31-33	31-35	43-46	116-126
	Control	10	30	28	37	105

Meanwhile, the most number of surviving plants (from three plants after the gamma + sorbitol treatment up to 30 plants after the FN + NaCl + sorbitol treatment) resulted in the cultivar Syr Suluy, with a PSR of up to 23.5%, followed by the cultivar Leader (from one plant after the FN + NaCl treatment up to 16 plants after the FN + NaCl + sorbitol treatment) with a PSR of up to 28.6%. The lowest mean of PSR after  $\gamma$ -irradiation and NaCl treatment was 0.0% (plants did not survive), with the least number coming from the cultivar Aikerim (from one plant after the gamma + sorbitol treatment up to seven plants after the FN + NaCl treatment) with a PSR of up to 15.4%. Germination rate also varies for different cultivars and treatments. The best germination percentage (72%) was evident for the cultivar Syr Suluy after the FN irradiation and NaCl treatment.

The results based on the phenological observations revealed almost all altered plant forms had interphase periods of either growth

and development prolongation or retardation, except the individual mutant plants of the cultivar Aikerim (Table 3). The observed high level of panicle emptiness and low plant growth confirm the manifestation of mutational processes under the influence of ionizing radiation. Thus, compared with control cultivars under the same conditions, the extension of interphase periods resulted in the elongation of the vegetation period for the cultivars Aikerim (3–12 days), Leader (1–9 days), and Syr Suluy (9–21 days).

The analysis of morphological and agronomic traits of the rice mutant lines resistant to salinization and drought indicated that, depending on the cultivar, irradiation type, and stress factor, the obtained mutants sharply differed (with KW-H = 87.9,  $P < 0.0001$ ). These outcomes are both from the original forms and among the irradiated for various traits (Table 4). Additional post-hoc comparisons using the Tukey HSD test indicated morphological and agronomic

**Table 4.** Descriptive statistics of morphological and agronomic traits of the rice M<sub>1</sub> lines obtained after ionizing radiations under salinity and drought stress conditions.

Traits	Means	Median	Minimum	Maximum	Variance	Std. Dev.	Coeff. of Var.	Std. Er.
Plant height (cm)	73.3	67.0	47.0	129.0	426.6	20.6	28.2	1.5
Bushiness (#)	6.9	6	1	22	15.7	4.0	57.6	0.29
Productive bushiness (#)	2.1	1	0	9	3.7	2.0	92.1	0.14
Panicle length (cm)	14.0	13	6.5	26	14.8	3.8	27.57	0.28
Number of grains (#)	38	19	0	162	1951.3	44.2	116.20	3.25
Puny grains (#)	39.0	32	0	130	759.2	27.5	70.80	2.2
Emptiness (%)	58.0	62.2	2.9	100	858.7	29.3	50.60	2.15
Grain weight (g)	1.1	0.55	0	5.1	1.6	1.3	113.00	0.09
Grain weight per plant (g)	4.2	0.95	0	29.3	52.7	7.2	174.00	0.53

characteristics, such as bushiness and puny grains, were nonsignificantly ( $p > 0.1$ ) different from the control, while other characteristics showed a significant difference ( $p < 0.0001$ ). A substantial number of resistant mutant plants to stress factors resulted in the cultivar Syr Suluy (98 plants), followed by Leader (44 plants) and Aikerim (16 plants). The number of mutant plants derived from  $\gamma$ -rays and FN irradiation was 10 and 11 for cultivar Aikerim, 14 and 30 for Leader, 24 and 74 for Syr Suluy, and a total of 43 and 115 plants, respectively.

In the plant population of various rice cultivars, those resistant plants to NaCl/sorbitol were in the cultivars Aikerim (10/3 plants), Leader (1/19 plants), and Syr Suluy (30/17 plants). However, the plants resistant to NaCl + sorbitol were in cultivars Aikerim (three plants), Leader (24 plants), and Syr Suluy (42 plants). A significant difference also occurred between the mutants and the parental cultivars in quantitative terms. The height of mutant plants of the cultivar Aikerim actually changed in both directions, and an increase of up to 127 cm and a decrease of up to 98 cm were notable. Meanwhile, in the cultivars Leader and Syr Suluy, the mutants were 11 to 41 cm lower than the parental genotypes.

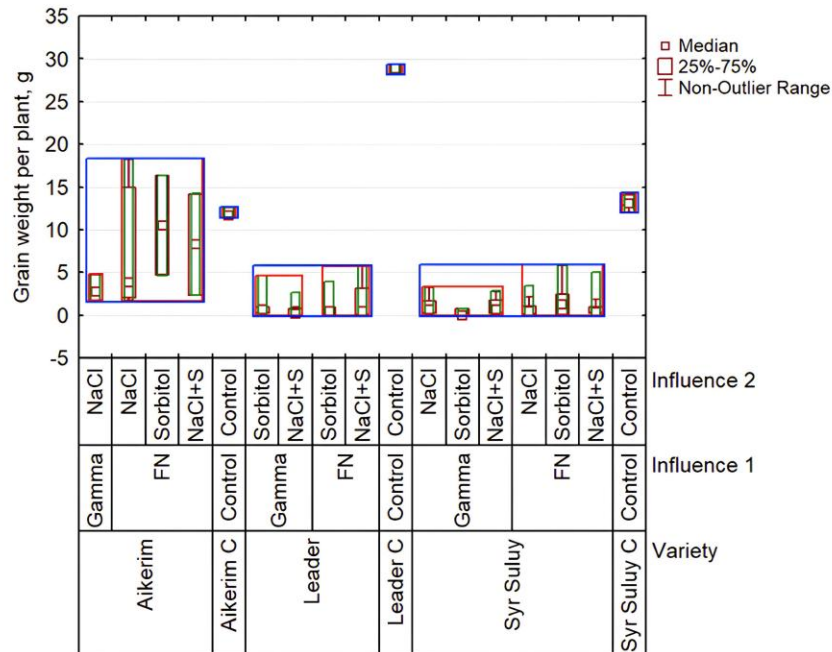
In mutants for general and productive tillering, the large fluctuation was remarkable. The total productivity of mutants for general and productive tillering ranged from 1 to 47 and 4.4–7.0, respectively, in initial plants. However, the mutants had very low rates of productive tillering (0–4) compared with the original cultivars (4.4–7.0). The main panicles

of the altered cultivar Aikerim plants appeared to be 0.4–6.4 cm longer than the parental form (19.6 cm). In the cultivars Leader and Syr Suluy, the main panicles were 0.6–6.1 and 3.0–11.5 cm, respectively, shorter than the parental types.

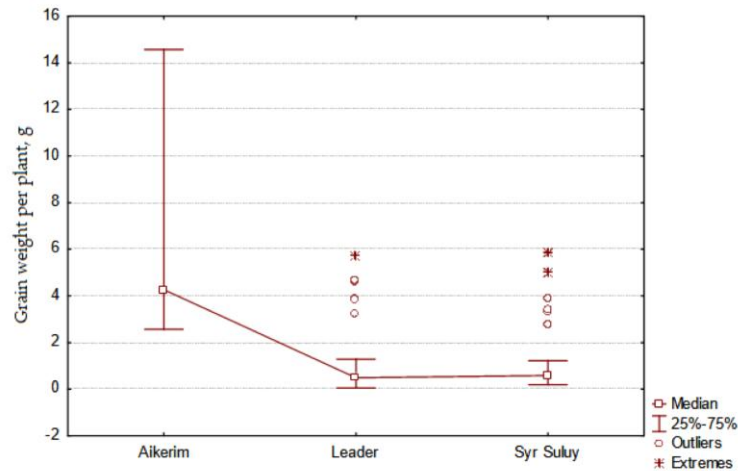
A very high percentage of grain desolation was also apparent in the initial forms (21.2%–28.9%). This was largely due to frequent interruptions in the supply of irrigation water during the flowering of rice plants, especially in mutant plants, which contributed to an acute grain fertility decrease. A similar pattern arose for the grain weight from all the panicles and a single plant.

The analysis further revealed that despite the cultivar Aikerim having the lowest level of survival, 31% of the matured plants had better productive traits than the control plants. The control (non-irradiated) plants have a higher grain weight per plant than the mutated ones, and this was noticeable in the cultivars Leader and Syr Suluy. For the cultivar Leader in the control, the average grain weight was  $28.76 \pm 0.13$  g; for the mutated plants, the value was  $1.15 \pm 0.23$  g; and in cultivar Syr Suluy, it was  $13.25 \pm 0.24$  and  $0.9 \pm 0.1$  g). For a third of the plants in the cultivar Aikerim, the weight was  $17.08 \pm 0.1$  g vs. the control ( $11.8 \pm 0.1$  g). The control groups of the cultivars Aikerim and Syr Suluy have similar productive levels (Figure 1).

Significant differences were evident among the different rice cultivars for grain weight per plant, with the KW-H = 32.6 and  $p < 0.0001$  (Figure 2). Based on Figures 1 and 2, one can conclude that cultivar Aikerim plants



**Figure 1.** Grain weight per plant obtained from the control and  $M_1$  plants of three rice cultivars treated with gamma and neutron irradiations under NaCl, sorbitol, and NaCl + sorbitol conditions.



**Figure 2.** Grain weight (g) per plant grown from the irradiated seeds and based on the rice cultivars.

had higher variability than other rice cultivars and their control plants. From this viewpoint, cultivar Aikerim seems very promising for further breeding. The coefficient of the grain mass index variation of one plant reaches

90.59%. In other cultivars, significant differences existed between the experimental and control plants (KW-H = 45.61;  $p < 0.01$ ) and among control cultivars (KW-H = 14.3;  $p < 0.01$ ). The experimental plants had



significantly lower productive indicators than the control. Within the control group, the cultivar Leader considerably differed from the rice cultivar Syr Suluy in terms of grain weight per plant.

One should also consider that the share of the exposure factor in combination with chemical pollutants in the total sample was massive. The value of the certain factors' prevalence ( $\eta^2 = 82.36$ ) means that irradiation and the chemical factors reliably affect the grain mass index per plant. For irradiation types, nonsignificant differences surfaced in the grain mass per plant between those irradiated with gamma radiation or FN (KW-H = 0.53;  $p < 0.05$ ). However, despite the unreliable values, it was obvious that plants irradiated with FN have numerous outliers and extremes (Figure 1). This means that among the entire sample of irradiated plants, one can meet a single specimen with a value of the grain mass index per plant comparable to the control samples. However, we would like to point out the lethality percentage was higher for the gamma-ray irradiation than for fast neutrons in all the cultivars, except Aikerim (Table 2).

## DISCUSSION

This study investigated three rice cultivars by subjecting them to irradiation under simulated conditions of soil salinity and desertification. The established median lethal dose of 100 Gy for  $\gamma$ -rays aligns with past findings (Abdelnour-Esquivel *et al.*, 2020), while for FN, there were no applicable data in the literature, and even with the wide range of FN energy, past research sometimes had no statements (FAO/IAEA, 2018).

For salinity levels, reports existed about NaCl concentration and identification of the promising tolerant lines. Tested salinity and drought concentrations matched reported thresholds, i.e., salt concentration varies with the range of 0.5%–10% (w/v) (Chen *et al.*, 2017; Abdelnour-Esquivel *et al.*, 2020). Reddy *et al.* (2017) also declared that different salt concentrations cause overexpression of some

genes, as well as showed different germination rates (Haque *et al.*, 2021). Similarly, optimal sorbitol levels for simulating drought effects differed, ranging from 5% to 36% (w/v) (Marssaro *et al.*, 2017; Abdelnour-Esquivel *et al.*, 2020).

All the rice cultivars showed better germination rates after treatment with NaCl + gamma (21.0%–63.8%) and NaCl + FN (47.0%–72.0%). The general plant survival was quite low; most of the seedlings died on the 20th day, and the highest survival rate resulted in Syr Suluy treated by FN + NaCl + sorbitol (10%). The cultivar Syr Suluy particularly showed resilience, yielding 98 stress-resistant mutant lines, followed by cultivars Leader (44 lines) and Aikerim (16 lines). These surviving plants exhibited significant morphological alterations, including extended vegetative periods, dwarfism (40–80 cm height), high panicle sterility (up to 100%), and notable variation in productive traits, which strongly suggested that these mutant types proved resistant to salinity, drought, and combined stress factors.

These promising plants underwent complete preservation until the end of harvesting. The obtained seeds entailed planting in the spring of 2024, and the M<sub>2</sub> generation will be further studied. Typically, the M<sub>2</sub> generation is studied because mutant phenotypes in the M<sub>1</sub> generation may result from physical damage caused by irradiation (FAO/IAEA, 2018). The mutant phenotypes in the M<sub>2</sub> generation highly occurred due to heritable effects. Therefore, M<sub>2</sub> plant phenotypes will be collected and cataloged. Their seeds will form the basis of a gamma and FN mutant library.

Plants grown from irradiated grains have a very high coefficient of productive trait variation, which makes it possible to count on considerable results of breeding work with these plants. The rice cultivar Aikerim warrants special attention due to its exceptional productive performance (90.59% grain mass variation) despite lower survival rates, suggesting a unique stress adaptation mechanism.

## CONCLUSIONS

Median lethal irradiation doses and stress factor concentrations were successful in their establishment, especially for the local rice cultivars, which will be beneficial in future work. The study successfully generated rice lines with proven resistance to regional abiotic stresses, providing valuable material for Kazakhstan's rice breeding programs. Further analysis of the M<sub>2</sub> generation will validate heritable traits for cultivar development. Particular promise emerged from the rice cultivar Aikerim mutants despite lower survival rates, warranting additional study due to their exceptional productivity traits.

## ACKNOWLEDGMENTS

The work proceeded within the framework of the program-targeted funding for scientific and technical programs for 2024–2026 of the Ministry of Agriculture of the Republic of Kazakhstan, "Breeding, seed production of grain crops to increase potential of productivity, quality, stress resistance in various soil-climatic zones of Kazakhstan," IRN BR24892821.

## REFERENCES

- Abdelnour-Esquivel A, Perez J, Rojas M, Vargas W, Gatica-Arias A (2020). Use of gamma radiation to induce mutations in rice (*Oryza sativa* L.) and the selection of lines with tolerance to salinity and drought. *In Vitro Cell. Dev. Biol. Plant* 56: 88-97. doi:10.1007/s11627-019-10015-5.
- Abikenova SM, Rau AG, Assanbekov BA, Zhanashev IZ, Kalybekova EM (2014). Research of the rice productivity on saline lands of rice systems in Kazakhstan Republic. *Life Sci. J.* 11: 356-361.
- Bado S *et al.* (2015). Plant mutation breeding: Current progress and future assessment. *Plant Breed. Rev.* 39: 23-88. doi:10.1002/9781119107743.ch02.
- Bakiruly K, Tautenov IA, Zhalbyrov AE (2022). Creation of source material for rice breeding by treating seeds with ionizing radiation. *Bull. Korkyt Ata Kyzylorda Univ.* 3(62): 55-64.
- Chen R, Cheng Y, Han S, Handel BV, Dong L, Li X, Xie X (2017). Whole genome sequencing and comparative transcriptome analysis of a novel seawater adapted, salt-resistant rice cultivar - sea rice 86. *BMC Genomics* 18: 655. doi:10.1186/s12864-017-4037-3.
- FAO (2025). Food Outlook – Biannual Report on Global Food Markets, June 2025. Retrieved from <https://www.fao.org/newsroom/detail/fao-food-outlook--global-output-of-key-food-commodity-crops-on-course-for-new-records/en>.
- FAO/IAEA (2018). Manual on Mutation Breeding. 3rd Ed. M.M. Spencer-Lopes, B.P. Forster, and L. Jankuloski (Eds.). Rome. 301 pp.
- FAO/IAEA-MVD (2023). Food and Agriculture Organization of the United Nations/International Atomic Energy Agency - Mutant Variety database. <https://nucleus.iaea.org/sites/mvd>.
- FAOSTAT (2025). Food and Agriculture Organization Statistics Database. Retrieved from <https://www.fao.org/faostat/en/#data/QCL>.
- Haque MA, Rafii MY, Yusoff MM, Ali NS, Yusuff O, Datta DR, Anisuzzaman M, Ikbali MF (2021). Advanced breeding strategies and future perspectives of salinity tolerance in rice. *Agronomy* 11(1631). doi:10.3390/agronomy11081631.
- Ichida H, Morita R, Shirakawa Y, Hayashi Y, Abe T (2019). Targeted exome sequencing of unselected heavy-ion beam-irradiated populations reveals less-biased mutation characteristics in the rice genome. *Plant J.* 98(2): 301-314. doi:10.1111/tpj.14213.
- Karlykhanov OK, Toktaganova GB (2016). The assessment of irrigated land salinization in the Aral Sea Region. *Int. J. Environ. Sci. Edu.* 11(15): 7946-7960.
- Kharitonov EM, Goncharova YK, Maliuchenko EA (2017). The genetics of the traits determining adaptability to abiotic stress in rice (*Oryza sativa* L.). *Russ. J. Genet. Appl. Res.* 7: 684-697. doi:10.1134/S2079059717060089.
- Krebs CJ (2014). Ecological Methodology. 3rd Ed. Univ. British Columbia.
- Kruglyak AI, Alekseenok YuV, Doroshkevich AS, Appazov NO, Bakiruly KB (2022). Obtaining a drought-resistant variety of rice culture as a result of mutagenesis induced by neutrons generated at the EG-5 installation at JINR. In: Proc. 1st Int. Conf. 'Genetic and Radiation Technologies in Agriculture,' Obninsk, Russia.
- Li F, Shimizu A, Nishio T, Tsutsumi N, Kato H (2019). Comparison and characterization of mutations induced by gamma-ray and carbon-ion irradiation in rice (*Oryza sativa* L.) using whole-genome resequencing. *G3 (Bethesda)*, 9(11): 3743-3751. doi:10.1534/g3.119.400555.

- Li G, Jain R, Chern M, Jain R, Martin JA, Schackwitz WS, Jiang L, Vega-Sánchez ME, Lipzen AM, Barry KW, Schmutz J, Ronald PC (2016). Genome-wide sequencing of 41 rice (*Oryza sativa* L.) mutated lines reveals diverse mutations induced by fast-neutron irradiation. *Mol. Plant.* 9(7): 1078-1081. doi:10.1016/j.molp.2016.03.009.
- Li G, Jain R, Chern M, Pham NT, Martin JA, Wei T, Schackwitz WS, Lipzen AM, Duong PQ, Jones KC, Jiang L, Ruan D, Bauer D, Peng Y, Barry KW, Schmutz J, Ronald PC (2017). The sequences of 1504 mutants in the model rice variety Kitaake facilitate rapid functional genomic studies. *Plant Cell*, 29(6): 1218-1231. doi:10.1105/tpc.17.00154.
- Marssaro AL, Morais-Lino LS, Cruz JL, Ledo CA, Santos-Serejo JA (2017). Simulation of in vitro water deficit for selecting drought-tolerant banana genotypes. *Pesq. Agropec. Bras.* 52: 1301-1304. doi:10.1590/s0100-204x2017001200021.
- Mondal M, Biswas B, Garai S, Adhikary S, Bandyopadhyay PK, Sarkar S, Banerjee H, Brahmachari K, Maitra S, Mandal TK, Gaber A, Althobaiti YS, Raafat BM, Hossain A (2021). Raising climate-resilient embolden rice (*Oryza sativa* L.) seedlings during the cool season through various types of nursery bed management. *Sustainability* 13: 12910. doi:10.3390/su132212910.
- Oladosu Y, Rafii MY, Abdullah N, Hussin G, Ramli A, Rahim HA et al. (2016). Principle and application of plant mutagenesis in crop improvement: A review. *Biotechnol. Biotechnol. Equip.* 30(1): 1-16. doi:10.1080/13102818.2015.1087333.
- Olzhabayeva AO, Rau AG, Sarkynov ES, Baimanov ZN, Shomantaev AA (2016). Effect of irrigation and fertilizers on rice yield in conditions of Kyzylorda irrigation array. *Biosci. Biotechnol. Res. Asia* 13(4): 2045-2053.
- Reddy INBL, Kim BK, Yoon IS, Kim KH, Kwon TR (2017). Salt tolerance in rice: Focus on mechanisms and approaches. *Rice Sci.* 24(3): 123-144. doi:10.1016/j.rsci.2016.09.004.
- Rice Growing Guide (2021). 2nd Ed. R. Ward (Ed.). NSW Dept. Primary Industries. 55 pp.
- Ruengphayak S, Ruanjaichon V, Saensuk C, Phromphan S, Tragoonrun S, Kongkachuichai R, Vanavichit A (2015). Forward screening for seedling tolerance to Fe toxicity reveals a polymorphic mutation in ferric chelate reductase in rice. *Rice* 8. doi:10.1186/s12284-014-0036-z.
- Schiocchet MA, Noldin JA, Raimondi JV, Neto AT, Marschalek R, Wickert E, Martins GN, Hickel E, Knoblauch R, Scheuermann KK, Eberhardt DS, Andrade A (2014). SCS118 Marques - new rice cultivar obtained through induced mutation. *Crop Breed. Appl. Biotechnol.* 14: 68-70. doi:10.1590/S1984-70332014000100012.
- Sikora P, Chawade A, Larsson M, Olsson J, Olsson O (2011). Mutagenesis as a tool in plant genetics, functional genomics, and breeding. *Int. J. Plant Genomics.* 2011: 1-13. doi:10.1155/2011/314829.
- Suska-Malawska M, Vyrakhamanova A, Ibraeva M, Poshanov M, Sulwinski M, Toderich K, Metrak M (2022). Spatial and in-depth distribution of soil salinity and heavy metals (Pb, Zn, Cd, Ni, Cu) in arable irrigated soils in Southern Kazakhstan. *Agronomy* 12(5): 1207.
- USDA (2018). The Grower's Guide to Conducting On-farm Variety Trials. USDA RMA, Organic Seed Alliance. [https://seedalliance.org/wp-content/uploads/2018/02/Growers-guide-on-farm-variety-trials\\_FINAL\\_Digital.pdf](https://seedalliance.org/wp-content/uploads/2018/02/Growers-guide-on-farm-variety-trials_FINAL_Digital.pdf).
- USDA/FAS IPAD. Kazakhstan Rice Area, Yield and Production (Marketing Years 2023/24–2024/25). <https://ipad.fas.usda.gov/countrysummary/Default.aspx?crop=Rice&id=KZ>.
- USEPA (2012). Ecological Effects Test Guidelines OCSP 850.4100: Seedling Emergence and Seedling Growth. <https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100IRBM.txt>.
- USEPA (2018). Part 158 Nontarget Plant Protection Data Requirements: Guidance for Calculating Percent Survival in Seedling Emergence Studies. [https://www.epa.gov/sites/default/files/2018-02/documents/arification\\_on\\_calculation\\_of\\_survival\\_seedling\\_emergence\\_study.pdf](https://www.epa.gov/sites/default/files/2018-02/documents/arification_on_calculation_of_survival_seedling_emergence_study.pdf).
- Viana VE, Pegoraro C, Busanello C, Costa-de-Oliveira A (2019). Mutagenesis in rice: The basis for breeding a new super plant. *Front. Plant Sci.* 10: 1326. doi:10.3389/fpls.2019.01326.
- World O Stats (2025). Rice Production by Country. Retrieved from <https://worldostats.com/country-stats/rice-production-by-country/>.