

SEEDLINGS AND VEGETATIVE STAGE OF RED RICE AS AFFECTED BY INDUCTION GAMMA-RAYS UNDER SALINE STRESS

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Abstract

The selection of plant varieties and the proper gamma dosage will affect germination and nutrient absorption. The goal studies were: (1) to obtain the salinity-tolerant red rice varieties and (2) to obtain the effect of varieties, gamma doses, and their interactions that could be improved the seedling characteristics and nutrient absorption in the vegetative phase of red rice plants under saline stress. The study was conducted at the greenhouse of Growth Center, LLDIKTI-1 Medan, from September until October 2020. This study used a Split Plot Design with the main plot of red rice varieties (V1 = MSP-17, V2 = Inpari-24 Gabusan, V3 = Pamara, V4 = Pamelen; and V5 = Inpara-7) and subplot was the gamma doses (0; 100; 200; 300; 400; 500; 600; and 700 Gy) within three replicates. The parameters were analyzed using ANOVA and set up by DMRT at $P < 0.05$ with IBM SPSS. The results showed that the varieties significantly affected seedling characteristics and nutrient absorption in the vegetative phase of red rice plants under saline stress. The MSP-17 variety had the greater vigor index, N, K⁺/Na⁺, and proline levels and it was classified as saline-tolerant. The low dose of gamma rays (100–200 Gy) could be improved root length and volume, as well as the levels of N, K, and proline of red rice plants were 3.77; 1.95; 8.62; 7.69; and 4.87% compared to the control. The interaction of the MSP-17 variety at low-dose gamma irradiation (0–300 Gy) enhances the greater performance of red rice plants under saline stress.

Keywords: gamma dosage, nutrients, proline, selection, varieties

INTRODUCTION

Rice (*Oryza sativa* L.) is widely grown in Asia, Latin America, and Africa and is a staple food for more than 50% of the world population (Lou *et al.*, 2012). Almost 90% of rice cultivated in Asia, such as China, India, and Pakistan contributed 30, 21, and 18%, respectively, while the remaining 30% was contributed by Thailand, Indonesia, Burma, and Japan (Khush, 2005). In addition to the type of white rice, several other types of rice have been cultivated in Indonesia, such as colored rice (black, brown,

and red). Large amounts of anthocyanin pigments cause the rice color on the rice husk (Chaudhary, 2003). The primary components of anthocyanins in red rice have been identified as having cyanidin-3-glucoside and peonidin-3-glucoside compounds (Hu *et al.*, 2003; Zhu *et al.*, 2010). In addition, red rice has also been reported to have carbohydrates ranging from 70.75 to 81.29 g, fat of 1.15–3.19 g, crude fiber of 0.28–0.61 g, protein of 7.16–10.85 g, anthocyanin was 0.35 mg/100 g, and total phenolic content of 118.47 mg/100 g (Sompong *et al.*, 2011).

Red rice is beneficial for health because it contains proanthocyanidins to treat type-2 diabetes, and modulates the inflammatory response to treat cardiovascular disease and several cancers (Chen *et al.*, 2016). Pengkumsri *et al.* (2015) also added that red rice contains the total phenolic, flavonoid, protocatechuic acid, and p-hydroxybenzoic acid were 36.14; 0.66; 0.03; and 0.34 mg/g, respectively.

Red rice planting is urgently needed to fulfill food sufficiency, and it was beneficial for health. However, red rice cultivation has weaknesses, such as a low yield of 2 to 3 tons/ha and a long harvest of 5 to 6 months (Suriyana, 2017). On the other hand, the rice harvested area in Indonesia decreased by 6.15% in 2019 compared to the previous year (Statistics Indonesia, 2020). Therefore, it should extend rice cultivation to coastal areas (saline conditions). Karolinoerita and Yusuf (2020) estimated that salinity-prone coastal areas cover 12.020 million ha or 6.20% of Indonesia's total area. This salinity stress is one of the leading causes of low yield in plants due to disruption of plant growth and development (Rachman *et al.*, 2018). Salinity stress can reduce the growth rate of rice, increase metabolic changes, and decrease the ability to absorb water and nutrients (Munns, 2002). In addition, salinity also inhibited grain development, especially inferior grains, and significantly reduced grain yield (Fu *et al.*, 2011; Zhang *et al.*, 2015).

Efforts to manage salinity stress to red rice plantings can grow and produce optimally, one of which is through plant breeding with gamma-ray induction techniques. Oladosu *et al.* (2016) stated that the initial stage of the plant breeding program could increase productivity and plant quality genetically through the induction of mutations in seeds and other plant parts both physically and chemically. Liu *et al.* (2012); Efendi *et al.* (2017) reported that mutation induction could be conducted to improve plants' agronomic character through the application of gamma-ray irradiation. Suliartini *et al.* (2015) said that mutations could produce populations with high levels of genetic diversity as the basis for selection. However, the intensity of gamma-ray irradiation affects plants' morphological, anatomical, biochemical, and physiological characteristics, such as changes in cellular structure, photosynthesis, antioxidant systems, and accumulation of phenolics (Kim *et al.*, 2004; Wi *et al.*, 2005). The optimal radiation dose for a crop improvement program is determined using the Lethal Dose 50 or LD₅₀ (Oldach, 2011; Ángeles-Espino *et al.*, 2013) and growth reduction 30/50 or GR₃₀/GR₅₀ (Khalil *et al.*, 2014). Mutants resulting from gamma-ray irradiation will produce the highest diversity around LD₂₀ and LD₅₀ (Human, 2012).

Thus, the germination and vegetative of the plant are appropriate initial phases for the development of gamma irradiation in red rice under salinity stress. Selvan and Thomas (1999) reported detecting irradiated seeds of plant species through seed germination and root extension of seedlings.

Chaudhuri (2002) added that a simple and reliable method for detecting gamma-irradiated seeds was germination efficiency and seedling growth tests. Dehpour *et al.* (2011) that the lowest percentage of germination and shoot length of rice was found in the gamma-irradiated less than 300 Gy with a salinity of 15 mmol/L. The researcher also said an increase in the proline content of rice seedlings along with the increase in the dose of gamma irradiation to 300 Gy and an increase in salinity to 25 mmol/L. Shereen *et al.* (2009) reported that a radiation dose of 150 Gy to be more effective in survival, increasing rice biomass production and nutrient response at higher salinity (75 mM NaCl). On the other hand, the gamma rays also affected rice plants in the absorption of nutrients such as N, P, K, Ca, Mg, and Na under saline stress (El-Beltagi *et al.*, 2013; Aly *et al.*, 2018).

However, gamma irradiation has not been reported on the seedling characteristics and nutrient absorption in the vegetative phase of red rice plants under saline stress from Indonesia, such as MSP-17, Inpari-24 Gabusan, Pamera, Pamelen, and Inpara-7 varieties. The goal studies were: (1) to obtain the salinity-tolerant red rice varieties and (2) to obtain the effect of varieties, gamma doses, and their interactions that could improve the seedling characteristics and nutrient absorption in the vegetative phase of red rice plants under saline stress.

MATERIALS AND METHODS

Source of Red Rice Seeds and Gamma Ray Induction

The red rice varieties in this study include MSP-17, Inpari-24 Gabusan, Pamera, Pamelen, and Inpara-7 obtained from Indonesia Center for Rice Research (ICRR) Sukamandi, Subang District, West Java Province, Indonesia. A total of 250 g seeds of red rice were conducted gamma rays-treated (Co⁶⁰ of Gamma Chambers) at the Isotope and Radiation Application Center, National Nuclear Energy Agency of Indonesia (PAIR-BATAN) Jakarta.

Saline Soil Collection and Seedlings Test

Saline soil was taken from Tanjung Rejo Village, Sei Tuan Subdistrict, Deli Serdang District, North Sumatra, Indonesia, and measured the salinity using a DHL meter. The salinity value was 6 mhos. 100 seeds of red rice-irradiated transferred into the seedling tray and arranged according to the study design. A portion of the saline soil was put into polybags with a size of 10 kg for the vegetative stage of red rice.

Study Area and Design

This seedling test was conducted at the greenhouse of Growth Center, Higher Education Service Institute of North Sumatra Region-I (LLDIKT-1) Medan from September to December 2020. This

study used a Split Plot Design (SPD) with the main plot being red rice varieties (MSP-17; Inpari-24 Gabusan; Pamera; Pamelen; Inpara-7) and the sub-plot was doses of gamma-ray irradiation (0; 100; 200; 300; 400; 500; 600; 700 Gy). The treatment combination was repeated in three replicates.

Vegetative Stage

After 21 days of germination, one seedling with homogeneous and successful growth was selected from each treatment and transplanted into polybags. Red rice plant cultivation is performed up to 8 weeks after transplanting.

Parameters and Data Analysis

The seedling parameters include maximum growth potential (MGP), germination percentage (GP), vigor index (VI), root length (RL), root volume (RV), and lethal doses 20 and 50 (LD₂₀ and LD₅₀). The MGP was calculated based on rice seeds that grow calculated from the third until the ninth day (equation 1). It calculated the GP based on normal seedlings (NS) from the third to the ninth day (equation 2). The VI was calculated based on the percentage of normal seedlings on the third day (equation 3). It measured the length and volume of rice roots at the end of the observation (21 days after seedling). Determination of the optimum dose of gamma irradiation was measured using LD₂₀ to LD₅₀ (Indrayanti *et al.*, 2011) through probit regression analysis based on the germination percentage.

$$\text{MGP} = \frac{\sum \text{Growing seeds}}{\sum \text{Planted seeds}} \times 100\% \quad (1)$$

$$\text{GP} = \frac{\sum \text{NS3} + \text{NS4} + \dots \text{NS9}}{\sum \text{Planted seeds}} \times 100\% \quad (2)$$

$$\text{VI} = \frac{\sum \text{Normal seedlings on the third day}}{\sum \text{Planted seeds}} \times 100\% \quad (3)$$

The vegetative parameters include nutrient content (N, P, K, Na, K⁺/Na⁺) and proline content. A 250 g of the

second leaf of the tip was analyzed. Measurement of N-content was conducted by the Titrimetric method, meanwhile, the P-content was carried out by the wet ashing method and measured using a UV-Vis spectrophotometer with a wavelength of 693 nm. The measurement of K and Na-contents were performed using the HCl-dry ashing method and was analyzed using an atomic absorption spectrophotometer. Proline content was estimated by the method of Bates *et al.* (1973). A 0.5 g of leaf sample was weighed and measured using a UV-Vis spectrophotometer at a wavelength of 520 nm. The results were expressed as µmol/g of proline equivalent to the fresh weight of the samples. The seedling and vegetative parameters were analyzed using ANOVA and set up by DMRT at $P < 0.05$ with IBM SPSS.

RESULTS

The gamma irradiation doses (R) significantly affected the all seedling and vegetative characteristics of red rice in this study, as well as their interactions except P and K contents in the leaves. The varieties of red rice significantly affected the maximum growth potential, vigor index, root length and volume, N and Na contents, K⁺/Na⁺, and proline content (Tab. I).

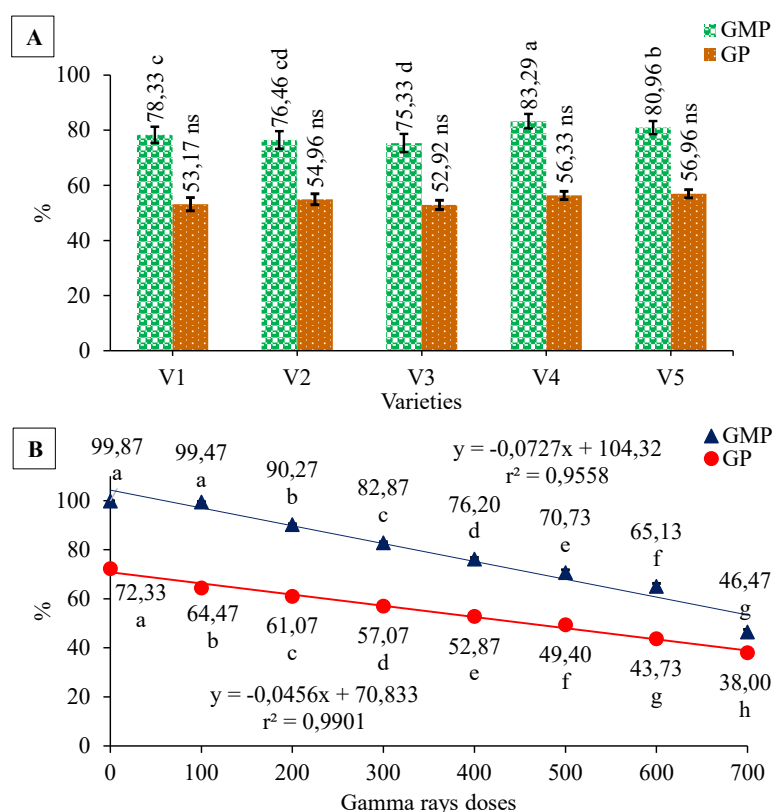
Maximum Growth Potential and Germination Percentage (%)

The Pamelen variety of red rice had the highest maximum growth potential of 83.29% in saline conditions compared to other varieties (Fig. 1A). It could be seen that the higher the doses of gamma rays up to 700 Gy resulting the lower the maximum growth potential and germination percentage of red rice plants under salinity conditions (Fig. 1B). The interaction of gamma doses from 0 to 100 Gy in the five varieties of red rice had a maximum growth potential that was significantly different. Likewise, the interaction of un-irradiated with the Inpara-7 variety significantly had the highest germination percentage of 74.67% compared to other interactions (Tab. II).

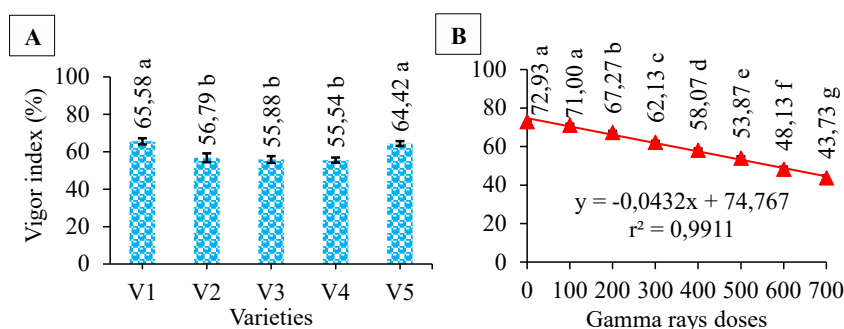
I: F-value of the gamma-ray doses effect on the seedling characteristics and nutrient absorption in the vegetative stage in five varieties of red rice under salinity conditions

Source	F-value										
	MGP	GP	VI	RL	RV	N-c	P-c	K-c	Na-c	K/Na	Prol
Block	0.21 ^{ns}	1.08 ^{ns}	1.23 ^{ns}	0.20 ^{ns}	2.30 ^{ns}	0.31 ^{ns}	1.62 ^{ns}	1.03 ^{ns}	13.37*	0.43 ^{ns}	0.27 ^{ns}
Main-plot											
Varieties (V)	8.10*	1.51 ^{ns}	8.20*	61.45*	31.96*	9.95*	3.13 ^{ns}	3.00 ^{ns}	118.53*	23.19*	300.19*
Sub-plot											
Gamma doses (G)	407.50*	204.63*	193.99*	90.02*	116.07*	24.88*	7.16*	10.73*	23.36*	17.18*	15.55*
Interaction (V × G)	3.08*	3.73*	3.28*	2.06*	5.44*	2.07*	0.24 ^{ns}	0.90 ^{ns}	3.22*	1.87*	14.57*

Note: F-value is significant at the 0.05 level (*) and ns = not significant. MGP = maximum growth potential; GP = germination percentage; VI = vigor index; RL = root length; RV = root volume; N-c = N content; P-c = P content; K-c = K content; Na-c = Na content; Prol = proline.



1: The maximum growth potential (GMP) and germination percentage (GP) of red rice due to the varieties (A) and gamma irradiation doses (B) under salinity conditions. V1 = MSP-17; V2 = Inpari-24 Gabusan; V3 = Pamera; V4 = Pamelen; V5 = Inpara-7.



2: The vigor index of red rice plants due to the varieties (A) and gamma irradiation doses (B) under salinity conditions. V1 = MSP-17; V2 = Inpari-24 Gabusan; V3 = Pamera; V4 = Pamelen; V5 = Inpara-7.

Vigor Index (%)

The MSP-17 variety had the highest vigor index of 65.58% compared to other varieties (Fig. 2A). There was a decrease in the vigor index of the red rice plant along with an increase in gamma-ray dose up to 700 Gy (Fig. 2B). The interaction of un-irradiated within the MSP-17 variety significantly had the highest vigor index of 77.67% compared to other interactions (Tab. II).

Root Length and Volume (cm and ml)

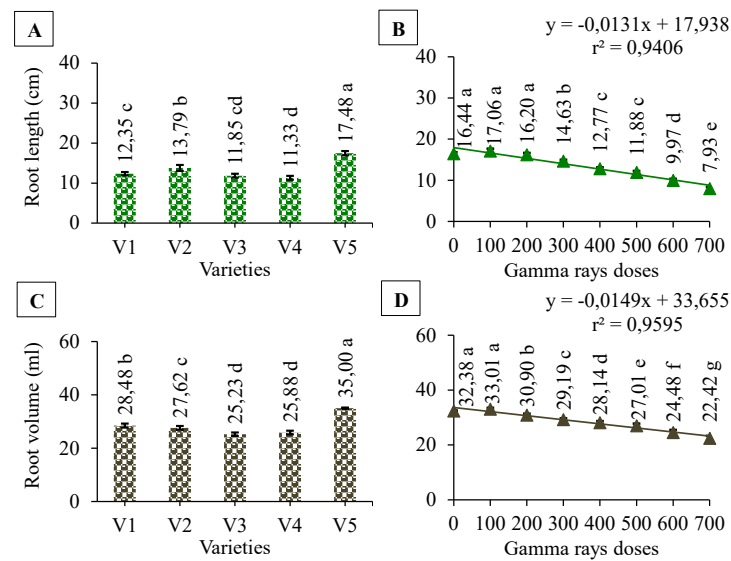
The Inpara-7 variety had the highest root length and volume of 17.48 cm and 35.00 ml compared to other varieties (Fig. 3A, C). The gamma-ray at 100 Gy significantly increased the highest root length and volume were 3.77 and 1.95%, respectively compared to the control and decreased along with the increase in the gamma irradiation dose up to 700 Gy (Fig. 3B, D). The interaction of gamma at a dose of 100 Gy with the Inpara-7 variety significantly increased the highest root length and volume by 22.91 cm and 37.81 ml compared to other interactions (Tab. II).

II: The interaction effect of varieties and gamma irradiation doses on the seedling characteristics and nutrient absorption in the vegetative stage of red rice under saline stress

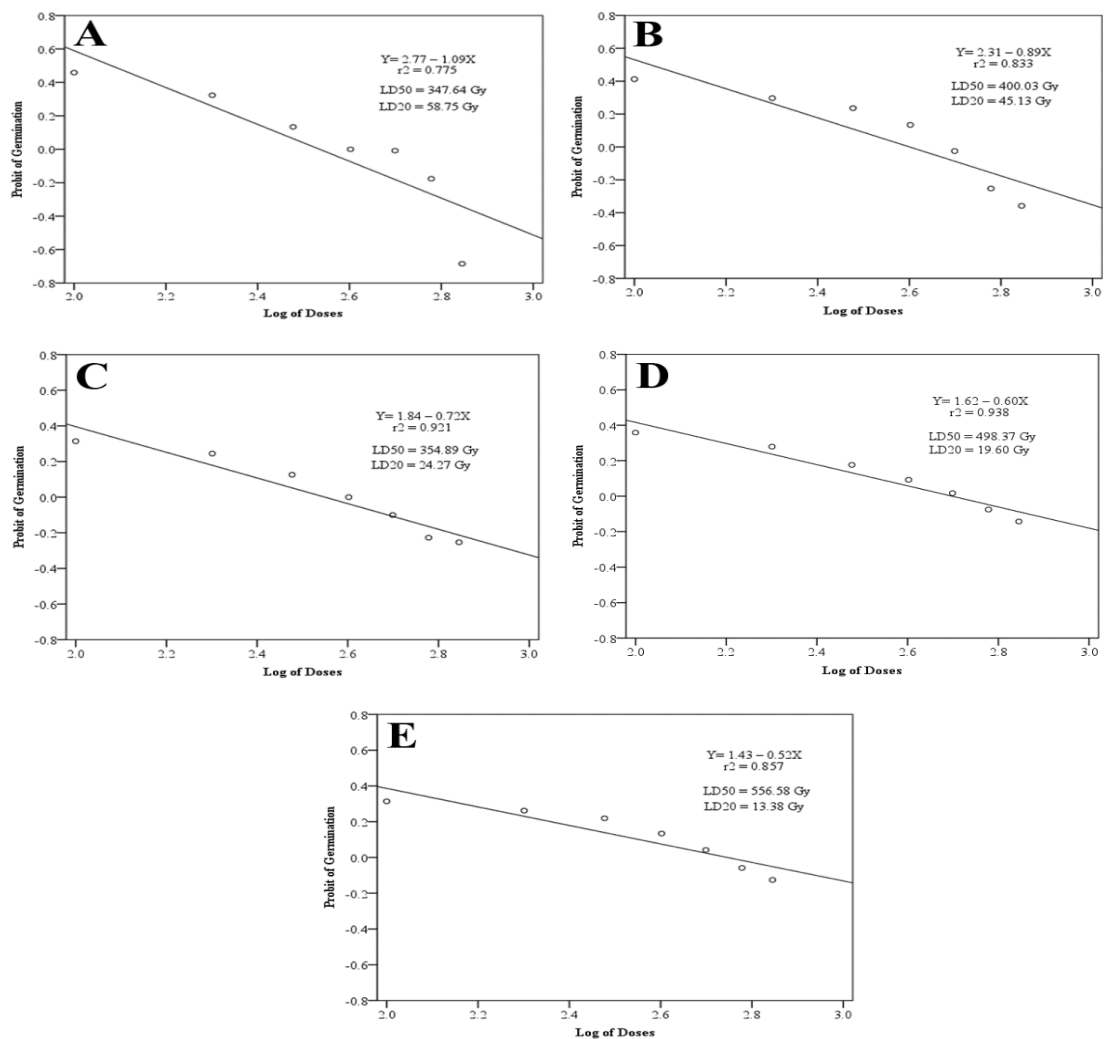
Red rice varieties	Gamma doses	Seedlings stage					Vegetative stage					Prol (μmol/g)
		MGP (%)	GP (%)	VI (%)	RL (cm)	RV (ml)	N-c (%)	P-c (%)	K-c (%)	Na-c (%)	K/Na ⁺	
MSP-17	0	100.00 a	72.33 abc	77.67 a	15.20 efg	34.24 b-e	0.58 d-i	0.49 ns	0.54 ns	3.15 h-k	0.18 c-h	83.09 e-h
	100	99.67 a	67.67 b-e	76.33 ab	15.50 def	34.48 b-e	0.70 a	0.43 ns	0.60 ns	2.31 op	0.26 a	112.41 ab
	200	91.33 b	62.67 d-g	72.33 a-e	14.92 e-h	31.81 e-h	0.69 ab	0.44 ns	0.55 ns	2.25 p	0.25 a	111.83 ab
	300	83.33 c-f	55.33 h-l	68.00 c-i	12.41 h-n	29.28 g-l	0.63 a-e	0.40 ns	0.55 ns	2.33 op	0.24 ab	113.74 a
	400	74.33 ijk	50.00 k-p	65.33 e-k	11.38 k-p	26.81 k-p	0.58 d-i	0.38 ns	0.54 ns	2.37 op	0.23 abc	112.24 ab
	500	67.67 k-o	49.67 l-p	62.00 g-n	11.11 l-q	25.67 m-q	0.53 f-k	0.39 ns	0.53 ns	3.14 i-k	0.17 d-j	113.37 ab
	600	62.67 nop	43.00 pqr	55.00 n-s	9.73 o-r	23.83 pqr	0.52 h-k	0.37 ns	0.56 ns	3.35 e-k	0.17 d-j	84.38 d-h
Inpari-24 Gabusan	700	47.67 r	24.67 t	48.00 stu	8.58 qrs	21.76 r-u	0.48 j-m	0.36 ns	0.45 ns	3.23 g-k	0.14 g-o	84.48 d-h
	0	100.00 a	72.33 abc	73.00 a-d	17.96 bcd	32.25 d-g	0.65 a-d	0.44 ns	0.47 ns	2.48 nop	0.19 b-g	83.15 e-h
	100	99.67 a	66.00 c-f	72.33 a-e	19.05 bc	32.48 c-f	0.67 abc	0.43 ns	0.50 ns	2.85 k-o	0.17 d-i	87.54 d-g
	200	87.67 b-e	61.67 e-i	69.00 b-g	18.24 bc	30.14 f-j	0.63 a-e	0.41 ns	0.50 ns	3.08 j-m	0.16 d-k	105.08 ab
	300	80.67 e-i	59.33 f-j	61.00 h-o	15.51 def	28.85 h-m	0.58 d-i	0.40 ns	0.53 ns	2.57 l-p	0.21 a-d	94.44 cd
	400	75.33 hij	55.33 h-l	57.00 m-r	11.77 l-p	28.21 i-o	0.56 e-j	0.38 ns	0.49 ns	3.01 j-n	0.16 d-k	76.15 hij
	500	67.00 l-o	49.00 l-p	49.00 st	12.67 g-m	27.13 j-o	0.54 f-k	0.38 ns	0.47 ns	3.00 j-n	0.16 d-l	74.68 h-k
Pamera	600	61.33 opq	40.00 rs	37.67 vw	9.25 pqr	22.14 rst	0.52 h-k	0.36 ns	0.41 ns	3.83 a-g	0.11 k-o	63.87 k-n
	700	40.00 s	36.00 s	35.33 w	5.88 t	19.75 tu	0.52 h-k	0.34 ns	0.43 ns	3.69 b-i	0.12 i-o	58.79 mn
	0	100.00 a	69.33 a-d	70.67 a-f	15.25 efg	30.41 f-i	0.50 i-l	0.49 ns	0.47 ns	2.32 op	0.21 a-e	82.45 e-h
	100	100.00 a	62.33 e-h	68.33 c-h	14.17 f-i	29.60 f-k	0.62 a-e	0.43 ns	0.58 ns	3.59 c-j	0.16 d-k	91.79 de
	200	89.00 bcd	59.67 f-j	64.00 f-m	14.24 f-i	27.88 i-o	0.61 b-g	0.43 ns	0.52 ns	3.47 d-j	0.16 d-m	87.92 d-g
	300	80.67 e-i	55.00 i-l	58.67 j-p	13.74 f-l	26.39 l-p	0.61 b-g	0.41 ns	0.49 ns	3.34 f-k	0.15 g-o	78.35 f-i
	400	70.67 j-m	50.00 k-p	53.00 p-s	11.47 j-p	25.27 opq	0.57 d-i	0.40 ns	0.51 ns	3.56 d-j	0.14 g-o	58.87 mn
	500	64.67 mno	46.00 n-r	49.67 rst	10.08 m-q	23.27 qrs	0.56 e-j	0.38 ns	0.48 ns	3.73 b-i	0.13 g-o	66.27 j-n
	600	57.33 pq	41.00 qrs	43.33 tuv	9.68 o-r	20.21 tu	0.55 e-j	0.34 ns	0.43 ns	3.82 a-g	0.11 j-o	59.04 mn
	700	40.33 s	40.00 rs	39.33 vw	6.18 st	18.84 u	0.50 i-l	0.36 ns	0.42 ns	3.97 a-d	0.11 k-o	56.50 n

Red rice varieties	Gamma doses	Seedlings stage					Vegetative stage					
		MGP (%)	GP (%)	VI (%)	RL (cm)	RV (ml)	N-c (%)	P-c (%)	K-c (%)	Na-c (%)	K ⁺ /Na ⁺	Prol (μmol/g)
Pamelen	0	100.00 a	73.00 ab	67.00 c-i	14.17 f-i	30.86 f-i	0.56 e-j	0.50 ns	0.51 ns	2.52 m-p	0.21 a-d	83.17 e-h
	100	99.33 a	64.00 d-g	64.67 f-l	13.66 f-j	30.69 f-i	0.58 d-i	0.47 ns	0.53 ns	3.73 b-i	0.14 g-o	58.78 mn
	200	93.33 ab	61.00 e-i	61.00 h-o	13.55 f-l	28.76 h-m	0.55 e-j	0.45 ns	0.49 ns	3.80 a-g	0.13 h-o	62.71 lmn
	300	87.00 b-f	57.00 g-k	57.33 l-q	13.41 f-l	26.71 k-p	0.50 i-m	0.45 ns	0.53 ns	3.92 a-f	0.14 g-o	56.98 mn
	400	80.33 f-i	53.67 j-m	54.33 o-s	12.17 i-o	26.21 l-q	0.52 h-k	0.44 ns	0.50 ns	3.95 a-f	0.13 h-o	60.16 mn
	500	82.00 e-h	50.67 k-o	50.67 prs	9.90 n-q	23.74 p-s	0.51 i-l	0.42 ns	0.48 ns	4.08 a-d	0.12 i-o	76.79 g-j
	600	76.00 g-j	47.00 m-q	47.67 stu	7.30 rst	20.75 stu	0.48 j-m	0.43 ns	0.42 ns	4.18 abc	0.10 mno	68.16 i-m
Inpara-7	700	48.33 r	44.33 o-r	41.67 uvw	6.50 st	19.29 tu	0.46 klm	0.41 ns	0.38 ns	4.21 ab	0.09 o	64.65 k-n
	0	99.33 a	74.67 a	76.33 ab	19.65 b	34.15 b-e	0.59 c-h	0.53 ns	0.59 ns	2.86 k-o	0.21 a-f	82.67 e-h
	100	98.67 a	62.33 e-h	73.33 abc	22.91 a	37.81 a	0.57 e-i	0.41 ns	0.59 ns	3.88 a-f	0.15 e-n	71.45i-l
	200	90.00 bc	60.33 f-j	70.00 b-f	20.05 b	35.93 ab	0.53 g-k	0.41 ns	0.56 ns	3.74 a-h	0.15 f-n	67.22 j-n
	300	82.67 d-g	58.67 g-j	65.67 d-j	18.08 bc	34.73 b-e	0.50 i-l	0.39 ns	0.53 ns	3.95 a-f	0.13 g-o	73.90 h-k
	400	80.33 f-i	55.33 h-l	60.67 i-o	17.07 cde	34.18 b-e	0.48 j-m	0.39 ns	0.50 ns	3.96 a-f	0.13 h-o	102.92 bc
	500	72.33 jkl	51.67 k-n	58.00 k-q	15.65 def	35.25 a-d	0.45 klm	0.38 ns	0.47 ns	3.97 a-e	0.12 i-o	87.83 d-g
CV (%)	600	68.33 k-n	47.67 m-q	57.00 m-r	13.88 f-k	35.49 abc	0.43 lm	0.39 ns	0.44 ns	4.19 abc	0.11 l-o	88.29 def
	700	56.00 q	45.00 n-r	54.33 o-s	12.52 h-m	32.48 c-f	0.42 m	0.38 ns	0.42 ns	4.35 a	0.10 no	84.78 d-h
		4.43	5.54	4.96	10.14	4.71	8.12	13.29	10.97	9.37	20.22	7.38

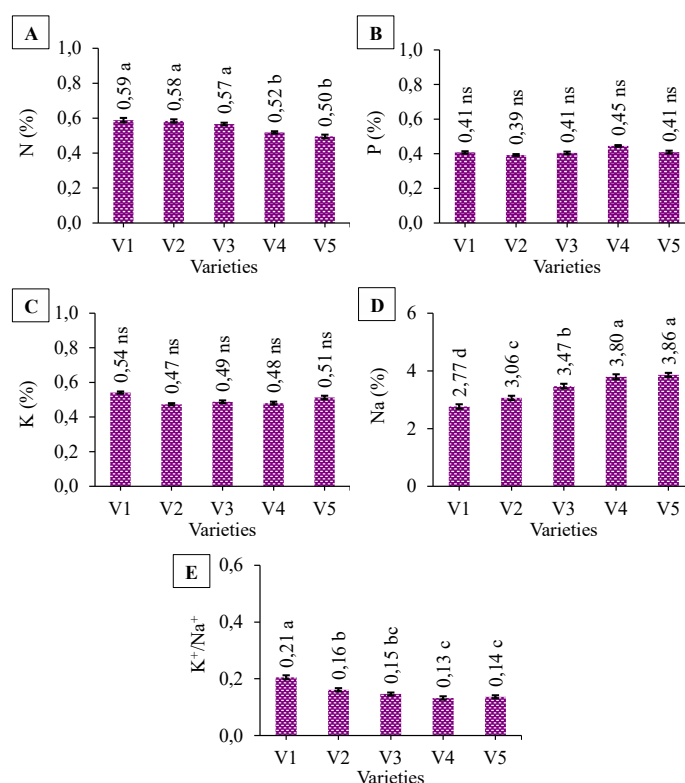
Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at P < 0.05. CV = coefficient of variation. MGP = maximum growth potential; GP = germination percentage; VI = vigor index; RL = root length; RV = root volume; N-c = N content; P-c = P content; K-c = K content; Na-c = Na content; Prol = proline.



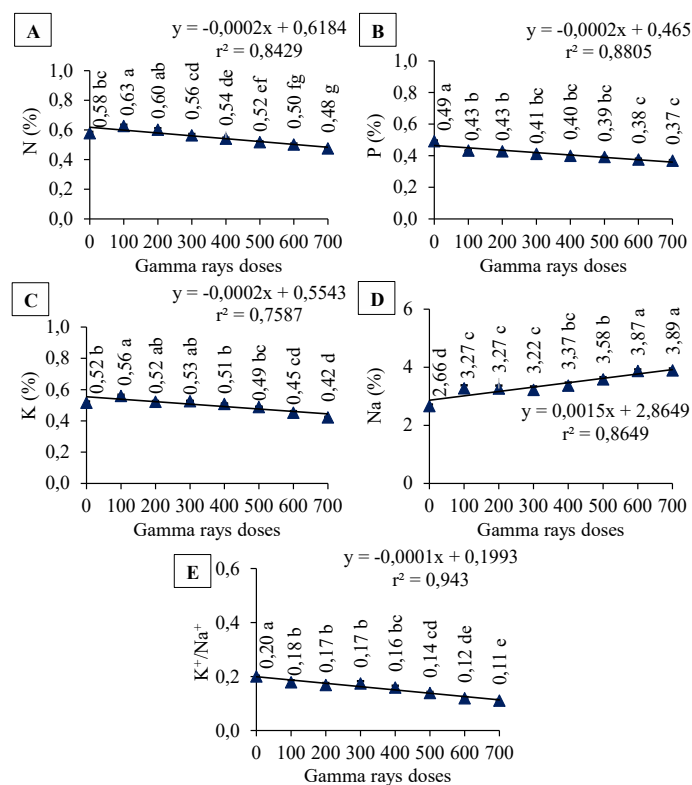
3: The root length and volume of red rice plants due to the varieties (A, C) and gamma irradiation doses (B, D) under salinity conditions. V1 = MSP-17; V2 = Inpari-24 Gabusan; V3 = Pamera; V4 = Pamelen; V5 = Inpara-7.



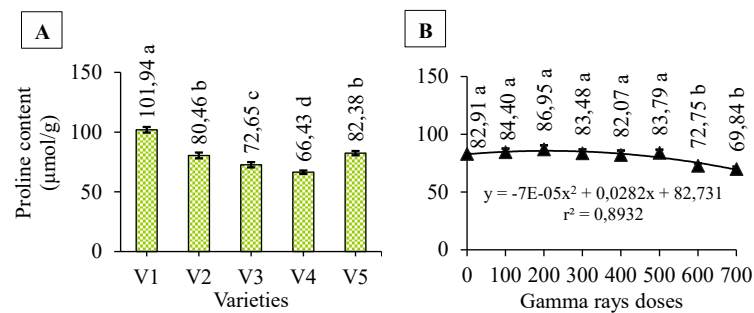
4: The lethal doses of 20–50 gamma rays from red rice varieties under saline. A = MSP-17; B = Inpari-24 Gabusan; C = Pamera; D = Pamelen; E = Inpara-7.



5: The effect of varieties on the nutrient content (A = nitrogen; B = phosphor; C = potassium; D = sodium; E = K⁺/Na⁺) under salinity conditions. V1 = MSP-17; V2 = Inpari-24 Gabusan; V3 = Pamera; V4 = Pamelen; V5 = Inpara-7.



6: The effect of gamma irradiation doses on the nutrient content (A = nitrogen; B = phosphor; C = potassium; D = sodium; E = K⁺/Na⁺) under salinity conditions



7: The proline content of red rice plants due to the varieties (A) and gamma irradiation doses (B) under salinity conditions. V1 = MSP-17; V2 = Inpara-24 Gabusan; V3 = Pamera; V4 = Pamelen; V5 = Inpara-7.

Lethal Dose 20–50 (LD_{20-50})

Lethal doses 20 and 50 from gamma irradiation in the five varieties of red rice under saline conditions could be seen in Fig. 4. The Inpara-7 variety had the highest radiosensitivity value (LD_{50}) of 556.58 Gy compared to other varieties. It was indicated that the Inpara-7 variety is more survivable at higher gamma doses and 556.58 Gy as the limit of 50% reduction seedlings. In addition, the optimum dose of gamma irradiation in the Inpara-7 variety is also relatively wide compared to other varieties. It was seen the value of LD_{20-50} in the Inpara-7 variety ranged from 13.38 to 556.58 Gy. Among these varieties tested, it was found that the Inpara-7 variety was classified as tolerant whereas the MSP-17 variety was classified as sensitive.

Nutrients Content

The MSP-17 variety had the highest N-content and K^+/Na^+ were 0.59% and 0.21 compared to other varieties (Fig. 5A, E) and the highest Na-content was found in Inpara-7 variety at 3.86% (Fig. 5D). The varieties were insignificantly affected the P and K-content in the leaves of red rice plants at the vegetative stage. The gamma-ray at 100 Gy significantly increased the highest N and K-content were 8.62 and 7.69%, respectively compared to the control and decreased along with the increase in the gamma irradiation dose up to 700 Gy (Fig. 6A, C). Likewise, the content of P and K^+/Na^+ were lower along with an increase in the doses of gamma rays (Fig. 6B, E). Vice versa, the Na-content was increased (Fig. 6D). The interaction of gamma at a dose of 100 Gy with the MSP-17 variety significantly increased the highest N-content and K^+/Na^+ were 0.70% and 0.26 compared to other interactions (Tab. II).

Proline Content (μmol/g)

The MSP-17 variety had the highest proline content of 101.94 μmol/g compared to other varieties (Fig. 7A). There was an increase in proline content up to 200 Gy gamma dose and decreased along with the increase in the gamma dose to 700 Gy (Fig. 7B). The interaction of gamma rays at 300 Gy with a MSP-17 variety significantly had the highest proline content of 113.74 μmol/g compared to other interactions (Tab. II).

DISCUSSION

Varieties Effect

Varieties significantly affected the maximum growth potential, vigor index, root length and volume, as well as the levels of N, Na, and proline in the leaves of red rice plants. However, it had an insignificant effect on the germination percentage, P-content, and K-content of red rice plants under saline soil. Among these varieties, MSP-17 and Inpara-7 were found to be dominantly superior in improving seedling characteristics and nutrient absorption in the vegetative phase under saline stress. The MSP-17 had the highest vigor index, N-content, K^+/Na^+ , and proline content were 65.58%; 0.59%; 0.21; and 101.94 μmol/g. Meanwhile, the Inpara-7 variety was superior in increasing root length, root volume, and N-content by 17.48 cm; 35.00 ml; and 3.86%. On the other hand, the Pamelen variety also had the highest maximum growth potential of 83.29%. Based on seedling characteristics, the optimum dose of gamma irradiation on the Inpara-7 variety also varied greatly from 13.38 to 556.58 Gy (Fig. 4E). The value LD_{20} and LD_{50} in the Inpara-7 variety of red rice an indicators of increased genetic diversity in seedling characteristics, such as root length and volume. This finding was supported by Indrayanti *et al.* (2011) that the radiosensitivity of plants to gamma-ray irradiation in increasing genetic diversity could be measured using the optimum dose in the range of LD_{20} to LD_{50} . Budi *et al.* (2019) reported that the percentage of seedling growth, plant height, and root length in the seedling phase, and the percentage of grain empty in mutant-1 (M1) of red rice obtained an irradiation dose from 200 to 300 Gy, which was effective in producing genetic diversity. Suliartini *et al.* (2020) added that the optimal dose of gamma irradiation for seedlings of lowland rice varieties include G10, G16, Baas Selem, and Inpago Unram-1 by 264; 398; 316; and 518 Gy, respectively. Gowthami *et al.* (2017) also found that the LD_{50} doses of gamma irradiation for varieties ADT-37 and ADT(R)-45 of rice were 300.03 and 300.00 Gy, respectively. However, the Inpara-7 variety highly accumulated sodium in the shoot than other varieties (Fig. 5D) and

could inhibit plant growth in the vegetative phase. It was indicated that this variety was classified as saline-sensitive. According to Akter and Oue, (2018) that saline-sensitive rice plants absorbed higher levels of Na in the shoot than the tolerant variety. Platten *et al.* (2013) also added that saline-tolerant plants usually accumulate low Na⁺ and high K⁺ compared to sensitive plants through selective uptake mechanisms.

In this study, the MSP-17 variety had higher K⁺/Na⁺ and proline content than other varieties (Fig. 5E and 7A). Both characters could be used as indicators of saline-tolerant plants. In addition, the N-content in MSP-17 variety was also higher (Fig. 5A). This finding was supported by Adams and Shin, (2014) that the Na⁺ and K⁺ are positively charged ions and compete to use the same channels to access the cell. Na⁺ had a strong effect on inhibiting K⁺ uptake in the cell. In addition, membrane depolarization caused by the large influx of Na⁺ in the cytosol results in increased K⁺ efflux. Akter and Oue, (2018) added that the Pokkali rice variety (saline-tolerant) had higher Na⁺/K⁺ in the roots than the leaves, which indicates that Na⁺ ions are mostly absorbed in the roots and then translocated to the plant shoot. Zhang *et al.* (2020) found that the saline-tolerant plants had lower Na⁺/K⁺ ratios in the leaves and had a higher ability to absorb N than saline-sensitive plants through increased N metabolism. According to Ashraf and Foolad (2007) that the proline produced in salinity-stressed plants will accumulate in plant tissues, and plays an important role in the regulation of osmotic pressure and antioxidants to reduce cell damage. Kibria *et al.* (2017) also reported that the proline content increased significantly in salt-tolerant rice genotypes with increasing saline concentration.

Scientifically, the impact of salinity can inhibit plant germination by disrupting the physiological and biochemical processes of seeds. Salinity affects the seed germination process through osmotic stress, ion-specific effects, and oxidative stress, characterized by reduced germination speed and longer germination time (Munns, 2002). Salinity can reduce water uptake during imbibition due to increased osmotic potential (Munns and Tester, 2008). Salinity can affect seed germination through the toxic effects of excess sodium and chloride ions on embryo viability (Jahromi *et al.*, 2008; Daszkowska-Golec, 2011). These toxic effects include disruption of enzymes and other macromolecular structures, damage to cell organelles and plasma membranes, and disruption of respiration, photosynthesis, and protein synthesis (Daszkowska-Golec, 2011; Parida and Das, 2005; Panda and Khan, 2009). Salinity could inhibit the absorption of K⁺, P, Mn²⁺, and Fe²⁺ nutrients in the leaves of saline-sensitive varieties (Farooq *et al.*, 2022) resulting in decreased germination and reduced biomass (Ologundudu *et al.*, 2014).

The Effect of Gamma Irradiation Doses

Gamma irradiation doses significantly affected the seedling characteristics and nutrient absorption

in the vegetative phase under saline stress. The gamma dose of 100 Gy significantly increased root length, root volume, N-content, and K-content of red rice plants under saline soils were 3.77; 1.95; 8.62; and 7.69%, respectively compared to the control. Likewise, an increase in gamma dose up to 200 Gy significantly increased proline levels in the shoot by 4.87% compared to un-treated, then decreased along with the increases in gamma dose to 700 Gy (Fig. 7B). These findings indicated that the low dose of gamma had a positive effect on improving the performance of red rice plants under saline stress. The results were supported by Dhakshanamoorthy *et al.* (2011) that low-dose gamma radiation can enhance plant germination by accelerating cell division in meristematic tissues. Kiani *et al.* (2022) noted that low-dose gamma radiation had an impact on genes that activate enzymes involved in the germination process and stimulating hormones. They also concluded that gamma irradiation doses of 200–300 Gy reduced plant growth by 30% such as fresh weight, dry weight, and plant height, and could increase the expression of antioxidant enzymes. Shereen *et al.* (2009) added that the K-content and chlorophyll (a, b, total) of the IR-8 rice plants increased at low doses (150 Gy) but decreased along with the increase of high gamma doses up to 250 Gy under saline stress at 50 mM NaCl. Baek *et al.* (2005) reported that there was an increase in germination, shoot length, total chlorophyll, superoxide dismutase, and ascorbate peroxidase in Ilpumbyeo and Sanghaehyanghyella varieties of rice plants at a gamma dose of 8 Gy (low-dose) compared to the control that was treated with 50 mM NaCl. Harding *et al.* (2012) found that increasing high doses of gamma irradiation (> 300 Gy) caused physiological damage and inhibited seedling height growth, survival, and tillering production. El-Beltagi *et al.* (2013) also added that irradiation of seeds with low-dose gamma rays (50 Gy) significantly increased plant growth, photosynthetic pigments, total carbohydrates, total phenols, proline, total free amino acids, and nutrient levels (N, P, and K) in roots and shoots compared to non-irradiated under salinity stress.

Gamma doses up to 700 Gy significantly inhibited the maximum growth potential, germination percentage, vigor index, K⁺/Na⁺, and P-content in the leaves of red rice plants under saline soils. According to Hameed *et al.* (2008) that high-dose of gamma irradiation on seeds can result in disruption of protein synthesis, hormone balance, leaf gas exchange, water exchange, and enzyme activity. This physiological and biochemical damage response due to high-dose of gamma irradiation had an impact on decreasing the seedlings rate and nutrient absorption in red rice plants. Similar studies have been reported by Kumar *et al.* (2013) that low-dose gamma irradiation of 200 Gy significantly increased the germination of the BPT-5204 variety of rice plants, but high doses ranging from 400 to 2000 Gy resulted in germination inhibition. Pujiyanti *et al.* (2021) found

that the gamma irradiation dose of 400 to 800 Gy on the Barak Cenana variety of red rice harmed seedlings growth. Macovei *et al.* (2014) added that there were a decrease in chlorophyll (a, b, total) and lipid peroxidation levels in rice seeds due to high-dose of gamma rays (100–200 Gy) compared to low-dose (25–50 Gy) under 100 mM NaCl salinity stress, but there was an increased hydrogen peroxide (H_2O_2), proline levels, enzymes of ascorbate peroxidase, catalase, and glutathione reductase. Volkova *et al.* (2020) said that there was an increase in hydrogen peroxide levels by 16.36% due to a high dose of gamma irradiation (100 Gy) compared to the control in plant shoots while at a low dose (15 Gy) caused a decrease of 14.81%. Aly *et al.* (2018) also added that there was an increase in N and chlorophyll levels at low-dose of gamma (100 Gy) compared to the control and then decreased along with the increase in gamma doses up to 300 Gy under saline stress.

The Interaction Effect

The interaction of varieties with gamma irradiation doses significantly affected all seedling characteristics and nutrient absorption in the vegetative phase of red rice plants under saline stress, except for P and K content. The gamma interaction of 0 to 100 Gy in the five varieties of red rice (MSP-17, Inpara-24 Gabusan, Pamara, Pamelen, Inpara-7) had a maximum growth potential significantly different compared to other interactions. Likewise, the interaction of MSP-17 variety with gamma rays at 0, 100, and 300 Gy had the highest vigor index, N-content, K^+/Na^+ , and proline level were 77.67%;

0.70%; 0.26; and 113.74 $\mu\text{mol/g}$, respectively (Tab. II). However, the interaction of the Inpara-7 variety with gamma rays at 0, 100, and 700 Gy had the highest germination percentage, root length and volume, and Na-content were 74.67%; 22.91 cm; 37.81 ml; and 4.35%, respectively. Saline-tolerant variety (MSP-17) at low-dose gamma irradiation (0–300 Gy) provided greater performance. Tolerant varieties will balance oxidative stress and have greater antioxidants that cause gamma-ray exposure to saline stress to have a synergistic effect, especially on increasing the levels of proline and nitrogen, as well as K^+/Na^+ . These findings were supported by Macovei *et al.* (2014) that gamma-irradiated plants under salt stress have an impact on oxidative stress and antioxidant regulatory mechanisms, thereby being compatible with plant survival. Kadhimi *et al.* (2016) found that gamma irradiation doses of 200 to 500 Gy decreased the seedling rate and height of MR269 and MRQ74 (local rice) varieties while doses exceeding 500 Gy resulted in physiological damage to seedling height and the absence of surviving varieties. Prabhandaru and Saputro (2017) also added that increasing the dose of irradiation can inhibit the germination and growth of SiGadis rice seedlings, while non-irradiation up to 100 Gy had a similar germination rate of 80%. Meanwhile, doses of 200 and 300 Gy reduced the germination rate by 70% and 60%, respectively. Based on this study, it was found that the release of the MSP-17 variety of red rice with gamma irradiation up to 300 Gy could enhance plant characteristics under salinity stress.

CONCLUSION

The MSP-17 variety was classified as saline-tolerant compared to other varieties of red rice. It was caused by higher characteristics of vigor index, N, K^+/Na^+ , and proline levels. The gamma dose of 100 Gy significantly increased root length, root volume, N-content, and K-content of red rice plants under saline stress were 3.77; 1.95; 8.62; and 7.69%, as well as the gamma dose up to 200 Gy significantly increased proline content in the shoot by 4.87%. The interaction of the MSP-17 variety at low-dose gamma irradiation (0–300 Gy) enhances the greater performance of red rice plants under saline stress.

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