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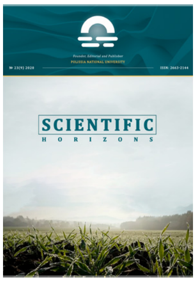

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Growth performance, yield, and proline content of two local cultivars of black rice irradiated by gamma rays for drought resistance

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Abstract. One of the critical effects of climate change on the agricultural environment is drought stress. This study aimed to determine the growth, yield performance, and proline content of black rice local cultivars Bantul, Boyolali mutant M2 at various doses of gamma rays and the proline content of mutant M3 during drought stress. The experiment used a Completely Randomized Design (CRD) with the factor dose of gamma-ray irradiation (0 Gy, 100 Gy, 200 Gy, and 300 Gy), applied in both paddy fields and polybags to observe and compare the growth, yield, and proline content of black rice plants with the control treatment (0 Gy). Laboratory test results showed that Boyolali and Bantul cultivars irradiated at 100 Gy and 200 Gy, had the highest proline content, which increased under drought stress conditions in both cultivars. At 100 Gy,

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the average plant height and the number of productive tillers of black rice of Boyolali and Bantul cultivars were the highest, and the diversity of the two local cultivars at this dose was higher than at other irradiation doses. Applying 100 Gy and 200 Gy gamma-ray irradiation doses on the Boyolali cultivar significantly affected the panicle length character and the number of seeds per panicle. For the Bantul cultivar, panicle length and the number of seeds per panicle at an irradiation dose of 200 Gy showed significantly different results. The conclusion is that a reduced amount of gamma-ray irradiation can enhance the growth, yield, and proline content of M2 black rice

Keywords: functional food; rice plant; water stress; agriculture; climate change

INTRODUCTION

Black rice (*Oryza sativa* L.) is a viable alternative to white rice and holds significant economic importance. It has gained widespread recognition due to the growing health consciousness among the public. Considered a functional food, black rice is rich in anthocyanin, fibre, and iron, making it beneficial for health (Rahim *et al.,* 2022). According to F. Agustia *et al.* (2019), black rice contains 7.5% dietary fibre and 5.8% hemicellulose. The fibre and hemicellulose in white rice are only 5.4% and 2.2%, respectively. Black rice has flavonoid content that is five times higher than white rice, and the calories of black rice are 362 kcal/100 grams lower than other rice (Aini *et al.,* 2023). However, local black rice still has the characteristics of a tall plant posture (>150 cm), a long life (>145 days), and low productivity, so farmers rarely cultivate black rice. Plants that are too tall will fall quickly. Boosting black rice productivity will become more profitable, if these weaknesses are resolved. Black rice M2 local cultivars Boyolali and Bantul have good characteristics from the M1 parents, namely low plant height and high yields seen from the weight of grain per clump. These good traits will be passed down and tested on M2 generation black rice, which is expected to be more stable than the elders. M2 hopes for higher genetic stability and will enter the advanced test stage to serve as a superior cultivar in the future. The genetic diversity of growth and yield characters displayed by M2 black rice is still high. According to R. Darmawan and D. Damanhuri (2019), plant breeding activities require a wide genetic diversity for the selection process to be more effective.

Today, global warming and the increasing human population pose problems in increasing water availability for agriculture. One of the effects of global warming is drought. It is a challenge in agriculture in maintaining plant growth and productivity in drought conditions. Over 4 million hectares of rainfed lowland and highland rice areas spread throughout the regions in Indonesia including Java, Sumatra, Kalimantan, Sulawesi, Bali, Nusa Tenggara, Maluku, and Papua. The area of rainfed rice fields, 2.07 million hectares, is divided between 33.8% of Java and other regions (Erythrina *et al.,* 2021). Drought tolerant rice cultivars that are tolerant to drought are needed to deal with water shortages in the future. It can use mutation breeding and selection activities to improve plant traits and maintain good plant traits. In addition, it is also expected to obtain new mutant lines that have the potential to be drought-tolerant and have high yields. According to Nandariyah *et al.* (2021), mutation breeding using gamma-ray irradiation can enhance the characteristics of black rice for the better. Mutations with gamma-ray irradiation have been widely applied to various types of plants to increase plant resistance to drought stress, such as soybeans (Oktavianus *et al.,* 2019), rice (Dama *et al.,* 2020), peanuts (Hemon *et al.,* 2020) and soybean cultivar namely kipas putih.

Plants undergo various metabolic adjustments to regulate morphological adaptations and physiology to environmental changes (Zaib *et al.,* 2023). Proline accumulation is an indicator of a plant's physiological response to drought. Proline acts as an osmolyte and maintains osmotic regulation in plant organs so plants remain protected when water potential is low (Ozturk *et al.,* 2020). M. Meena *et al.* (2019) added that proline contributes to counteracting ROS and sustaining the structure of membranes and proteins. The response of the cempo ireng rice line to drought stress by increasing the leaf proline content (Patmi *et al.,* 2020). Drought tolerant plants will accumulate more proline than sensitive plants. According to A. Furlan *et al.* (2020), the elevated levels of proline content in plants indicate a relationship with drought tolerance. The growth, yield, and proline content information of Boyolali and Bantul M2 black rice cultivars are limited. It must know the research on the tolerance level of local cultivars of black rice Boyolali and Bantul M2 before selecting individual drought-tolerant plants. The proline content in each black rice cultivar indicates the tolerance level to drought stress. Therefore, it is necessary to research to examine more deeply the growth performance, yield, and proline content of local cultivars of black rice Boyolali and Bantul M2 that are irradiated at various doses of gamma-ray irradiation. This study aimed to determine the growth and yield performance and the proline content of black rice local cultivars of Boyolali and Bantul M2 due to gamma irradiation. Furthermore, observation of M3 black rice proline content at various doses of gamma rays when subjected to drought stress treatment.

MATERIALS AND METHODS

The research was conducted in 2023 and carried out in two places, namely the planting of M2 black rice in the rice fields of Jati Village, Jaten District, Karanganyar

Regency, and M3 black rice planting in Kaloran Village, Wonogiri District, Wonogiri Regency in polybags. The proline content test was carried out at the Biochemistry Laboratory, Universitas Gadjah Mada. Implementation in the rice fields of Jaten Karanganyar used a single factor completely randomized design (CRD), namely the dose of gamma-ray irradiation (0 Gy, 100 Gy, 200 Gy, and 300 Gy) without replication because the experimental unit is homogeneous. The materials used were black rice seeds selected by M1 from Boyolali and Bantul irradiated with 100 Gy, 200 Gy, and 300 Gy gamma rays and control (0 Gy). Other materials used were urea fertilizer, ponskha, SP-36, pesticides, and chemicals to test the leaf proline content. In this study, the growth, yield, and proline content of individual black rice irradiated by gamma rays were observed and compared with the control treatment (0 Gy).

The second experiment was carried out in polybags with drought stress treatment. The material used was black rice produced by local cultivars M3 Boyolali and Bantul irradiated by gamma rays. The study used a completely randomized design (CRD) with gamma-ray irradiation factors (irradiation levels of 100 Gy, 200 Gy, and 300 Gy) subjected to drought stress and without drought. This second experiment tests the proline content of local cultivars of black rice Boyolali and Bantul due to gamma-ray irradiation. According to T. Handayani and H. Kurniawan (2018), the procedure for analyzing leaf proline content is to smooth black rice leaf samples of each black rice cultivar irradiated with gamma rays and without irradiation with 3% sulfosalicylic acid. The smooth leaves were filtered with Whatman #2 filter paper, then the filtrate was added with ninhydrin acid and glacial acetic acid at 100°C. One hour later, it was extracted with toluene to form two phases. It took the upper phase (chromophore) and measured it at an absorbance of 520 nm – calculation of proline concentration with a standard curve.

Observation variables included number of productive tillers, plant height, panicle length, number of seeds per panicle, the weight of 100 seeds, and proline content of black rice planted in paddy fields and polybags. The growth and yield components of local cultivars of black rice from Boyolali and Bantul that were exposed to gamma-ray irradiation at levels of 100 Gy, 200 Gy, and 300 Gy were compared descriptively with those of black rice that was not irradiated. The growth and yield data were further analyzed using a T-test to determine the significance of the differences between the two cultivars of gamma-irradiated with black rice (0 Gy). The proline content data were analyzed descriptively by comparing the proline content of each cultivar under different doses of gamma-ray irradiation and a control group (not exposed to gamma-ray irradiation). Data were presented in tabular form. The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

The T-test indicated that the mean height of rice plants (Table 1) and the number of productive tillers (Table 2) were significantly different between black rice cultivars from Boyolali and Bantul irradiated with 100 Gy gamma rays compared to non-irradiated control black rice (0 Gy). The average plant height of Boyolali and Bantul cultivars has the highest dose of 100 Gy, with the highest standard deviation in each black rice cultivar. It showed that the black rice's two local cultivars exhibited greater diversity in plant height and number of productive tillers at a irradiation dose of 100 Gy compared to other doses.

***Table 1**. Plant height of M2 mutant black rice cultivars Boyolali and Bantul irradiated by gamma rays with various doses of irradiation*

Cultivars	Doses (Gy)	Lowest		Highest		Range	Average (cm)
		Plant Code	Height (cm)	Plant Code	Height (cm)		
Boyolali	0	Control	104.5	Control	139	104.5-139	122.5±8.47
	100	M2-BY1-1-40	112	M2-BY1-1-12	155.5	112-155.5	132.6±13.29*
	200	M2-BY2-2-35	109	M2-BY2-2-03	149	109-149	125.6±11.40
	300	M2-BY3-7-07	96.5	M2-BY3-7-17	121.5	96.5-121.5	117.7±10.66
Bantul	0	Control	104	Control	144	104-144	117.6±10.76
	100	M2-BT1-4-10	112.2	M2-BT1-4-17	160.3	112.2-160.3	135.5±12.8*
	200	M2-BT2-5-21	106	M2-BT2-5-06	153.5	106.5-153.5	123.3±11.37
	300	M2-BT3-8-22	101	M2-BT3-8-20	168	101-168	119.7±11.96

***Note:** the numbers are followed by sign (*) were significantly different from the control based on the T-test $\alpha = 0.05$*
***Source:** laboratory test results*

Table 2*. Number of productive tillers of M2 mutant black rice Boyolali and Bantul cultivars irradiated with gamma rays with various doses of irradiation*

Cultivars	Doses (Gy)	The Lowest		The Most		Range	Average (Stem)
		Plant Code	Total (Stem)	Plant Code	Total (Stem)		
Boyolali	0	Control	8	Control	21	8-21	12.2±3.28
	100	M2-BY1-1-35	9	M2-BY1-1-1	23	9-23	15.3±3.79*
	200	M2-BY2-2-36	7	M2-BY2-2-30	21	7-21	11.9±3.79
	300	M2-BY3-7-09	9	M2-BY3-7-13	20	9-20	13.5±3.15
Bantul	0	Control	6	Control	21	6-21	13.8±4.15
	100	M2-BT1-4-06	10	M2-BT1-4-08	28	10-28	16.6±4.66*
	200	M2-BT2-5-06	5	M2-BT2-5-17	26	5-26	13.9±4.32
	300	M2-BT3-8-14	8	M2-BT3-8-08	21	8-21	15.0±3.40

Note: *the numbers are followed by sign (*) were significantly different from the control based on the T-test $\alpha = 0.05$*
Source: *laboratory test results*

The panicle length and the number of seeds per panicle were significantly affected by the application of gamma-ray irradiation doses of 100 Gy and 200 Gy on the Boyolali cultivar, as shown in Table 3 and Table 4. Meanwhile, black rice of the Bantul cultivar shown significantly different in panicle length (Table 3) and the number of seeds per panicle (Table 4) when subjected to an irradiation dose of 200 Gy. The mean value and standard deviation indicated the highest diversity in panicle length for the Boyolali cultivar at a dose of 200 Gy (Table 3). The Bantul cultivar, plant code M2-BT1-4-17 (Table 4), produced the greatest number of seeds per panicle whe exposed to 100 Gy gamma-ray irradiation. The Boyolali cultivar treated with 100 Gy irradiation showed significant differences from the control plants on the 100-grain weight, while the Bantul cultivar treated with 200 Gy irradiation (Table 4).

Table 3*. Panicle length of mutant black rice M2 Boyolali and Bantul cultivars irradiated by gamma rays with various doses of irradiation*

Cultivars	Doses (Gy)	The Shortest		The Length		Range	Average
		Plant Code	Panicle Lenght	Plant Code	Panicle Lenght		
Boyolali	0	Control	20.96	Control	26.48	20.96-26.48	23.4±1.61
	100	M2-BY1-2-29	22.52	M2-BY1-1-43	26.78	22.52-26.78	24.7±1.17*
	200	M2-BY2-2-28	20.6	M2-BY2-2-02	27.68	20.6-27.68	24.7±1.67*
	300	M2-BY3-7-03	19.7	M2-BY3-7-05	26	19.7-26	22.8±1.39
Bantul	0	Control	20.3	Control	26.42	20.3-26.42	22.9±1.38
	100	M2-BT1-4-07	20.8	M2-BT1-4-28	27.4	20.8-27.4	23.8±1.82
	200	M2-BT2-5-12	17.85	M2-BT2-5-06	24.56	17.85-24.56	22.2±1.63*
	300	M2-BT3-8-11	18.56	M2-BT3-8-20	26.68	18.56-26.68	23.4±1.77

Note: *the numbers are followed by sign (*) were significantly different from the control based on the T-test $\alpha = 0.05$*
Source: *laboratory test results*

Table 4*. Weight of 100 grains of mutant black rice M2 Boyolali and Bantul cultivars irradiated with gamma rays with various doses of irradiation*

Cultivars	Doses (Gy)	The Lowest		The Highest		Range	Average (g)
		Plant Code	Weight (g)	Plant Code	Weight (g)		
Boyolali	0	Control	2.17	Control	3.04	2.17-3.04	2.6±0.20
	100	M2-BY1-2-07	2.45	M2-BY1-1-35	3.18	2.45-3.18	2.8±0.16*
	200	M2-BY2-2-13	2.08	M2-BY2-2-30	3.2	2.08-3.2	2.8±0.25
	300	M2-BY3-7-19	2.1	M2-BY3-7-15	2.71	2.1-2.71	2.7±0.19
Bantul	0	Control	1.9	Control	3.29	1.9-3.29	2.6±0.22
	100	M2-BT1-4-06	2	M2-BT143-02	3.35	2-3.35	2.6±0.37
	200	M2-BT2-5-05	2.25	M2-BT2-5-19	3.08	2.25-3.08	2.7±0.23*
	300	M2-BT3-8-16	2.43	M2-BT3-8-12	3.08	2.43-3.08	2.7±0.12

Note: *the numbers are followed by sign (*) were significantly different from the control based on the T-test $\alpha = 0.05$*
Source: *laboratory test results*

Laboratory test results showed that Boyolali 100 Gy and Bantul 200 Gy cultivars had the highest proline content (Table 5). The proline content increased under drought stress conditions in both Boyolali and Bantul cultivars (Table 6). The highest proline accumulation was in Boyolali and Bantul cultivars when exposed to 100 Gy gamma rays in dry conditions (Table 6).

***Table 5**. Proline content of mutant black rice M2 in Boyolali and Bantul cultivars irradiated with gamma rays with various doses of irradiation in an experiment in paddy fields*

Dose (Gy)	Repetition	Proline Content	
		Boyolali	Bantul
0	I	9.57	6.14
100		20.14	8.70
200		17.64	17.24
300		1.33	2.36
0	II	15.63	8.703
100		19.42	10.11
200		16.61	10.16
300		1.62	2.03

***Source:** laboratory test results*

***Table 6**. Proline content of mutant black rice M3 in Boyolali and Bantul cultivars irradiated by gamma rays with various doses of irradiation under drought stress*

Dose (Gy)	Treatment	Proline Content	
		Boyolali	Bantul
0	Control	4.15	3.99
100		9.49	7.74
200		5.91	6.14
300		3.81	3.70
0	Drought Stress	5.13	4.33
100		11.72	9.06
200		6.36	6.83
300		4.21	4.33

***Source:** laboratory test results*

The shortest plant heights in Boyolali and Bantul cultivars are 112 cm and 112.2 cm, respectively. Plant codes M2-BY1-1-40 and M2-BT-1-4-10 (Table 1). The mean value and standard deviation of black rice plant height of Boyolali cultivar irradiated with 100 Gy gamma rays were the highest, namely 132.6 cm and 13.29, respectively, as well as the Bantul cultivar with an average plant height of 135.5 cm and a standard deviation of 12.18 (Table 3). It is in line with the statement A. Gaur *et al.* (2018), that exposure to higher doses of gamma rays is inhibitory, while lower doses of radiation are sometimes stimulants. Free radicals produced by gamma rays can be destructive to affect the morphophysiology, anatomy, and biochemistry of plants depending on the level of radiation. Co-60 gamma-ray irradiation changes and increases genetic diversity so that the success rate of plant breeding is greater (Riviello-Flores *et al.*, 2022).

The number of productive tillers directly affects grain yield. Productive tillers produce panicles that produce grain so that which is associated with increased rice productivity. Gamma-ray radiation of 100 Gy led greater number of productive tillers in Boyolali and Bantul black rice cultivars with wider genetic diversity compared to other treatments (Table 2). The value of high genetic diversity in the number of productive tillers during the M2 generation allows selection for that generation. M. Shabani *et al.* (2022), also reported gamma-ray irradiation led to an increase in genetic diversity, resulting in higher number of productive tillers and yields per wheat plant. The greatest amount of productive tillers was in Bantul 100 Gy is 28 with code M2-BT1-4-8, while Boyolali 100 Gy had the greatest amount of productive tillers 23 at M2-BY1-1-11 (Table 2). These findings align with research W. Hanifah *et al.* (2020), that exposure to 100 Gy gamma-ray irradiation resulted in an increase in productive tillers. However, it depends on the individual plant because the results of irradiation in plants occur randomly.

Panicle length of M2 mutant black rice Boyolali cultivar at irradiation doses of 100 Gy and 200 Gy, respectively 22.52-26.78 cm and 20.6-27.68 cm longer than the control (20.96-26.48 cm) (Table 3). The plant M2-BY2-2-02 had the longest panicle among individuals measuring an average of 24.7 cm, compared to the control plants, which had an average panicle length of 23.4 cm. According to M. Huang *et al.* (2020), long panicles allow higher yield per unit area because more grain is produced. Even though the dose of gamma-ray irradiation was consistent, the observed growth and yield of each plant varied. Each plant is affected uniquely by its environmental adaptability as it grows. The genetic potential of each plant is different. T. Begna (2021), added that different genetic potentials cause growth diversity and individual yields to be different.

Longer panicles tend to increase the number of seeds per panicle, indicating a positive correlation between panicle length and seed yield (Mai *et al.,* 2021). The Boyolali 200 Gy cultivar, specifically plant code M2-BY2-2-09, had the greatest seeds number per panicle, reaching 166.2 seeds (Table 4), although it was still lower than the control. The number of productive tillers also affects the number of seeds per panicle. The greater the productivity of tillers, the higher the number of panicles per unit area, leading to the formation of grains in the panicles (Asis *et al.,* 2021) also added that the grain yield was high in long panicles if accompanied by many secondary panicle branches. The weight of 100 grains determines the yield potential of a rice cultivar. The weight of 100 grain in the Boyolali cultivar was highest at gamma-ray irradiation of 200 Gy, which was 3.2 g, while in the Bantul cultivar at a dose of 100 Gy, the highest weight of 100 grain reached 3.35 g (Table 4). According to M. Syafi'ie and D. Damanhuri (2018), the weight of grain can be influenced by the shape and size of the grain so that the weight value of each plant will be different. The process of panicle formation and environmental conditions related to nutrient uptake significantly affect grain weight. D. Novitasari *et al.* (2021) also added that the value of seed mass is determined by the dry matter content of the assimilate used in filling rice seeds.

Exposing Boyolali cultivar black rice to gamma-ray irradiation at a dose of 100 Gy resulted in an increase in proline content, reaching an average of 19.78. In Bantul cultivar, the highest was in gamma-ray irradiation of 200 Gy with an average proline content of 13.70 (Table 5). A. Aly *et al.* food sterilization and medicinal healing. In the present study irradiation techniques were applied to investigate the effect of gamma irradiation on germination and physiological aspects of pigeon pea seedlings. Pigeon pea (Var. BSMR 736(2018) also reported that exposure to gamma radiation alone, significantly increased proline accumulation. Gamma-rays, a form of ionizing radiation, create free radicals within cells, which impact the structure and metabolic processes of plant cells. This includes expanding thylakoid membranes, influencing photosynthesis, regulating antioxidant systems, and increasing the accumulation of phenolic compounds (Hong *et al.,* 2022). Gamma irradiation causes an increase in the proline content of black rice under drought stress. The highest increase in proline content in Boyolali and Bantul cultivars at an irradiation dose of 100 Gy was 10.5% and 7.85%, respectively (Table 6). An increase in significant proline also occurred in seedlings *Arabidopsis* irradiated at a dose of 50 Gy under heat stress conditions, in contrast to those without irradiation (Wang *et al.,* 2022).

This study investigated the effects of gamma-ray irradiation on various traits of black rice cultivars from Boyolali and Bantul. Results indicate significant differences in plant height, number of productive tillers, panicle length, seed yield, and proline content in response to different doses of irradiation, with implications for genetic diversity and plant productivity. These findings underscore the complex interplay between gamma-ray exposure, genetic variability, and physiological responses in black rice, contributing to a nuanced understanding of its potential for crop improvement and stress tolerance.

CONCLUSIONS

Gamma-ray irradiation increased the average number of productive tillers, plant height, panicle length, number of seeds per panicle, the weight of 100 M2 black rice grains of both Boyolali and Bantul cultivars. It showed that the genetic diversity of M2 black rice exposed to gamma rays is broader compared to those not exposed. Gamma-ray irradiation at 100 Gy resulted in a greater number of productive tillers in both cultivars. The M2 mutant black rice Boyolali cultivar showed longer panicle length and higher number of seeds per panicle at irradiation doses of 100 Gy and 200 Gy. The weight of 100 grains was highest in Boyolali at 200 Gy and in Bantul at 100 Gy. The highest proline content on black rice was planted in paddy fields, namely Boyolali cultivars produced by gamma-ray irradiation of 100 Gy and Bantul cultivars of 200 Gy during drought stress, the proline content in Boyolali and Bantul black rice cultivars increased following irradiation with 100 Gy gamma rays. A reduced dose of gamma-ray irradiation can enhance the growth, yield, and proline content of M2 black rice.

These findings highlight the potential of gamma-ray irradiation to induce genetic variability and enhance traits associated with crop productivity and stress tolerance. Additionally, the study underscores the importance of understanding the complex interactions between radiation, genetic diversity, and physiological responses in plants. Further research in this area could explore the specific genetic changes responsible for these observed effects, contribute to the development of novel breeding strategies for improving crop resilience and yield in the face of environmental challenges.

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CONFLICT OF INTEREST

None.

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Ростові показники, врожайність та вміст проліну двох місцевих сортів чорного рису, опромінених гамма-променями для підвищення посухостійкості

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Анотація. Одним із критичних наслідків зміни клімату для сільськогосподарського середовища є стрес від посухи. Метою дослідження було визначити ріст, урожайність та вміст проліну у чорного рису місцевого сорту Бантул, мутанта Бойолалі М2 за різних доз гамма-опромінення та вміст проліну у мутанта М3 під час посушливого стресу. В експерименті використовувався повністю рандомізований дизайн (CRD) з факторною дозою гамма-опромінення (0 Гр, 100 Гр, 200 Гр і 300 Гр), що застосовувався як на рисових полях, так і в поліетиленових мішках для спостереження і порівняння росту, врожайності та вмісту проліну в рослинах чорного рису з контрольною обробкою (0 Гр). Результати лабораторних досліджень показали, що сорти Бойолалі і Бантул, опромінені в дозах 100 Гр і 200 Гр, мали найвищий вміст проліну, який збільшився в умовах стресового впливу посухи в обох сортах. При дозі 100 Гр середня висота рослин і кількість продуктивних стебел чорного рису сортів Бойолалі і Бантул були найвищими, а різноманітність двох місцевих сортів при цій дозі була вищою, ніж при інших дозах опромінення. Застосування доз гамма-опромінення 100 Гр і 200 Гр на сорті Бойолалі суттєво вплинуло на характер довжини волоті та кількість насінин у волоті. Для сорту Бантул довжина волоті та кількість насінин у волоті при дозі опромінення 200 Гр суттєво відрізнялися. Зроблено висновок, що зменшена кількість гамма-опромінення може покращити ріст, врожайність та вміст проліну в чорному рисі сорту М2

Ключові слова: функціональні продукти харчування; рослина рис; водний стрес; сільське господарство; зміна клімату
