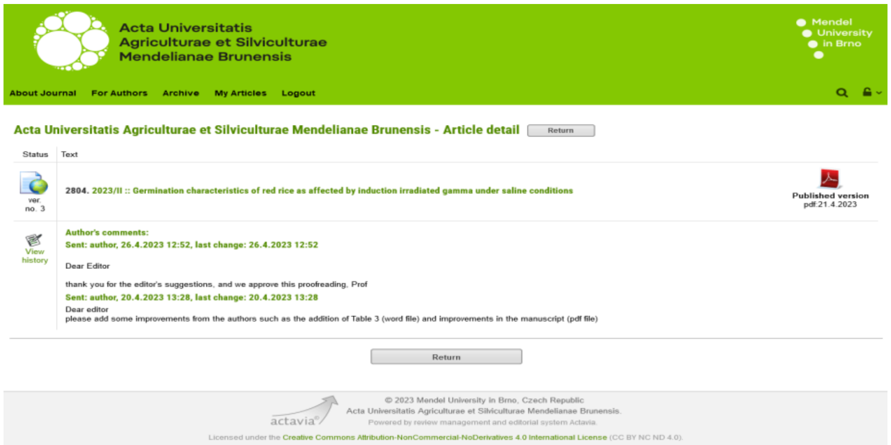
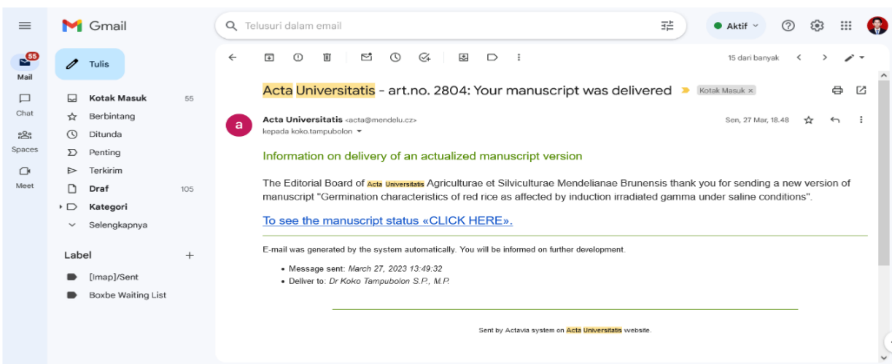
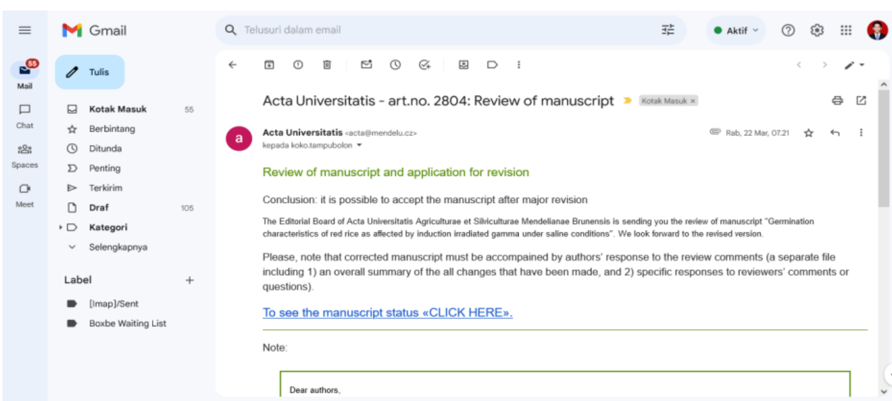
Seedlings and vegetative stage of red rice as affected by induction gamma-rays under saline stress.







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TITLE PAGE

Title: Germination characteristics of red rice as affected by induction irradiated gamma under saline conditions

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Abstract

The goal studies were: (1) to obtain the salinity-tolerant red rice varieties at the germination phase and (2) to find the lethal dose 20-50 of gamma that could support red rice germination under salinity. The germination test was conducted at the greenhouse of Growth Center, LLDIKTI-1 Medan, from September until October 2020. This study was used a Split Plot Design with the main-plot of red rice varieties (V1 = MSP-17, V2= Inpari-24 Gabusan, V3= Pamera, V4= Pamelen; V5= Inpara-7) and sub-plot was the gamma doses (R0= 0; R1= 100; R2= 200; R3= 300; R4= 400; R5= 500; R6= 600; R7= 700 Gy) within three replicates. The results showed that the variety significantly affected germination characteristics, except germination percentage. The gamma doses and their interaction significantly affected all germination characteristics of red rice under salinity. The Inpara-7 variety at the gamma-exposed was more tolerant, and wider LD_{20-50} ranged from 13.38 to 556.58 Gy under salinity. Gamma dose of 100 Gy can increase roots length

and volume (3.77 and 1.95%) compared to untreated and decrease with increasing gamma dose until 700 Gy. The interaction of 100 Gy with the Inpara-7 could support the red rice germination under salinity.
Keywords: gamma doses; lethal dose 50; NaCl; red rice varieties; seedlings

MAIN TEXT

Introduction

Rice (*Oryza sativa* L.) is widely grown in Asia, Latin America, Africa and is a staple food for more than 50% of the world population (Lou et al., 2012). Almost 90% of rice is 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; Calpe, 2006). 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 to 3.19 g, crude fiber by 0.28 to 0.61 g, the 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, 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 was 36.14 mg/g; 0.66 mg/g; 0.03 mg/g; 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 (Suriany, 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 the coastal area is susceptible to a salinity area of 12.020 million ha or 6.20% of the total area of Indonesia. 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 inhibits grain development, especially inferior grains, significantly reducing 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) stated 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 LD_{50} (Oldach, 2011; Ángeles-Espino et al., 2013) and Growth Reduction 30/50 or GR_{30}/GR_{50} (Khalil et al., 2014). Mutants resulting from gamma-ray irradiation will produce the highest diversity around LD_{20} and LD_{50} (Human, 2012).

Thus, the germination phase is the initial stage for the development of gamma irradiation in red rice plants under salinity stress conditions. Selvan & 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. Hameed et al. (2008) reported that high doses of gamma-ray radiation applied to seeds could interfere with protein synthesis, hormone balance, leaf air exchange, water exchange, and enzyme activity. It has been reported by Dehpour et al. (2011) that the lowest percentage of germination and shoot length of rice was found in the gamma irradiation treatment <300 Gy with a salinity of 15 mmosh/l. The researcher also said an increase in the proline content of rice seedling along with the increase in the dose of gamma irradiation to 300 Gy and an increase in salinity to 25 mmosh/l. Shereen et al. (2009) reported that it found a radiation dose of 150 Gy to be more effective to survive, increasing rice biomass production and nutrient response at higher salinity conditions (75 mM NaCl).

However, gamma irradiation has not been reported on the germination of superior red rice plants from Indonesia, such as MSP-17, Inpari-24 Gabusan, Pamera, Pamelen, and Inpara-7 varieties under salinity conditions. The goal studies were: (1) to obtain the salinity-tolerant red rice varieties at the germination phase and (2) obtain the LD_{20-50} of gamma irradiation that could support red rice germination under salinity conditions.

Materials and Methods

Source of Red Rice Seeds

The varieties of red rice were 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.

Gamma Ray Induction

A total of 250 g seeds of red rice was taken from the treatment combination, and gamma irradiation was conducted at the Isotope and Radiation Application Center, National Nuclear Energy Agency of Indonesia (PAIR-BATAN) Jakarta, in March 2020. Gamma radiation as a source was Co^{60} of Gamma Chambers.

Location and Design

This germination test was conducted at the greenhouse of Growth Center, Higher Education Service Institute of North Sumatra Region-I (LLDIKTI-1) Medan from September to October 2020. This study used a Split Plot Design (SPD) with the main-plot was red rice varieties (V1= MSP-17; V2= Inpari-24 Gabusan; V3= Pamera; V4= Pamelen; V5= Inpara-7) and sub-plots was doses of gamma-ray irradiation (R0= 0; R1= 100 Gy; R2= 200 Gy; R3= 300 Gy; R4= 400 Gy; R5= 500 Gy; R6= 600 Gy; R7= 700 Gy). The treatment combination was repeated in three replicates.

Saline Soil Collection and Seedlings Test

Saline soil was taken from Paluh Merbau Sub-village, Tanjung Rejo Village, Sei Tuan Sub-district, Deli Serdang District, North Sumatra, Indonesia, and measured the level of salinity using a DHL meter. The measurement results showed that the level of soil salinity was 5 to 6 mhos. It has taken 100 seeds of red rice that have been irradiated by gamma rays and then planted into the seedling tray and arranged according to the study design.

Parameters and Data Analysis

The parameters of this study included maximum growth potential (MGP), germination percentage (GP), vigor index (VI), root length (RL), root volume (RV), Lethal Dose 20 (LD_{20}), LD_{50}, and correlation analysis. 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 seedling (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 Lethal Dose 20 (LD_{20}) to LD_{50} (Indrayanti et al., 2011) through probit regression analysis of the germination percentage.

$$MGP = \frac{\Sigma \text{ Growing seeds}}{\Sigma \text{ Planted seeds}} \text{ x } 100\% \qquad (1)$$

$$GP = \frac{\Sigma \text{ NS } 3 + \text{NS } 4 + \text{........} + \text{NS } 9}{\Sigma \text{ Planted seeds}} \text{ x } 100\% \qquad (2)$$

$$VI = \frac{\Sigma \text{ Normal seedlings on the third day}}{\Sigma \text{ Planted seeds}} \text{ x } 100\% \qquad (3)$$

The relationship between gamma irradiation doses and the germination character of red rice varieties was measured using Pearson correlation analysis. The data of this study were analyzed using analysis of variance (ANOVA) and set up by DMRT at the level of 5% using IBM SPSS Statistics v.20 software.

Results

The gamma irradiation doses (R) and their interaction significantly affect maximum growth potential, germination percentage, vigor index, root length, and root volume of red rice plants under salinity conditions, as well as the varieties (V) except for the germination percentage (Tabel 1).

Table 1. F-value of the effect of gamma-ray doses on five varieties of red rice under salinity conditions.

Source	F-value				
	MGP	GP	VI	RL	RV
Block	0.21^{ns}	1.08^{ns}	1.23^{ns}	0.20^{ns}	2.30^{ns}
Main-plots					
Varieties (V)	8.10^{*}	1.51^{ns}	8.20^{*}	61.45^{*}	31.96^{*}
Sub-plots					
Gamma doses (R)	407.50^{*}	204.63^{*}	193.99^{*}	90.02^{*}	116.07^{*}
Interaction (V×R)	3.08^{*}	3.73^{*}	3.28^{*}	2.06^{*}	5.44^{*}

Note: F-value is significant at the 0.05 level (*) and not significant (ns). MGP= maximum growth potential, GP= germination percentage, VI= vigor index, RL= root length, RV= root volume.

Maximum Growth Potential (%)

The effect of varieties, gamma irradiation doses, and interaction significantly affects red rice's maximum growth potential under salinity conditions (Table 2). The results showed that the Pamelen variety of red rice had the highest maximum growth potential of 83.29% in saline conditions compared to other varieties. It can be seen that the higher the doses of gamma irradiation up to 700 Gy resulting the lower the maximum growth potential of red rice, but the gamma dose of 100 Gy had the maximum growth potential, which was not different from the un-irradiated (control). The interaction of gamma doses from 0 to 100 Gy in the five varieties of red rice (MSP-17, Inpari-24 Gabusan, Pamera, Pamelen, Inpara-7) had a maximum growth potential that was significantly different compared to other interactions.

Table 2. The effect of varieties, gamma irradiation doses, and its interaction on the maximum growth potential of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	100.00 a	100.00 a	100.00 a	100.00 a	99.33 a	99.87 a
100	99.67 a	99.67 a	100.00 a	99.33 a	98.67 a	99.47 a
200	91.33 b	87.67 b-e	89.00 bcd	93.33 ab	90.00 bc	90.27 b
300	83.33 c-f	80.67 e-i	80.67 e-i	87.00 b-f	82.67 d-g	82.87 c
400	74.33 ijk	75.33 hij	70.67 j-m	80.33 f-i	80.33 f-i	76.20 d
500	67.67 k-o	67.00 l-o	64.67 mno	82.00 e-h	72.33 jkl	70.73 e
600	62.67 nop	61.33 opq	57.33 pq	76.00 g-j	68.33 k-n	65.13 f
700	47.67 r	40.00 s	40.33 s	48.33 r	56.00 q	46.47 g
Average	78.33 c	76.46 cd	75.33 d	83.29 a	80.96 b	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at *P*<0.05.

Germination Percentage (%)

The effect of gamma irradiation doses and their interaction significantly affected the germination percentage of red rice under salinity conditions, but varieties had an insignificant effect (Table 3). The higher the dose of gamma irradiation up to 700 Gy, the lower the germination percentage of red rice under saline conditions. The highest germination percentage of red rice was found un-irradiated at 72.33% compared to other doses. The interaction of un-irradiated within the Inpara-7 variety significantly had the highest germination percentage of 74.67% compared to other interactions.

Table 3. The effect of varieties, gamma irradiation doses, and interaction on the germination percentage of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	72.33 abc	72.33 abc	69.33 a-d	73.00 ab	74.67 a	72.33 a
100	67.67 b-e	66.00 c-f	62.33 e-h	64.00 d-g	62.33 e-h	64.47 b
200	62.67 d-g	61.67 e-i	59.67 f-j	61.00 e-i	60.33 f-j	61.07 c
300	55.33 h-l	59.33 f-j	55.00 i-l	57.00 g-k	58.67 g-j	57.07 d
400	50.00 k-p	55.33 h-l	50.00 k-p	53.67 j-m	55.33 h-l	52.87 e
500	49.67 l-p	49.00 l-p	46.00 n-r	50.67 k-o	51.67 k-n	49.40 f
600	43.00 pqr	40.00 rs	41.00 qrs	47.00 m-q	47.67 m-q	43.73 g
700	24.67 t	36.00 s	40.00 rs	44.33 o-r	45.00 n-r	38.00 h
Average	53.17 ns	54.96 ns	52.92 ns	56.33 ns	56.96 ns	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at *P*<0.05. not significant (ns).

Vigor Index (%)

The effect of gamma irradiation doses, varieties, and their interaction significantly affected the vigor index of red rice under salinity conditions (Table 4). The results showed that red rice varieties of MSP-17 and Inpara-7 had a vigor index of 65.58%, and 64.42% were significantly different from other varieties. The higher the doses of gamma irradiation up to 700 Gy, the lower the vigor index of red rice under salinity conditions. The gamma doses of 0 to 100 Gy had the vigor index of red rice significantly different than other doses, and the highest was found in un-irradiated by 72.93%. The interaction of un-irradiated within the MSP-17 variety significantly had the highest vigor index of 77.67% compared to other interactions.

Table 4. The effect of varieties, gamma irradiation doses, and its interaction on the vigor index of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	77.67 a	73.00 a-d	70.67 a-f	67.00 c-i	76.33 ab	72.93 a
100	76.33 ab	72.33 a-e	68.33 c-h	64.67 f-l	73.33 abc	71.00 a
200	72.33 a-e	69.00 b-g	64.00 f-m	61.00 h-o	70.00 b-f	67.27 b
300	68.00 c-i	61.00 h-o	58.67 j-p	57.33 l-q	65.67 d-j	62.13 c
400	65.33 e-k	57.00 m-r	53.00 p-s	54.33 o-s	60.67 i-o	58.07 d
500	62.00 g-n	49.00 st	49.67 rst	50.67 prs	58.00 k-q	53.87 e
600	55.00 n-s	37.67 vw	43.33 tuv	47.67 stu	57.00 m-r	48.13 f
700	48.00 stu	35.33 w	39.33 vw	41.67 uvw	54.33 o-s	43.73 g
Average	65.58 a	56.79 b	55.88 b	55.54 b	64.42 a	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at P<0.05.

Root Length (cm)

The effect of gamma irradiation doses, varieties, and their interaction significantly affects the root length of red rice under salinity conditions (Table 5).

Table 5. The effect of varieties, gamma irradiation doses, and its interaction on the root length of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	15.20 efg	17.96 bcd	15.25 efg	14.17 f-i	19.65 b	16.44 a
100	15.50 def	19.05 bc	14.17 f-i	13.66 f-j	22.91 a	17.06 a
200	14.92 e-h	18.24 bc	14.24 f-i	13.55 f-l	20.05 b	16.20 a
300	12.41 h-n	15.51 def	13.74 f-l	13.41 f-l	18.08 bc	14.63 b
400	11.38 k-p	11.77 i-p	11.47 j-p	12.17 i-o	17.07 cde	12.77 c
500	11.11 l-q	12.67 g-m	10.08 m-q	9.90 n-q	15.65 def	11.88 c
600	9.73 o-r	9.25 pqr	9.68 o-r	7.30 rst	13.88 f-k	9.97 d
700	8.58 qrs	5.88 t	6.18 st	6.50 st	12.52 h-m	7.93 e
Average	12.35 c	13.79 b	11.85 cd	11.33 d	17.48 a	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at P<0.05.

The results showed that the Inpara-7 variety of red rice had a root length of 17.48 cm was significantly different from other varieties. The gamma-ray of 100 Gy increased the root length of red rice by 3.77% compared to the control and decreased along with the increase in the gamma irradiation dose up to 700 Gy. The gamma doses of 0 to 200 Gy had the root length of red rice significantly different from other doses. The interaction of gamma at a dose of 100 Gy with the Inpara-7 variety significantly had the highest root length of 22.91 cm compared to other interactions.

Root Volume (ml)

The effect of gamma irradiation doses, varieties, and their interaction significantly affected the root volume of red rice under salinity conditions (Table 6). The Inpara-7 variety of red rice with a root volume of 35.00 ml was significantly different from other varieties. The application of 100 Gy gamma rays increased the root volume of red rice by 1.95% compared to the control and decreased along with the increase in gamma irradiation dose up to 700 Gy. The gamma doses of 0 to 100 Gy had the root volume of red rice significantly different from other doses. The interaction of 100 Gy gamma within the Inpara-7 variety significantly had the highest root volume of 37.81 ml compared to other interactions.

Table 6. The effect of varieties, gamma irradiation doses, and its interaction on the root volume of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	34.24 b-e	32.25 d-g	30.41 f-i	30.86 f-i	34.15 b-e	32.38 a
100	34.48 b-e	32.48 c-f	29.60 f-k	30.69 f-i	37.81 a	33.01 a
200	31.81 e-h	30.14 f-j	27.88 i-o	28.76 h-m	35.93 ab	30.90 b
300	29.28 g-l	28.85 h-m	26.39 l-p	26.71 k-p	34.73 b-e	29.19 c
400	26.81 k-p	28.21 i-o	25.27 opq	26.21 l-q	34.18 b-e	28.14 d
500	25.67 m-q	27.13 j-o	23.27 qrs	23.74 p-s	35.25 a-d	27.01 e
600	23.83 pqr	22.14 rst	20.21 tu	20.75 stu	35.49 abc	24.48 f
700	21.76 r-u	19.75 tu	18.84 u	19.29 tu	32.48 c-f	22.42 g
Average	28.48 b	27.62 c	25.23 d	25.88 d	35.00 a	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at *P*<0.05.

Lethal Dose 20 dan 50 ($LD_{20\text{-}50}$)

Lethal doses 20 and 50 from gamma irradiation in the five varieties of red rice under saline conditions can be seen in Figure 1. The Inpara-7 variety had the highest radiosensitivity value (LD_{50}) of 556.58 Gy compared to other varieties. The dose of gamma irradiation could be determined to reduce 50% seedling of Inpara-7 variety under saline conditions, namely 556.58 Gy. 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 $LD_{20\text{-}50}$ in the Inpara-7 variety ranged from 13.38 to 556.58 Gy. Results also showed that the Inpara-7 variety was classified as tolerant caused it had the higher LD_{50}. The MSP-17 variety was more sensitive because it had the lowest LD_{50} than other varieties based on germination percentage.

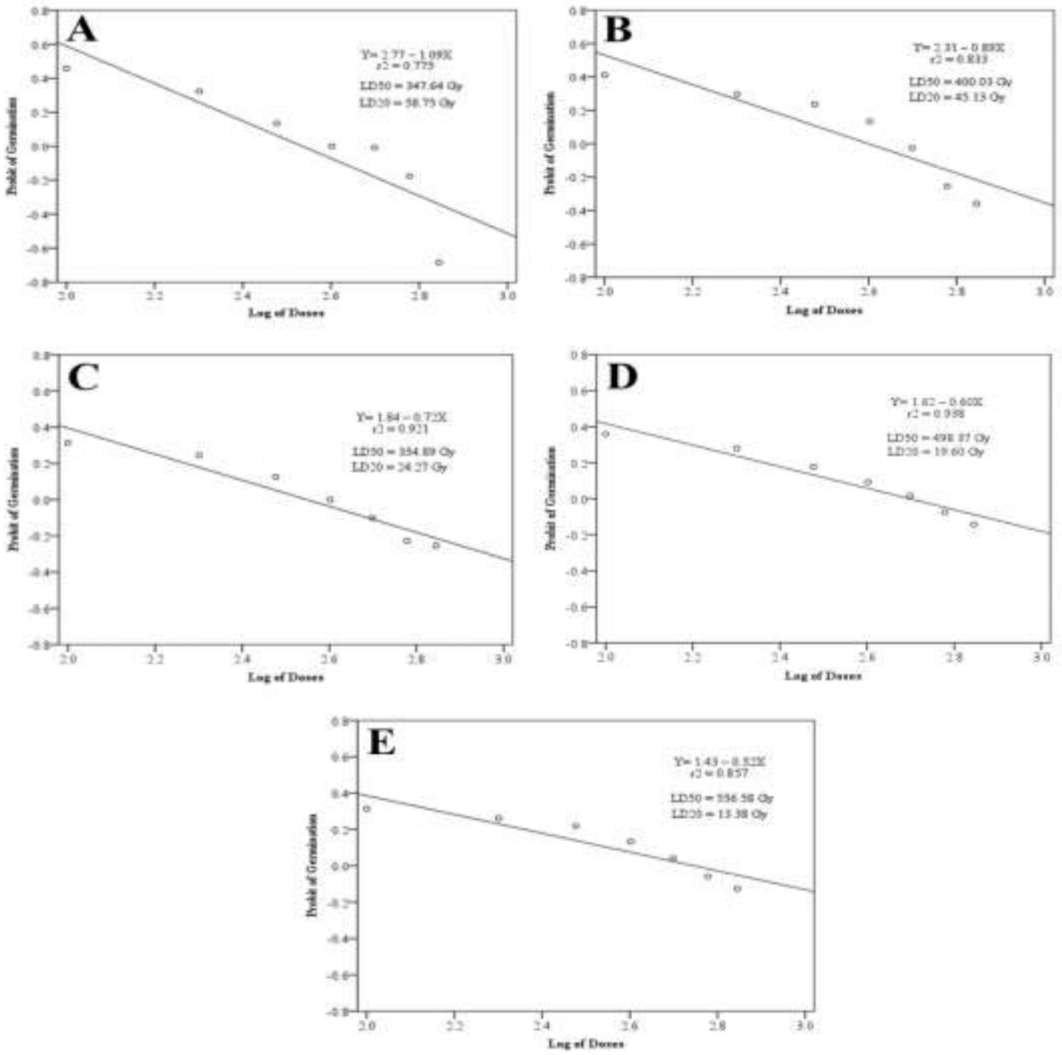

Figure 1. The lethal doses 20 and 50 of gamma irradiation from red rice varieties under saline. A= MSP-17; B= Inpari-24 Gabusan; C= Pamera; D= Pamelen; E= Inpara-7.

Correlation Analysis of Germination Characteristics

Correlation analysis between gamma irradiation doses on the germination characteristics of the red rice varieties under saline conditions can be seen in Table 7.

Table 7. Correlation analysis between gamma irradiation doses on the germination characteristics of the red rice varieties under saline conditions

Red rice varieties	Germination characteristics				
	MGP	GP	VI	RL	RV
MSP-17	-0.966**	-0.926**	-0.944**	-0.863**	-0.918**
Inpari-24 Gabusan	-0.970**	-0.954**	-0.956**	-0.917**	-0.901**
Pamera	-0.973**	-0.943**	-0.911**	-0.904**	-0.974**
Pamelen	-0.891**	-0.952**	-0.948**	-0.883**	-0.883**
Inpara-7	-0.968**	-0.930**	-0.915**	-0.886**	-0.380^{ns}

Note: Correlation is significant at the 0.01 level (2-tailed) and not significant (ns). MGP= maximum growth potential, GP= germination percentage, VI= vigor index, RL= root length, RV= root volume.

The relationship between gamma irradiation doses and germination characteristics (maximum growth potential, germination percentage, vigor index, root length, and root volume) in the five varieties of red rice were significantly negatively correlated, except for root volume in the Inpara-7 variety. The higher gamma irradiation doses resulting, the lower or inhibited germination characteristics of red rice under salinity conditions. Therefore, the application of gamma irradiation must be based on the LD_{50} to support red rice germination under salinity conditions.

Discussion

The effect of varieties on red rice germination under salinity

Varieties significantly affect maximum growth potential, vigor index, root length, and volume, but they had an insignificant effect on the germination percentage of red rice under salinity conditions. The Inpara-7 variety had the highest root length and volume of 17.48 cm and 35.00 ml, respectively (Tables 5-6) and the vigor index by 64.42%, significantly different from other varieties (Table 4). On the other hand, the Pamelen variety had the highest maximum growth potential of 83.29%, and the MSP-17 variety also had the highest vigor index of 65.58%. It was significantly different from other varieties. Thus, the Inpara-7 variety in gamma-exposed was more tolerant than other red rice varieties under saline conditions. The higher LD_{50} supported this result in the Inpara-7 variety of 556.58 Gy compared to other varieties (Figure 1). Besides, the optimum dose of gamma irradiation on the Inpara-7 variety was also wide ranged from 13.38 to 556.58 Gy (Figure 1). The range value of LD_{20} and LD_{50} in the Inpara-7 variety of red rice as an indicator of increased genetic diversity in germination characteristics, such as root length, root volume, and vigor index, tends to be higher than other rice varieties. In addition, gamma-ray irradiation can affect the physiology of rice plants resulting in greater rice growth at certain gamma doses under salinity conditions. It was confirmed by Shereen et al. (2009) that the total chlorophyll content of Shua-92, Sarshar, and IR-8 mutants of rice increased with increasing irradiation doses compared to non-saline conditions. Under saline conditions, the amount of chlorophyll b was relatively higher than chlorophyll a. It found higher chlorophyll content at a radiation dose of 150 Gy in 50 and 75 mM NaCl salinity levels. Baek et al. (2005) also reported that an increase in chlorophyll, superoxide dismutase (SOD), and ascorbate peroxidase (APX) in rice seedlings of Ilpumbyeo and Sanghaehyanghyella varieties at gamma doses of 8 Gy with the salinity of 50 and 100 mM compared to the control.

Therefore, the Inpara-7 variety was more tolerant and potential than other gamma irradiation varieties in increasing germination in saline soils. Indrayanti et al. (2011) reported that the radiosensitivity of plants to gamma-ray irradiation in increasing genetic diversity could be measured using the optimum dose in the ranged of LD_{20} to LD_{50}. Suliartini et al. (2015) stated that the lower the LD_{50} value, the more sensitivity to gamma irradiation, and vice versa. Harding et al. (2012) reported that the percentage of survival of rice seedlings in the laboratory decreased significantly with increasing doses of gamma radiation up to 600 Gy, whereas at doses of more than 300 Gy resulted in a decrease in seedling height and survival of the M1 generation in the field. Suliartini et al. (2020) reported that the optimal dose of gamma irradiation for germination of lowland rice varieties include G10, G16, Baas Selem, and Inpago Unram-1 was 264; 398; 316; and 518 Gy, respectively, and resulted in the G10 variety was classified as the most sensitive and the Inpago Unram-1 variety was classified as tolerant. Budi et al. (2019) added 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. Gowthami et al. (2017) also reported 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, and ADT(R)-45 variety was more sensitive compared to the ADT-37 variety in the first generation (M1).

The effect of gamma irradiation doses on red rice germination under salinity

Gamma irradiation doses significantly affected maximum growth potential, germination percentage, vigor index, root length, and volume of red rice under salinity conditions. The higher the amount of gamma irradiation up to 700 Gy, the lower the maximum growth potential, germination percentage, and vigor index of red rice, but differ in the character of root length and volume. The gamma dose of 100 Gy had the maximum red rice growth potential and vigor index, insignificantly different from un-irradiated (control). Gamma irradiation of 100 Gy could be increased the root length and volume of red rice by 3.77% and 1.95%, respectively, compared to control and decreased with increasing doses of gamma irradiation up to 700 Gy. The gamma dose of 0 to 200 Gy had the red rice root length significantly different from other doses. It was supported by the relationship of gamma irradiation doses with germination characteristics (maximum growth potential, germination, vigor index, root length, and root volume) in the five varieties of red rice that were significantly negatively correlated (Table 7).

It has been reported by Kumar et al. (2013); Olakunle et al. (2016) that high doses of gamma irradiation affect electrons in the nucleus, which convert normal ions into positive and release energy (ionization) as it passes through the tissue, and it had an impact on the formation of reactive hydroxyl radicals. The radical reaction could be affected by chemical changes and directly react with all organic molecules structurally and functionally, resulting in metabolism and germination. Kumar et al. (2013) also added that gamma irradiation could be reduced the α-amylase activity, resulting in the inhibition of germination. Pujiyanti et al. (2021) reported that the gamma irradiation dose of 400 to 800 Gy on the Barak Cenana variety of red rice had a negative effect on germination growth and obtained two mutants (A3 and C6) at radiation doses of 100 and 400 Gy, which had the greater characters, include shorter plant height, shorter harvest period and higher amount of grain-filled compared to the wild type.

The interaction effect of varieties and gamma irradiation doses

Interaction of varieties with gamma irradiation doses significantly affected all germination characteristics of red rice plants under salinity conditions. The gamma interaction of 0 to 100 Gy in the five varieties of red rice (MSP-17, Inpari-24 Gabusan, Pamera, Pamelen, Inpara-7) had a maximum growth potential significantly different compared to other interactions. It was found that interaction un-irradiated (control) with Inpara-7 and MSP-17 varieties had the highest germination percentage and vigor index of 74.67% and 77.67%, respectively. Likewise, the interaction of gamma 100 Gy with the Inpara-7 variety had the highest root length and volume of 22.91 cm and 37.81 ml, respectively. It was shown that the interaction of 100 Gy gamma irradiation with the Inpara-7 variety could be applied in salinity conditions. The Inpara-7 variety caused it to be tolerant on exposure to gamma (higher LD_{50}) compared to the other varieties (Figure 1), and gamma irradiation dose insignificant negative correlation to the root volume parameter in the Inpara-7 variety (Table 7). According to Kadhimi et al. (2016), that gamma irradiation dose of 200 to 500 Gy could be showed a decrease in the seedling rate and height of MR269 and MRQ74 varieties (local rice), doses exceeding 500 Gy caused physiological damage to the seedling height and not found varieties that survive. It was found that the LD_{50} for MR269 and MRQ74 varieties of local rice were 351 and 365 Gy, respectively. Prabhandaru and Saputro (2017) reported that increasing the irradiation dose could decrease germination, and the simultaneous growth of SiGadis rice seeds and un-irradiation and 100 Gy treatments had similar results (80% germination). Meanwhile, doses of 200 and 300 Gy decreased the germination rate sequentially by 70% and 60%.

Conclusions

Varieties significantly affected all germination characteristics of red rice, except for germination percentage. However, the gamma irradiation doses and their interaction significantly affected all germination characteristics of red rice under salinity conditions. The Inpara-7 variety that gamma-exposed was more tolerant than other varieties of red rice under saline conditions. Gamma dose of 100 Gy had the maximum growth potential and vigor index of red rice insignificantly different from un-irradiated. It could increase root length and volume (3.77% and 1.95%) compared to control and decrease with increasing gamma irradiation doses up to 700 Gy. The interaction of 100 Gy gamma with the Inpara-7 variety showed greater germination characteristics compared to other interactions. Among the red rice varieties tested, it was found in the Inpara-7 variety had a wider optimum dose of gamma irradiation ($LD_{20\text{-}50}$) ranged from 13.38 to 556.58 Gy.

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Germination characteristics of red rice as affected by induction irradiated gamma under saline conditions

Reviews ver. 1

Review no. 1: Recommended major revision

revision with the addition of advanced stages with K/Na parameters and proline content

1. Originality of the contribution *good*	2. Importance in its field *good*
3. Style, overall presentation & understandability *good*	4. Awareness of related work *good*
5. English language *good*	6. Does the title represent manuscript's contents? *yes*
7. Is the Abstract accurate and concise? *yes*	8. Are the objectives/goals clearly defined? *yes*
9. Are the approach/methods properly described? *yes*	10. Are the conclusions and interpretations sound? *yes*
11. Are the references properly cited and presented? *yes*	12. Is this a new/original/confirmatory contribution? *yes*
13. Are all images and tables necessary? *yes*	14. Paper type: *Research article*

Review no. 2: Recommended minor revision

The research article entitled "Germination characteristics of red rice as affected by induction irradiated 4 gamma under saline conditions". The theme of the study is interesting and the draft is well compiled.

My general comments/suggestions are as under,
In abstract section: Add problem statement. Summarize the key results. If possible, add the numerical description of most important results. Be consistent regarding units.
Introduction section contains various unnecessary statements. Remain focused on the topic. Begin with the broadest scope and get progressively narrower, leading steadily to the statement of objectives. Be clear regarding objectives.
Discussion should be merely based on the observed findings. Not just a review of literature. Answer the question posed in introduction and correlate your finding with the existing knowledge.
Conclusion: Just report the key findings… it should not be a detailed summary of the work done. It seems lengthy.
Majority of the references are too old. Add few recent studies.
Language needs substantial improvement. There are several grammatical and typo mistakes throughout the manuscript.
All the tables and figures should be self-explanatory. Define all the abbreviations in the table foot note/figure captions.

1. Originality of the contribution *good*	2. Importance in its field *fair*
3. Style, overall presentation & understandability *good*	4. Awareness of related work *fair*
5. English language *fair*	6. Does the title represent manuscript's contents? *yes*
7. Is the Abstract accurate and concise? *yes*	8. Are the objectives/goals clearly defined? *yes*
9. Are the approach/methods properly described? *yes*	10. Are the conclusions and interpretations sound? *yes*
11. Are the references properly cited and presented? *yes*	12. Is this a new/original/confirmatory contribution? *yes*
13. Are all images and tables necessary? *yes*	14. Paper type: *Research article*

TITLE PAGE

Title: Germination characteristics of red rice as affected by induction irradiated gamma under saline conditions

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Abstract

The goal studies were: (1) to obtain the salinity-tolerant red rice varieties at the germination phase and (2) to find the lethal dose 20-50 of gamma that could support red rice germination under salinity. The germination test was conducted at the greenhouse of Growth Center, LLDIKTI-1 Medan, from September until October 2020. This study was used a Split Plot Design with the main-plot of red rice varieties (V1 = MSP-17, V2= Inpari-24 Gabusan, V3= Pamera, V4= Pamelen; V5= Inpara-7) and sub-plot was the gamma doses (R0= 0; R1= 100; R2= 200; R3= 300; R4= 400; R5= 500; R6= 600; R7= 700 Gy) within three replicates. The results showed that the variety significantly affected germination characteristics, except germination percentage. The gamma doses and their interaction significantly affected all germination characteristics of red rice under salinity. The Inpara-7 variety at the gamma-exposed was more tolerant, and wider LD_{20-50} ranged from 13.38 to 556.58 Gy under salinity. Gamma dose of 100 Gy can increase roots length and volume (3.77 and 1.95%) compared to untreated and decrease with increasing gamma dose until 700 Gy. The interaction of 100 Gy with the Inpara-7 could support the red rice germination under salinity.

Keywords: gamma doses; lethal dose 50; NaCl; red rice varieties; seedlings

MAIN TEXT

Introduction

Rice (*Oryza sativa* L.) is widely grown in Asia, Latin America, Africa and is a staple food for more than 50% of the world population (Lou et al., 2012). Almost 90% of rice is 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; Calpe, 2006). 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 to 3.19 g, crude fiber by 0.28 to 0.61 g, the 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, 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 was 36.14 mg/g; 0.66 mg/g; 0.03 mg/g; 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 (Suriany, 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 the coastal area is susceptible to a salinity area of 12.020 million ha or 6.20% of the total area of Indonesia. 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 inhibits grain development, especially inferior grains, significantly reducing 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) stated 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 LD_{50} (Oldach, 2011; Ángeles-Espino et al., 2013) and Growth Reduction 30/50 or GR_{30}/GR_{50} (Khalil et al., 2014). Mutants resulting from gamma-ray irradiation will produce the highest diversity around LD_{20} and LD_{50} (Human, 2012).

Thus, the germination phase is the initial stage for the development of gamma irradiation in red rice plants under salinity stress conditions. Selvan & 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. Hameed et al. (2008) reported that high doses of gamma-ray radiation applied to seeds could interfere with protein synthesis, hormone balance, leaf air exchange, water exchange, and enzyme activity. It has been reported by Dehpour et al. (2011) that the lowest percentage of germination and shoot length of rice was found in the gamma irradiation treatment <300 Gy with a salinity of 15 mmosh/l. The researcher also said an increase in the proline content of rice seedling along with the increase in the dose of gamma irradiation to 300 Gy and an increase in salinity to 25 mmosh/l. Shereen et al. (2009) reported that it found a radiation dose of 150 Gy to be more effective to survive, increasing rice biomass production and nutrient response at higher salinity conditions (75 mM NaCl).

However, gamma irradiation has not been reported on the germination of superior red rice plants from Indonesia, such as MSP-17, Inpari-24 Gabusan, Pamera, Pamelen, and Inpara-7 varieties under salinity conditions. The goal studies were: (1) to obtain the salinity-tolerant red rice varieties at the germination phase and (2) obtain the LD_{20-50} of gamma irradiation that could support red rice germination under salinity conditions.

Materials and Methods

Source of Red Rice Seeds

The varieties of red rice were 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.

Gamma Ray Induction

A total of 250 g seeds of red rice was taken from the treatment combination, and gamma irradiation was conducted at the Isotope and Radiation Application Center, National Nuclear Energy Agency of Indonesia (PAIR-BATAN) Jakarta, in March 2020. Gamma radiation as a source was Co^{60} of Gamma Chambers.

Location and Design

This germination test was conducted at the greenhouse of Growth Center, Higher Education Service Institute of North Sumatra Region-I (LLDIKTI-1) Medan from September to October 2020. This study used a Split Plot Design (SPD) with the main-plot was red rice varieties (V1= MSP-17; V2= Inpari-24 Gabusan; V3= Pamera; V4= Pamelen; V5= Inpara-7) and sub-plots was doses of gamma-ray irradiation (R0= 0; R1= 100 Gy; R2= 200 Gy; R3= 300 Gy; R4= 400 Gy; R5= 500 Gy; R6= 600 Gy; R7= 700 Gy). The treatment combination was repeated in three replicates.

Saline Soil Collection and Seedlings Test

Saline soil was taken from Paluh Merbau Sub-village, Tanjung Rejo Village, Sei Tuan Sub-district, Deli Serdang District, North Sumatra, Indonesia, and measured the level of salinity using a DHL meter. The measurement results showed that the level of soil salinity was 5 to 6 mhos. It has taken 100 seeds of red rice that have been irradiated by gamma rays and then planted into the seedling tray and arranged according to the study design.

Parameters and Data Analysis

The parameters of this study included maximum growth potential (MGP), germination percentage (GP), vigor index (VI), root length (RL), root volume (RV), Lethal Dose 20 (LD_{20}), LD_{50}, and correlation analysis. 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 seedling (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 Lethal Dose 20 (LD_{20}) to LD_{50} (Indrayanti et al., 2011) through probit regression analysis of the germination percentage.

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

$$GP = \frac{\Sigma \text{ NS 3} + \text{NS 4} + \ldots\ldots.. + \text{NS 9}}{\Sigma \text{ Planted seeds}} \text{ x } 100\% \quad (2)$$

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

The relationship between gamma irradiation doses and the germination character of red rice varieties was measured using Pearson correlation analysis. The data of this study were analyzed using analysis of variance (ANOVA) and set up by DMRT at the level of 5% using IBM SPSS Statistics v.20 software.

Results

The gamma irradiation doses (R) and their interaction significantly affect maximum growth potential, germination percentage, vigor index, root length, and root volume of red rice plants under salinity conditions, as well as the varieties (V) except for the germination percentage (Tabel 1).

Table 1. F-value of the effect of gamma-ray doses on five varieties of red rice under salinity conditions.

Source	F-value				
	MGP	GP	VI	RL	RV
Block	0.21^{ns}	1.08^{ns}	1.23^{ns}	0.20^{ns}	2.30^{ns}
Main-plots					
Varieties (V)	8.10^{*}	1.51^{ns}	8.20^{*}	61.45^{*}	31.96^{*}
Sub-plots					
Gamma doses (R)	407.50^{*}	204.63^{*}	193.99^{*}	90.02^{*}	116.07^{*}
Interaction (V×R)	3.08^{*}	3.73^{*}	3.28^{*}	2.06^{*}	5.44^{*}

Note: F-value is significant at the 0.05 level (*) and not significant (ns). MGP= maximum growth potential, GP= germination percentage, VI= vigor index, RL= root length, RV= root volume.

Maximum Growth Potential (%)

The effect of varieties, gamma irradiation doses, and interaction significantly affects red rice's maximum growth potential under salinity conditions (Table 2). The results showed that the Pamelen variety of red rice had the highest maximum growth potential of 83.29% in saline conditions compared to other varieties. It can be seen that the higher the doses of gamma irradiation up to 700 Gy resulting the lower the maximum growth potential of red rice, but the gamma dose of 100 Gy had the maximum growth potential, which was not different from the un-irradiated (control). The interaction of gamma doses from 0 to 100 Gy in the five varieties of red rice (MSP-17, Inpari-24 Gabusan, Pamera, Pamelen, Inpara-7) had a maximum growth potential that was significantly different compared to other interactions.

Table 2. The effect of varieties, gamma irradiation doses, and its interaction on the maximum growth potential of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	100.00 a	100.00 a	100.00 a	100.00 a	99.33 a	99.87 a
100	99.67 a	99.67 a	100.00 a	99.33 a	98.67 a	99.47 a
200	91.33 b	87.67 b-e	89.00 bcd	93.33 ab	90.00 bc	90.27 b
300	83.33 c-f	80.67 e-i	80.67 e-i	87.00 b-f	82.67 d-g	82.87 c
400	74.33 ijk	75.33 hij	70.67 j-m	80.33 f-i	80.33 f-i	76.20 d
500	67.67 k-o	67.00 l-o	64.67 mno	82.00 e-h	72.33 jkl	70.73 e
600	62.67 nop	61.33 opq	57.33 pq	76.00 g-j	68.33 k-n	65.13 f
700	47.67 r	40.00 s	40.33 s	48.33 r	56.00 q	46.47 g
Average	78.33 c	76.46 cd	75.33 d	83.29 a	80.96 b	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at $P<0.05$.

Germination Percentage (%)

The effect of gamma irradiation doses and their interaction significantly affected the germination percentage of red rice under salinity conditions, but varieties had an insignificant effect (Table 3). The higher the dose of gamma irradiation up to 700 Gy, the lower the germination percentage of red rice under saline conditions. The highest germination percentage of red rice was found un-irradiated at 72.33% compared to other doses. The interaction of un-irradiated within the Inpara-7 variety significantly had the highest germination percentage of 74.67% compared to other interactions.

Table 3. The effect of varieties, gamma irradiation doses, and interaction on the germination percentage of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	72.33 abc	72.33 abc	69.33 a-d	73.00 ab	74.67 a	72.33 a
100	67.67 b-e	66.00 c-f	62.33 e-h	64.00 d-g	62.33 e-h	64.47 b
200	62.67 d-g	61.67 e-i	59.67 f-j	61.00 e-i	60.33 f-j	61.07 c
300	55.33 h-l	59.33 f-j	55.00 i-l	57.00 g-k	58.67 g-j	57.07 d
400	50.00 k-p	55.33 h-l	50.00 k-p	53.67 j-m	55.33 h-l	52.87 e
500	49.67 l-p	49.00 l-p	46.00 n-r	50.67 k-o	51.67 k-n	49.40 f
600	43.00 pqr	40.00 rs	41.00 qrs	47.00 m-q	47.67 m-q	43.73 g
700	24.67 t	36.00 s	40.00 rs	44.33 o-r	45.00 n-r	38.00 h
Average	53.17 ns	54.96 ns	52.92 ns	56.33 ns	56.96 ns	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at $P<0.05$. not significant (ns).

Vigor Index (%)

The effect of gamma irradiation doses, varieties, and their interaction significantly affected the vigor index of red rice under salinity conditions (Table 4). The results showed that red rice varieties of MSP-17 and Inpara-7 had a vigor index of 65.58%, and 64.42% were significantly different from other varieties. The higher the doses of gamma irradiation up to 700 Gy, the lower the vigor index of red rice under salinity conditions. The gamma doses of 0 to 100 Gy had the vigor index of red rice significantly different than other doses, and the highest was found in un-irradiated by 72.93%. The interaction of un-irradiated within the MSP-17 variety significantly had the highest vigor index of 77.67% compared to other interactions.

Table 4. The effect of varieties, gamma irradiation doses, and its interaction on the vigor index of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	77.67 a	73.00 a-d	70.67 a-f	67.00 c-i	76.33 ab	72.93 a
100	76.33 ab	72.33 a-e	68.33 c-h	64.67 f-l	73.33 abc	71.00 a
200	72.33 a-e	69.00 b-g	64.00 f-m	61.00 h-o	70.00 b-f	67.27 b
300	68.00 c-i	61.00 h-o	58.67 j-p	57.33 l-q	65.67 d-j	62.13 c
400	65.33 e-k	57.00 m-r	53.00 p-s	54.33 o-s	60.67 i-o	58.07 d
500	62.00 g-n	49.00 st	49.67 rst	50.67 prs	58.00 k-q	53.87 e
600	55.00 n-s	37.67 vw	43.33 tuv	47.67 stu	57.00 m-r	48.13 f
700	48.00 stu	35.33 w	39.33 vw	41.67 uvw	54.33 o-s	43.73 g
Average	65.58 a	56.79 b	55.88 b	55.54 b	64.42 a	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at $P<0.05$.

Root Length (cm)

The effect of gamma irradiation doses, varieties, and their interaction significantly affects the root length of red rice under salinity conditions (Table 5).

Table 5. The effect of varieties, gamma irradiation doses, and its interaction on the root length of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	15.20 efg	17.96 bcd	15.25 efg	14.17 f-i	19.65 b	16.44 a
100	15.50 def	19.05 bc	14.17 f-i	13.66 f-j	22.91 a	17.06 a
200	14.92 e-h	18.24 bc	14.24 f-i	13.55 f-l	20.05 b	16.20 a
300	12.41 h-n	15.51 def	13.74 f-l	13.41 f-l	18.08 bc	14.63 b
400	11.38 k-p	11.77 i-p	11.47 j-p	12.17 i-o	17.07 cde	12.77 c
500	11.11 l-q	12.67 g-m	10.08 m-q	9.90 n-q	15.65 def	11.88 c
600	9.73 o-r	9.25 pqr	9.68 o-r	7.30 rst	13.88 f-k	9.97 d
700	8.58 qrs	5.88 t	6.18 st	6.50 st	12.52 h-m	7.93 e
Average	12.35 c	13.79 b	11.85 cd	11.33 d	17.48 a	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at $P<0.05$.

The results showed that the Inpara-7 variety of red rice had a root length of 17.48 cm was significantly different from other varieties. The gamma-ray of 100 Gy increased the root length of red rice by 3.77% compared to the control and decreased along with the increase in the gamma irradiation dose up to 700 Gy. The gamma doses of 0 to 200 Gy had the root length of red rice significantly different from other doses. The interaction of gamma at a dose of 100 Gy with the Inpara-7 variety significantly had the highest root length of 22.91 cm compared to other interactions.

Root Volume (ml)

The effect of gamma irradiation doses, varieties, and their interaction significantly affected the root volume of red rice under salinity conditions (Table 6). The Inpara-7 variety of red rice with a root volume of 35.00 ml was significantly different from other varieties. The application of 100 Gy gamma rays increased the root volume of red rice by 1.95% compared to the control and decreased along with the increase in gamma irradiation dose up to 700 Gy. The gamma doses of 0 to 100 Gy had the root volume of red rice significantly different from other doses. The interaction of 100 Gy gamma within the Inpara-7 variety significantly had the highest root volume of 37.81 ml compared to other interactions.

Table 6. The effect of varieties, gamma irradiation doses, and its interaction on the root volume of red rice under salinity conditions.

Gamma doses (Gy)	Red rice varieties					Average
	MSP-17	Inpari-24 Gabusan	Pamera	Pamelen	Inpara-7	
0	34.24 b-e	32.25 d-g	30.41 f-i	30.86 f-i	34.15 b-e	32.38 a
100	34.48 b-e	32.48 c-f	29.60 f-k	30.69 f-i	37.81 a	33.01 a
200	31.81 e-h	30.14 f-j	27.88 i-o	28.76 h-m	35.93 ab	30.90 b
300	29.28 g-l	28.85 h-m	26.39 l-p	26.71 k-p	34.73 b-e	29.19 c
400	26.81 k-p	28.21 i-o	25.27 opq	26.21 l-q	34.18 b-e	28.14 d
500	25.67 m-q	27.13 j-o	23.27 qrs	23.74 p-s	35.25 a-d	27.01 e
600	23.83 pqr	22.14 rst	20.21 tu	20.75 stu	35.49 abc	24.48 f
700	21.76 r-u	19.75 tu	18.84 u	19.29 tu	32.48 c-f	22.42 g
Average	28.48 b	27.62 c	25.23 d	25.88 d	35.00 a	

Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at $P<0.05$.

Lethal Dose 20 dan 50 (LD_{20-50})

Lethal doses 20 and 50 from gamma irradiation in the five varieties of red rice under saline conditions can be seen in Figure 1. The Inpara-7 variety had the highest radiosensitivity value (LD_{50}) of 556.58 Gy compared to other varieties. The dose of gamma irradiation could be determined to reduce 50% seedling of Inpara-7 variety under saline conditions, namely 556.58 Gy. 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. Results also showed that the Inpara-7 variety was classified as tolerant caused it had the higher LD_{50}. The MSP-17 variety was more sensitive because it had the lowest LD_{50} than other varieties based on germination percentage.

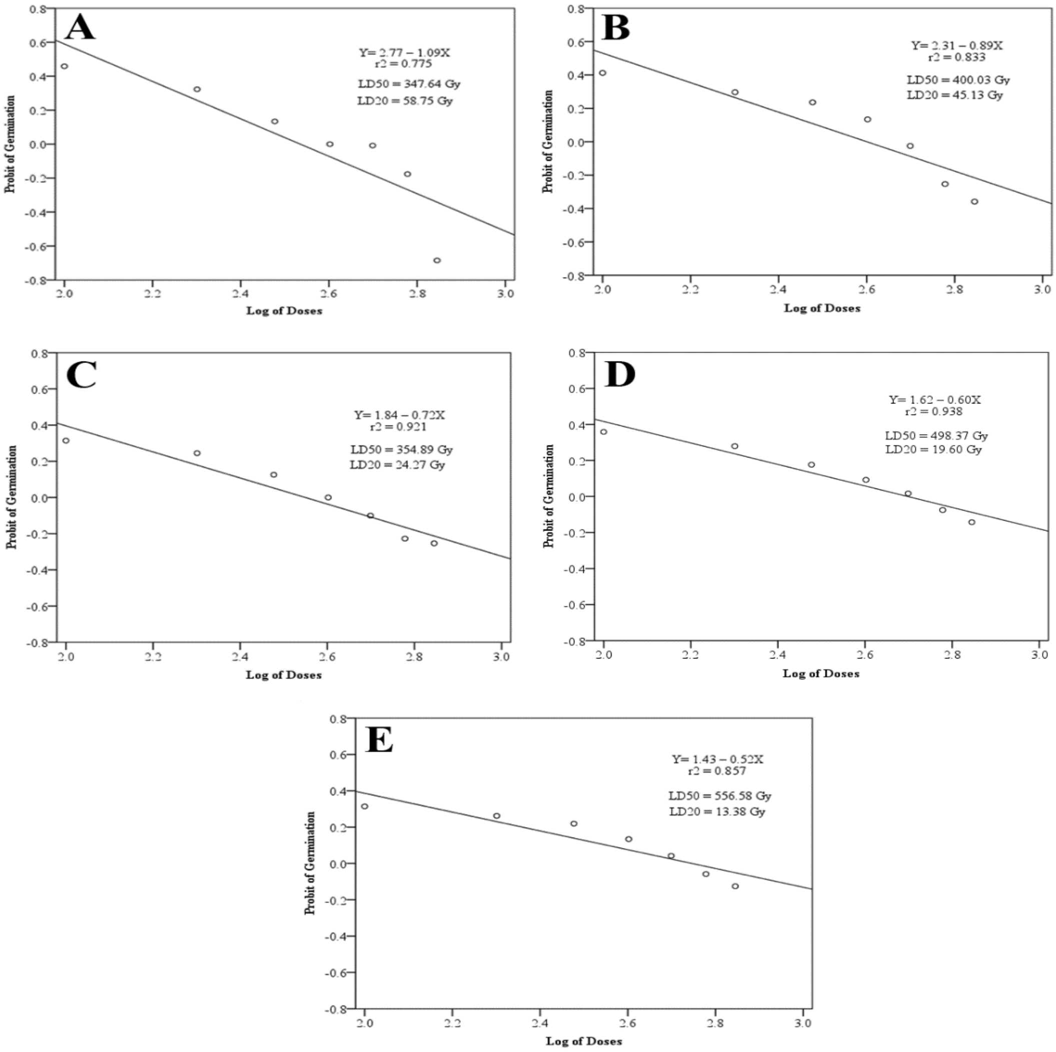

Figure 1. The lethal doses 20 and 50 of gamma irradiation from red rice varieties under saline. A= MSP-17; B= Inpari-24 Gabusan; C= Pamera; D= Pamelen; E= Inpara-7.

Correlation Analysis of Germination Characteristics

Correlation analysis between gamma irradiation doses on the germination characteristics of the red rice varieties under saline conditions can be seen in Table 7.

Table 7. Correlation analysis between gamma irradiation doses on the germination characteristics of the red rice varieties under saline conditions

Red rice varieties	Germination characteristics				
	MGP	GP	VI	RL	RV
MSP-17	-0.966**	-0.926**	-0.944**	-0.863**	-0.918**
Inpari-24 Gabusan	-0.970**	-0.954**	-0.956**	-0.917**	-0.901**
Pamera	-0.973**	-0.943**	-0.911**	-0.904**	-0.974**
Pamelen	-0.891**	-0.952**	-0.948**	-0.883**	-0.883**
Inpara-7	-0.968**	-0.930**	-0.915**	-0.886**	-0.380^{ns}

Note: Correlation is significant at the 0.01 level (2-tailed) and not significant (ns). MGP= maximum growth potential, GP= germination percentage, VI= vigor index, RL= root length, RV= root volume.

The relationship between gamma irradiation doses and germination characteristics (maximum growth potential, germination percentage, vigor index, root length, and root volume) in the five varieties of red rice were significantly negatively correlated, except for root volume in the Inpara-7 variety. The higher gamma irradiation doses resulting, the lower or inhibited germination characteristics of red rice under salinity conditions. Therefore, the application of gamma irradiation must be based on the LD_{50} to support red rice germination under salinity conditions.

Discussion

The effect of varieties on red rice germination under salinity

Varieties significantly affect maximum growth potential, vigor index, root length, and volume, but they had an insignificant effect on the germination percentage of red rice under salinity conditions. The Inpara-7 variety had the highest root length and volume of 17.48 cm and 35.00 ml, respectively (Tables 5-6) and the vigor index by 64.42%, significantly different from other varieties (Table 4). On the other hand, the Pamelen variety had the highest maximum growth potential of 83.29%, and the MSP-17 variety also had the highest vigor index of 65.58%. It was significantly different from other varieties. Thus, the Inpara-7 variety in gamma-exposed was more tolerant than other red rice varieties under saline conditions. The higher LD_{50} supported this result in the Inpara-7 variety of 556.58 Gy compared to other varieties (Figure 1). Besides, the optimum dose of gamma irradiation on the Inpara-7 variety was also wide ranged from 13.38 to 556.58 Gy (Figure 1). The range value of LD_{20} and LD_{50} in the Inpara-7 variety of red rice as an indicator of increased genetic diversity in germination characteristics, such as root length, root volume, and vigor index, tends to be higher than other rice varieties. In addition, gamma-ray irradiation can affect the physiology of rice plants resulting in greater rice growth at certain gamma doses under salinity conditions. It was confirmed by Shereen et al. (2009) that the total chlorophyll content of Shua-92, Sarshar, and IR-8 mutants of rice increased with increasing irradiation doses compared to non-saline conditions. Under saline conditions, the amount of chlorophyll b was relatively higher than chlorophyll a. It found higher chlorophyll content at a radiation dose of 150 Gy in 50 and 75 mM NaCl salinity levels. Baek et al. (2005) also reported that an increase in chlorophyll, superoxide dismutase (SOD), and ascorbate peroxidase (APX) in rice seedlings of Ilpumbyeo and Sanghaehyanghyella varieties at gamma doses of 8 Gy with the salinity of 50 and 100 mM compared to the control.

Therefore, the Inpara-7 variety was more tolerant and potential than other gamma irradiation varieties in increasing germination in saline soils. Indrayanti et al. (2011) reported that the radiosensitivity of plants to gamma-ray irradiation in increasing genetic diversity could be measured using the optimum dose in the ranged of LD_{20} to LD_{50}. Suliartini et al. (2015) stated that the lower the LD_{50} value, the more sensitivity to gamma irradiation, and vice versa. Harding et al. (2012) reported that the percentage of survival of rice seedlings in the laboratory decreased significantly with increasing doses of gamma radiation up to 600 Gy, whereas at doses of more than 300 Gy resulted in a decrease in seedling height and survival of the M1 generation in the field. Suliartini et al. (2020) reported that the optimal dose of gamma irradiation for germination of lowland rice varieties include G10, G16, Baas Selem, and Inpago Unram-1 was 264; 398; 316; and 518 Gy, respectively, and resulted in the G10 variety was classified as the most sensitive and the Inpago Unram-1 variety was classified as tolerant. Budi et al. (2019) added 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. Gowthami et al. (2017) also reported 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, and ADT(R)-45 variety was more sensitive compared to the ADT-37 variety in the first generation (M1).

The effect of gamma irradiation doses on red rice germination under salinity

Gamma irradiation doses significantly affected maximum growth potential, germination percentage, vigor index, root length, and volume of red rice under salinity conditions. The higher the amount of gamma irradiation up to 700 Gy, the lower the maximum growth potential, germination percentage, and vigor index of red rice, but differ in the character of root length and volume. The gamma dose of 100 Gy had the maximum red rice growth potential and vigor index, insignificantly different from un-irradiated (control). Gamma irradiation of 100 Gy could be increased the root length and volume of red rice by 3.77% and 1.95%, respectively, compared to control and decreased with increasing doses of gamma irradiation up to 700 Gy. The gamma dose of 0 to 200 Gy had the red rice root length significantly different from other doses. It was supported by the relationship of gamma irradiation doses with germination characteristics (maximum growth potential, germination, vigor index, root length, and root volume) in the five varieties of red rice that were significantly negatively correlated (Table 7).

It has been reported by Kumar et al. (2013); Olakunle et al. (2016) that high doses of gamma irradiation affect electrons in the nucleus, which convert normal ions into positive and release energy (ionization) as it passes through the tissue, and it had an impact on the formation of reactive hydroxyl radicals. The radical reaction could be affected by chemical changes and directly react with all organic molecules structurally and functionally, resulting in metabolism and germination. Kumar et al. (2013) also added that gamma irradiation could be reduced the α-amylase activity, resulting in the inhibition of germination. Pujiyanti et al. (2021) reported that the gamma irradiation dose of 400 to 800 Gy on the Barak Cenana variety of red rice had a negative effect on germination growth and obtained two mutants (A3 and C6) at radiation doses of 100 and 400 Gy, which had the greater characters, include shorter plant height, shorter harvest period and higher amount of grain-filled compared to the wild type.

The interaction effect of varieties and gamma irradiation doses

Interaction of varieties with gamma irradiation doses significantly affected all germination characteristics of red rice plants under salinity conditions. The gamma interaction of 0 to 100 Gy in the five varieties of red rice (MSP-17, Inpari-24 Gabusan, Pamera, Pamelen, Inpara-7) had a maximum growth potential significantly different compared to other interactions. It was found that interaction un-irradiated (control) with Inpara-7 and MSP-17 varieties had the highest germination percentage and vigor index of 74.67% and 77.67%, respectively. Likewise, the interaction of gamma 100 Gy with the Inpara-7 variety had the highest root length and volume of 22.91 cm and 37.81 ml, respectively. It was shown that the interaction of 100 Gy gamma irradiation with the Inpara-7 variety could be applied in salinity conditions. The Inpara-7 variety caused it to be tolerant on exposure to gamma (higher LD_{50}) compared to the other varieties (Figure 1), and gamma irradiation dose insignificant negative correlation to the root volume parameter in the Inpara-7 variety (Table 7). According to Kadhimi et al. (2016), that gamma irradiation dose of 200 to 500 Gy could be showed a decrease in the seedling rate and height of MR269 and MRQ74 varieties (local rice), doses exceeding 500 Gy caused physiological damage to the seedling height and not found varieties that survive. It was found that the LD_{50} for MR269 and MRQ74 varieties of local rice were 351 and 365 Gy, respectively. Prabhandaru and Saputro (2017) reported that increasing the irradiation dose could decrease germination, and the simultaneous growth of SiGadis rice seeds and un-irradiation and 100 Gy treatments had similar results (80% germination). Meanwhile, doses of 200 and 300 Gy decreased the germination rate sequentially by 70% and 60%.

Conclusions

Varieties significantly affected all germination characteristics of red rice, except for germination percentage. However, the gamma irradiation doses and their interaction significantly affected all germination characteristics of red rice under salinity conditions. The Inpara-7 variety that gamma-exposed was more tolerant than other varieties of red rice under saline conditions. Gamma dose of 100 Gy had the maximum growth potential and vigor index of red rice insignificantly different from un-irradiated. It could increase root length and volume (3.77% and 1.95%) compared to control and decrease with increasing gamma irradiation doses up to 700 Gy. The interaction of 100 Gy gamma with the Inpara-7 variety showed greater germination characteristics compared to other interactions. Among the red rice varieties tested, it was found in the Inpara-7 variety had a wider optimum dose of gamma irradiation (LD_{20-50}) ranged from 13.38 to 556.58 Gy.

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TITLE PAGE

Title: 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= Pamera, V4= Pamelen; and V5= Inpara-7) and sub-plot 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

MAIN TEXT

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 (Suriany, 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 LD_{50} (Oldach, 2011; Ángeles-Espino et al., 2013) and growth reduction 30/50 or GR_{30}/GR_{50} (Khalil et al., 2014). Mutants resulting from gamma-ray irradiation will produce the highest diversity around LD_{20} and LD_{50} (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 mmosh/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 mmosh/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 (Co^{60} 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 (LLDIKTI-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 (LD_{20} and LD_{50}). 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 LD_{20} to LD_{50} (Indrayanti et al., 2011) through probit regression analysis based on the germination percentage.

$$\text{MGP} = \frac{\Sigma\ \text{Growing seeds}}{\Sigma\ \text{Planted seeds}} \text{ x } 100\% \qquad (1)$$

$$\text{GP} = \frac{\Sigma\ \text{NS3} + \text{NS4} + \ldots\ldots + \text{NS9}}{\Sigma\ \text{Planted seeds}} \text{ x } 100\% \qquad (2)$$

$$\text{VI} = \frac{\Sigma\ \text{Normal seedlings on the third day}}{\Sigma\ \text{Planted seeds}} \text{ x } 100\% \qquad (3)$$

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 (Table 1).

Table 1. F-value of the gamma-ray doses effect on the seedling characteristics 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 (R)	407.50*	204.63*	193.99*	90.02*	116.07*	24.88*	7.16*	10.73*	23.36*	17.18*	15.55*
Interaction (V×R)	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.

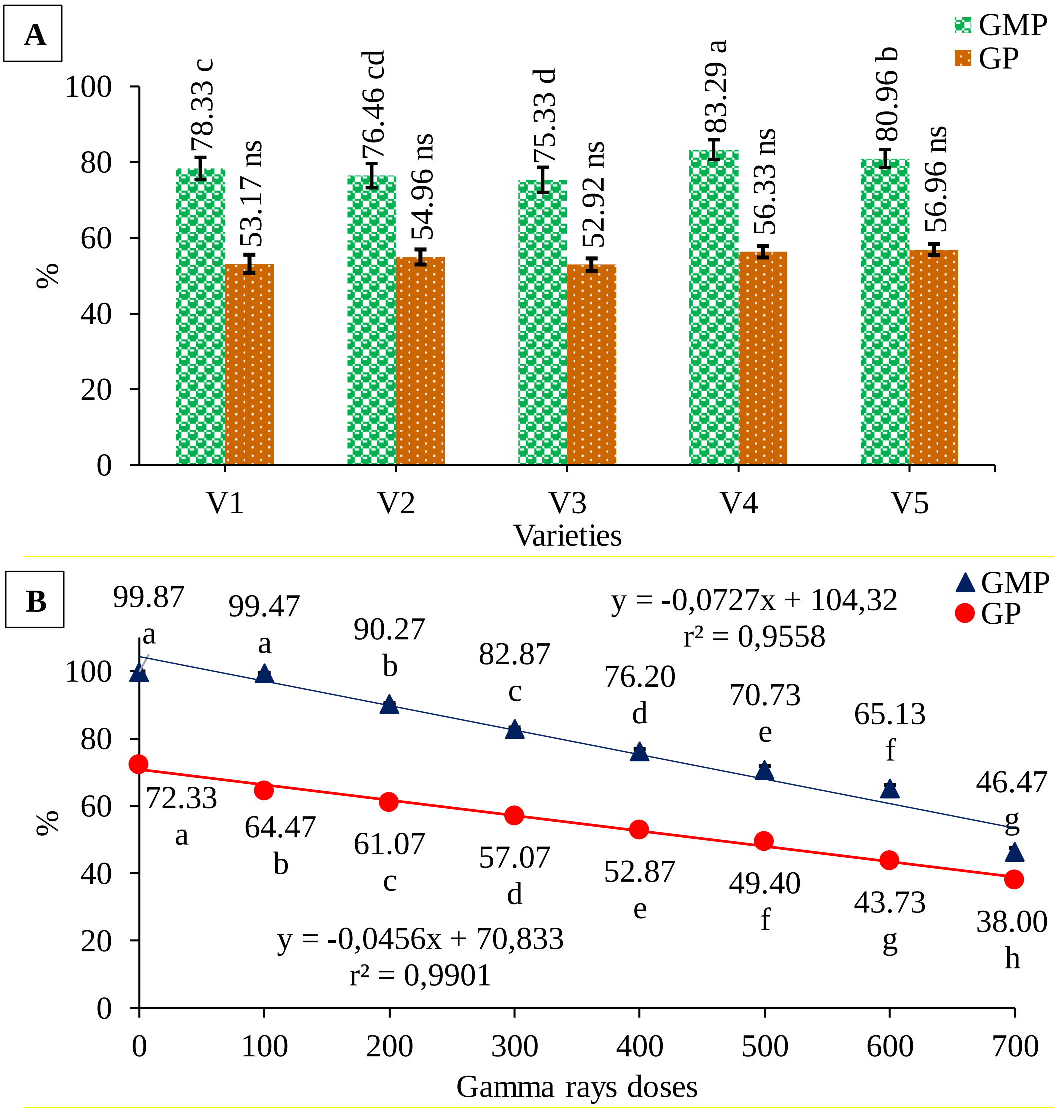

Figure 1. The maximum growth potential (GMP) and germination percentage (GP) of red rice due to the varieties (A) and gamma irradiation doses (B) under salinity conditions. V1= MSP-17; V2= Inpari-24 Gabusan; V3= Pamera; V4= Pamelen; V5= Inpara-7.

Table 2. 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					
		MGP (%)	GP (%)	VI (%)	RL (cm)	RV (ml)	N-c (%)	P-c (%)	K-c (%)	Na-c (%)	K^+/Na^+	Prol (µmol/g)
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
	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
Inpari-24 Gabusan	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 i-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
	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
Pamera	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
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
	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
Inpara-7	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
	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
CV (%)		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.

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 (Figure 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 (Figure 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 (Table 2).

Vigor Index (%)

The MSP-17 variety had the highest vigor index of 65.58% compared to other varieties (Figure 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 (Figure 2B). The interaction of un-irradiated within the MSP-17 variety significantly had the highest vigor index of 77.67% compared to other interactions (Table 2).

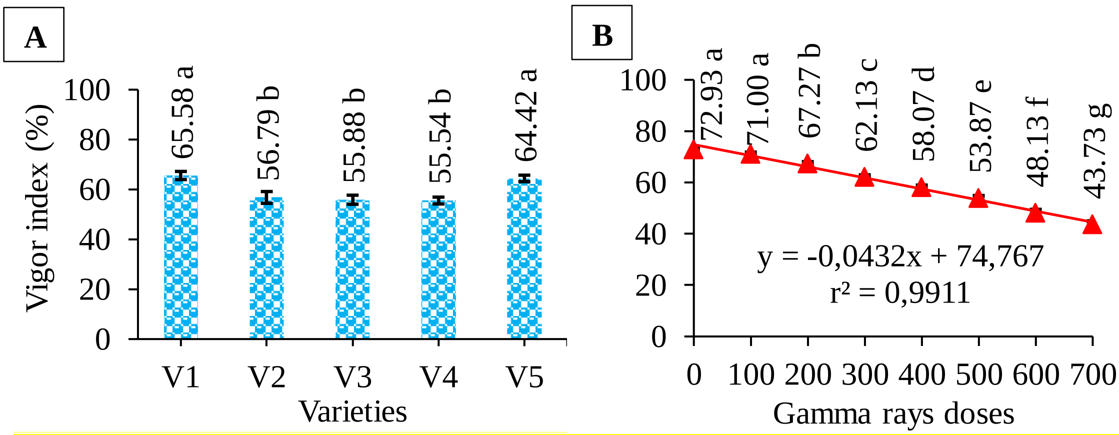

Figure 2. The vigor index of red rice plants due to the varieties (A) and gamma irradiation doses (B) under salinity conditions. V1= MSP-17; V2= Inpari-24 Gabusan; V3= Pamera; V4= Pamelen; V5= Inpara-7.

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 (Figure 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 (Figure 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 (Table 2).

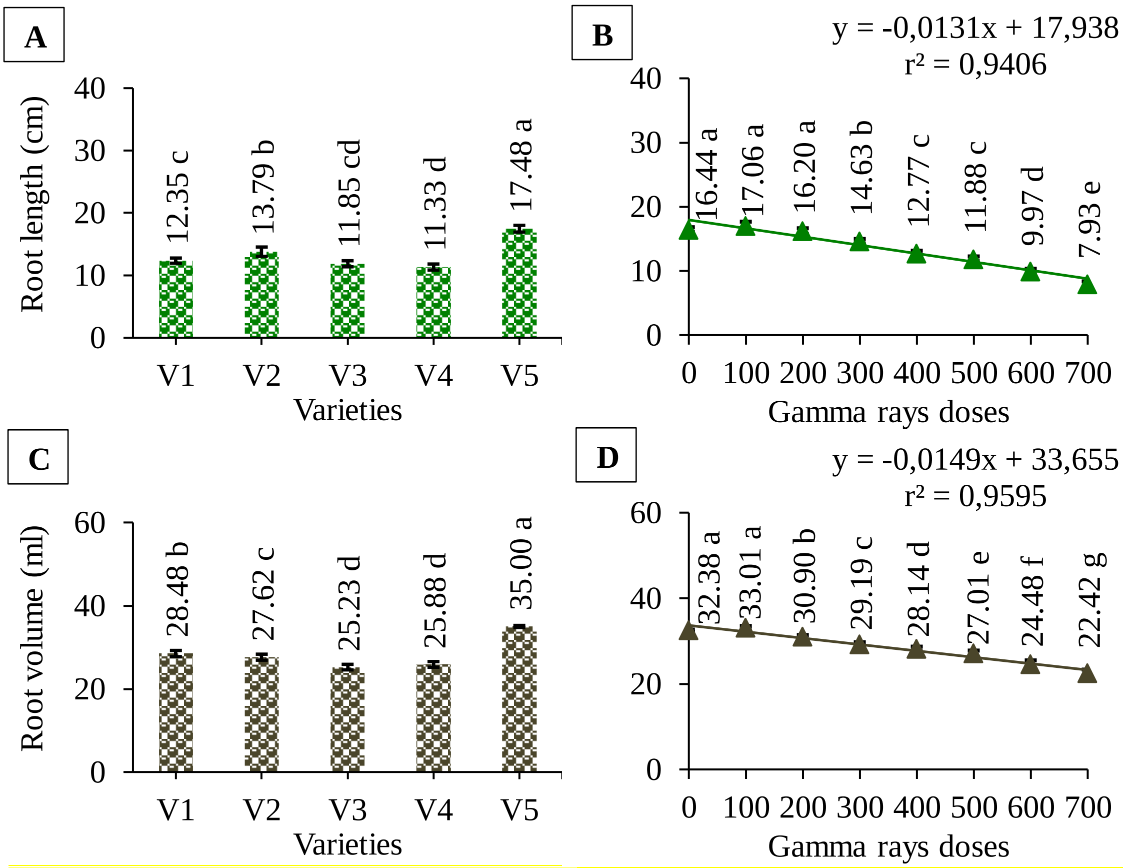

Figure 3. The root length and volume of red rice plants due to the varieties (A, C) and gamma irradiation doses (B, D) under salinity conditions. V1= MSP-17; V2= Inpari-24 Gabusan; V3= Pamera; V4= Pamelen; V5= Inpara-7.

Lethal Dose 20-50 ($LD_{20\text{-}50}$)

Lethal doses 20 and 50 from gamma irradiation in the five varieties of red rice under saline conditions could be seen in Figure 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 $LD_{20\text{-}50}$ 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.

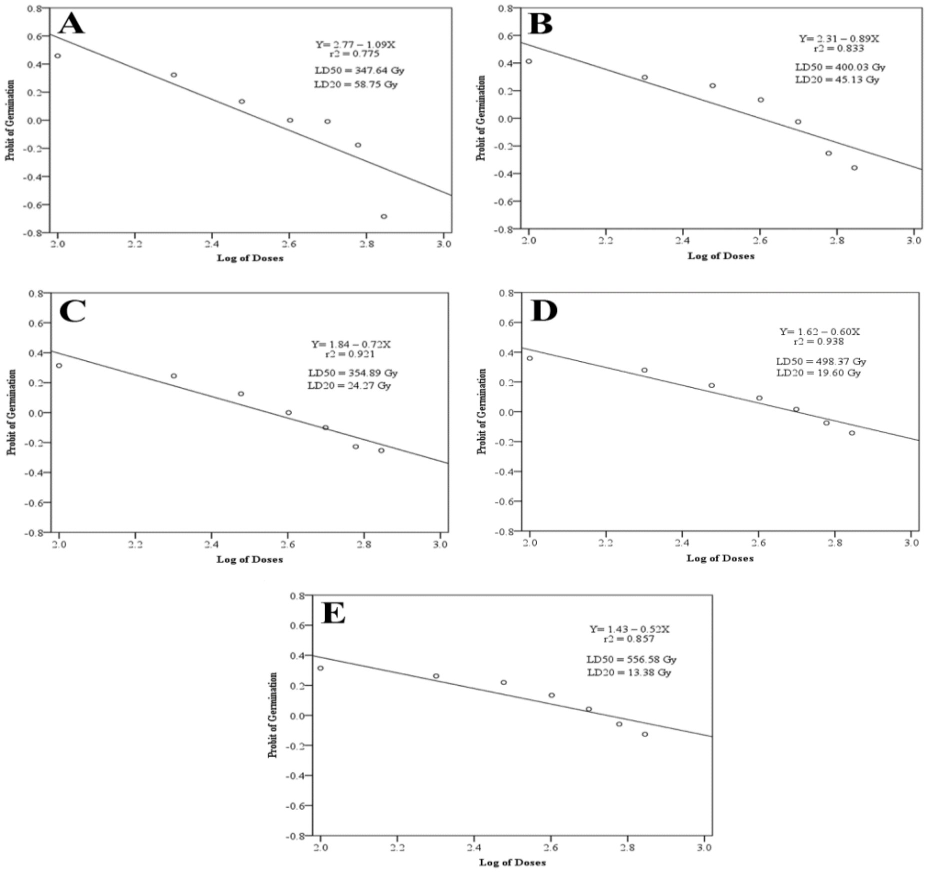

Figure 4. The lethal doses of 20-50 gamma rays from red rice varieties under saline. A= MSP-17; B= Inpari-24 Gabusan; C= Pamera; D= Pamelen; E= Inpara-7.

Nutrients Content

The MSP-17 variety had the highest N-content and K^+/Na^+ were 0.59% and 0.21 compared to other varieties (Figure 5A, E) and the highest Na-content was found in Inpara-7 variety at 3.86% (Figure 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 (Figure 6A, C). Likewise, the content of P and K^+/Na^+ were lower along with an increase in the doses of gamma rays (Figure 6B, E). Vice versa, the Na-content was increased (Figure 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 (Table 2).

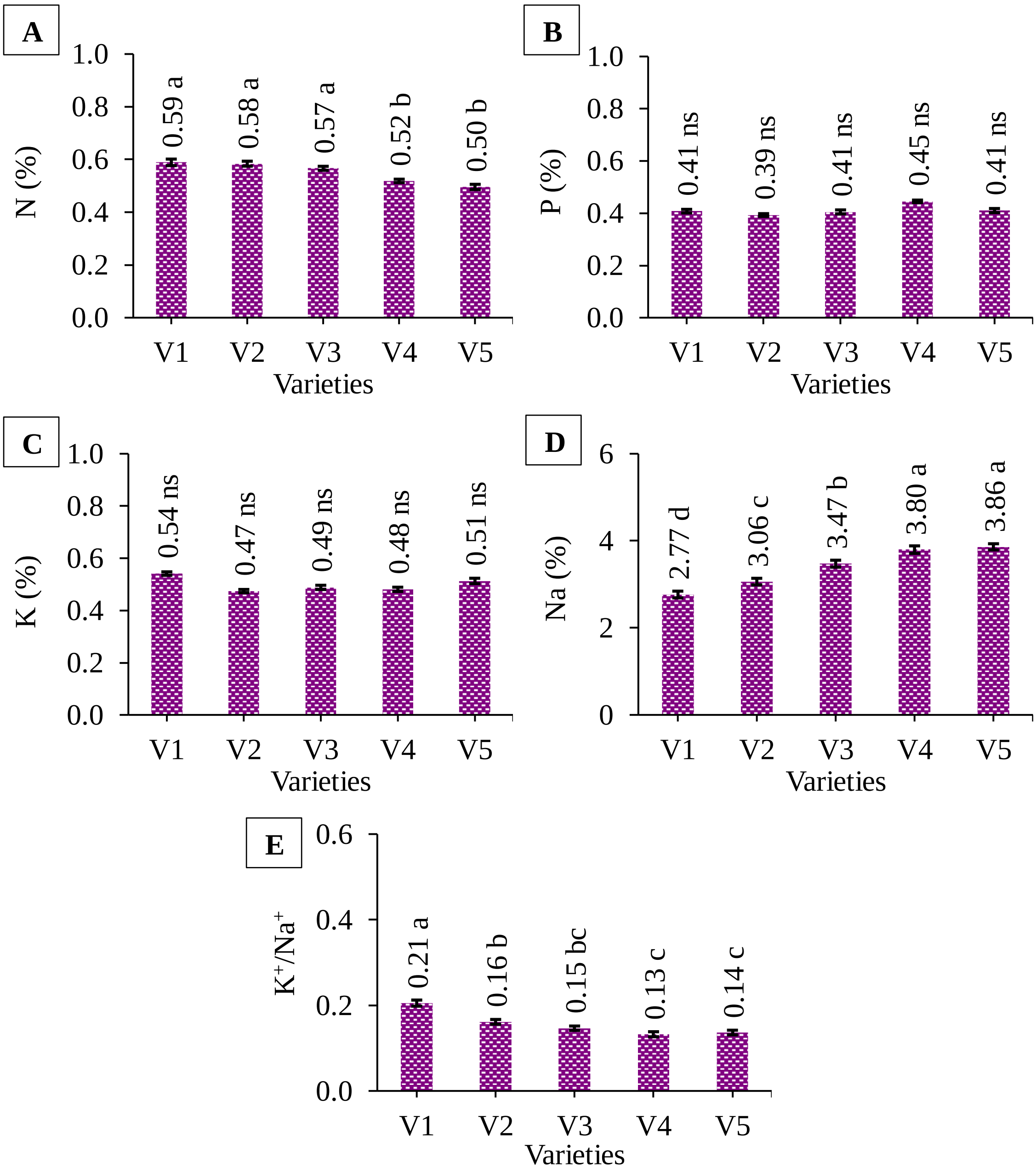

Figure 5. The effect of varieties on the nutrient content (A= nitrogen; B= phosphor; C= potassium; D= sodium; E=K^+/Na^+) under salinity conditions. V1= MSP-17; V2= Inpari-24 Gabusan; V3= Pamera; V4= Pamelen; V5= Inpara-7.

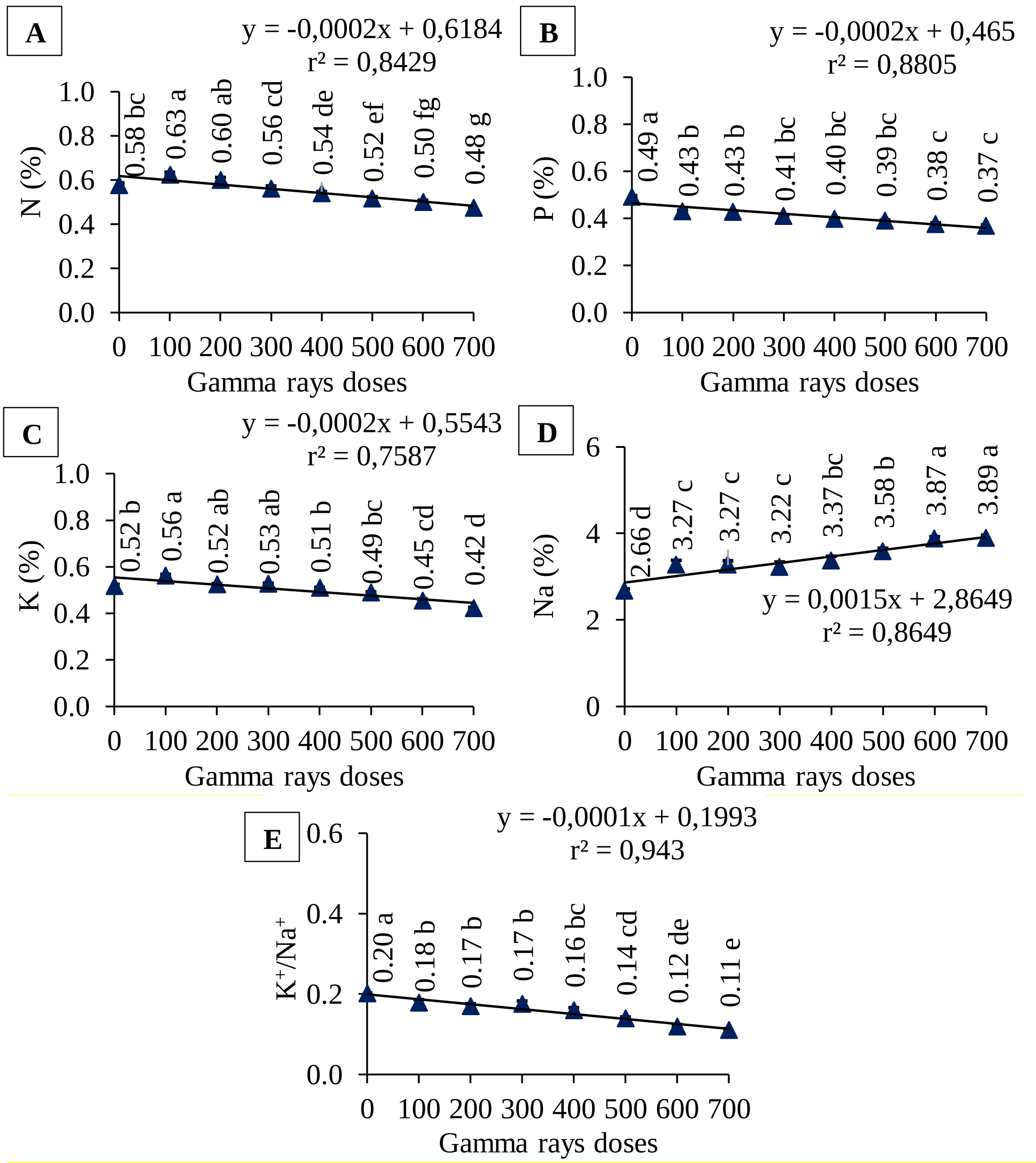

Figure 6. The effect of gamma irradiation doses on the nutrient content (A= nitrogen; B= phosphor; C= potassium; D= sodium; E=K^+/Na^+) under salinity conditions.

Proline Content (µmol/g)

The MSP-17 variety had the highest proline content of 101.94 µmol/g compared to other varieties (Figure 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 (Figure 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 (Table 2).

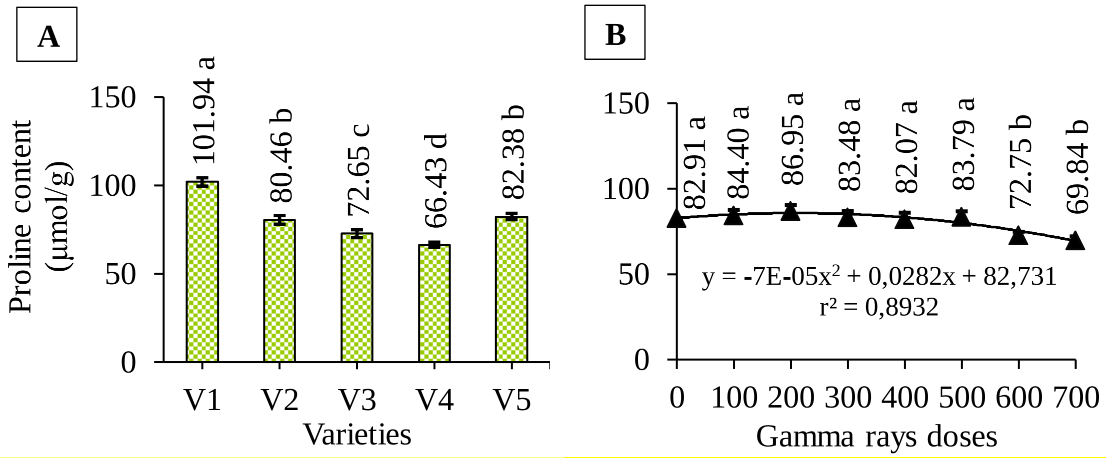

Figure 7. The proline content of red rice plants due to the varieties (A) and gamma irradiation doses (B) under salinity conditions. V1= MSP-17; V2= Inpari-24 Gabusan; V3= Pamera; V4= Pamelen; V5= Inpara-7.

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 (Figure 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 (Figure 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 (Figures 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 (Figure 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, Mn^{2+}, and Fe^{2+} 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 (Figure 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 peroxide, 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, Inpari-24 Gabusan, Pamera, 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 μmol/g, respectively (Table 2). 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.

CONCLUSIONS

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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Germination characteristics of red rice as affected by induction irradiated gamma under saline conditions

Reviews ver. 3

Review no. 1: Recommended acceptance

The improvements made have fulfilled the reviewer's suggestions and can be used as a scientific reference in the future.

1. Originality of the contribution *excellent*	2. Importance in its field *excellent*
3. Style, overall presentation & understandability *excellent*	4. Awareness of related work *good*
5. English language *excellent*	6. Does the title represent manuscript's contents? *yes*
7. Is the Abstract accurate and concise? *yes*	8. Are the objectives/goals clearly defined? *yes*
9. Are the approach/methods properly described? *yes*	10. Are the conclusions and interpretations sound? *yes*
11. Are the references properly cited and presented? *yes*	12. Is this a new/original/confirmatory contribution? *yes*
13. Are all images and tables necessary? *yes*	14. Paper type: *Research article*

Review no. 2: Recommended acceptance

The authors worked on my suggestions and comments, I accept this article for publication.

Thanks

1. Originality of the contribution *good*	2. Importance in its field *good*
3. Style, overall presentation & understandability *good*	4. Awareness of related work *good*
5. English language *good*	6. Does the title represent manuscript's contents? *yes*
7. Is the Abstract accurate and concise? *yes*	8. Are the objectives/goals clearly defined? *yes*
9. Are the approach/methods properly described? *yes*	10. Are the conclusions and interpretations sound? *yes*
11. Are the references properly cited and presented? *yes*	12. Is this a new/original/confirmatory contribution? *yes*
13. Are all images and tables necessary? *yes*	14. Paper type: *Research article*

ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

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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 = Pamera, V4 = Pamelen; and V5 = Inpara-7) and sub-plot 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 (Suriany, 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 LD_{50} (Oldach, 2011; Ángeles-Espino et al., 2013) and growth reduction 30/50 or GR_{30}/GR_{50} (Khalil et al., 2014). Mutants resulting from gamma-ray irradiation will produce the highest diversity around LD_{20} and LD_{50} (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 mmosh/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 mmosh/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 (Co^{60} 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 (LLDIKTI-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 (LD_{20} and LD_{50}). 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 LD_{20} to LD_{50} (Indrayanti et al., 2011) through probit regression analysis based on the germination percentage.

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

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

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

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).

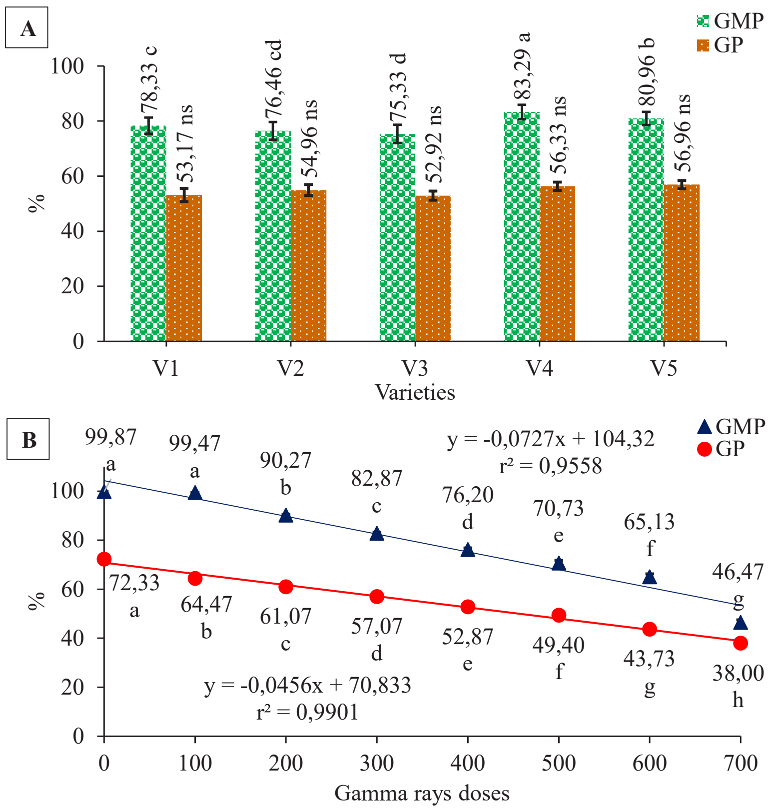
Maximum Growth Potential and Germination Percentage (%)

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).

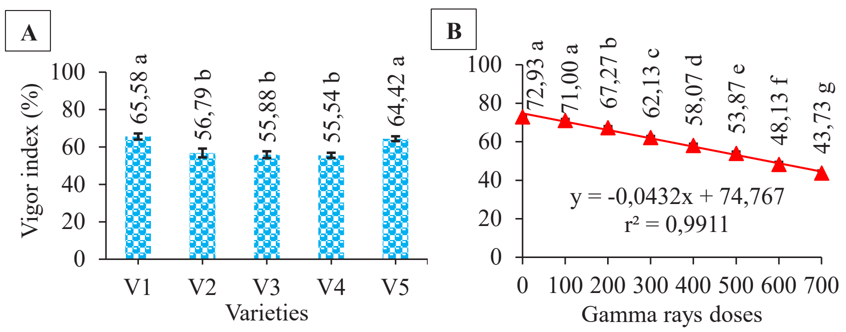
I: F-value of the gamma-ray doses effect on the seedling characteristics in five varieties of red rice under salinity conditions

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 (R)	407.50^{*}	204.63^{*}	193.99^{*}	90.02^{*}	116.07^{*}	24.88^{*}	7.16^{*}	10.73^{*}	23.36^{*}	17.18^{*}	15.55^{*}
Interaction (V × R)	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)

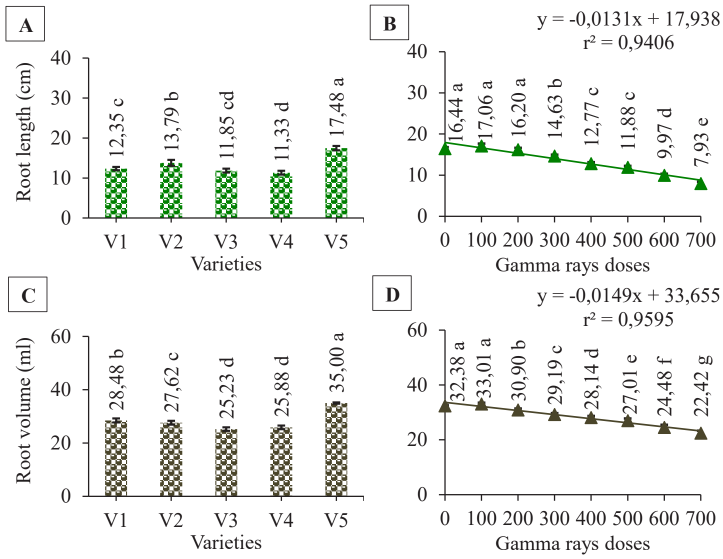
The Inpara-7 variety had the highest root length and volume of 17.48 cm and 35.00 ml compared to other varieties (Fig. 3A, C). The gamma-ray at 100 Gy significantly increased the highest root length and volume were 3.77 and 1.95%, respectively compared to the control and decreased along with the increase 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

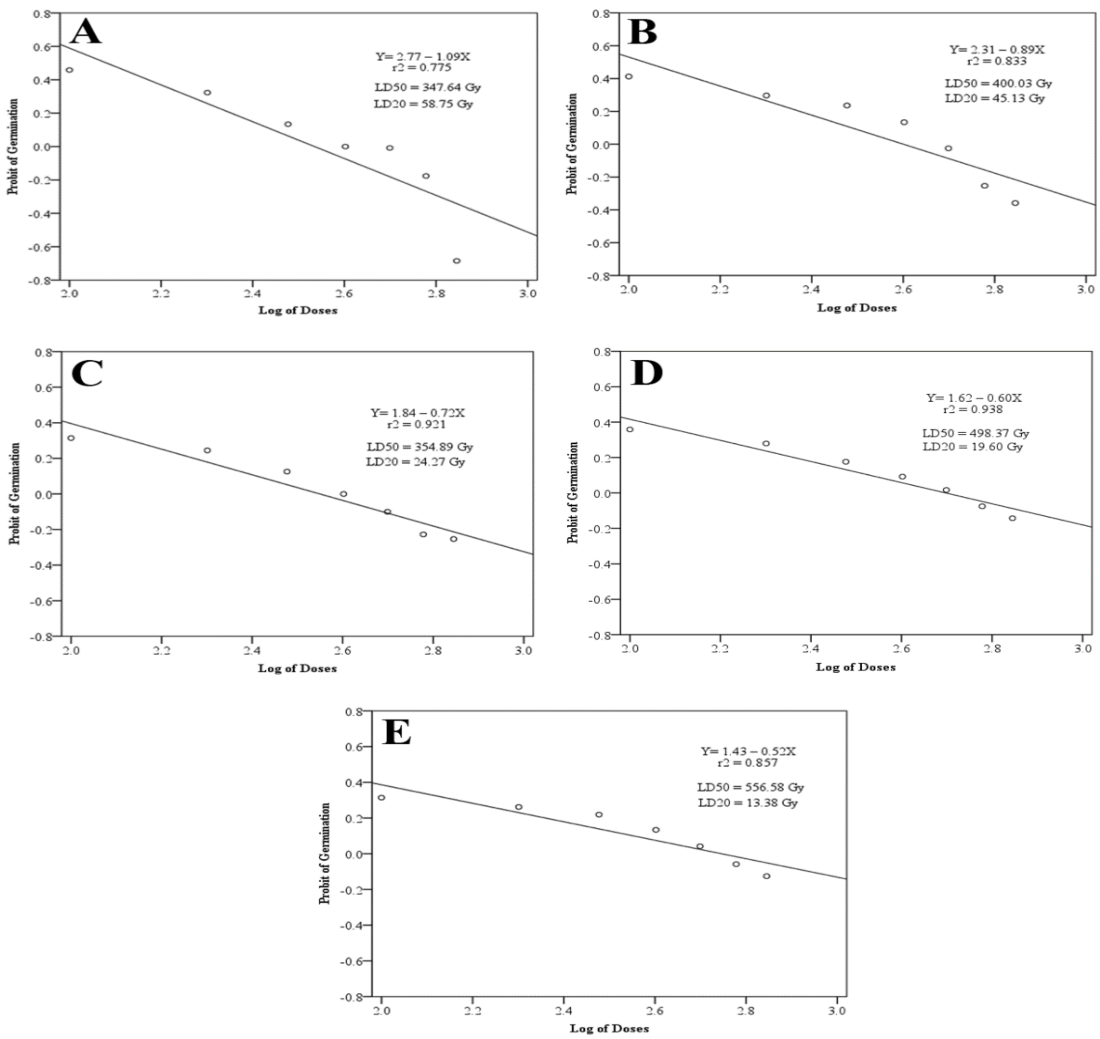
Red rice varieties	Gamma doses	Seedlings stage					Vegetative stage					
		MGP (%)	GP (%)	VI (%)	RL (cm)	RV (ml)	N-c (%)	P-c (%)	K-c (%)	Na-c (%)	K^+/Na^+	Prol (μmol/g)
MSP-17	0	100.00 a	72.33 abc	77.67 a	15.20 efg	34.24 b-e	0.58 d-i	0.49 ns	0.54 ns	3.15 h-k	0.18 c-h	83.09 e-h
	100	99.67 a	67.67 b-e	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
	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
Inpari-24 Gabusan	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 i-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
	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
Pamera	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
	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
Inpara-7	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
	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
CV (%)		4.43	5.54	4.96	10.14	4.71	8.12	13.29	10.97	9.37	20.22	7.38

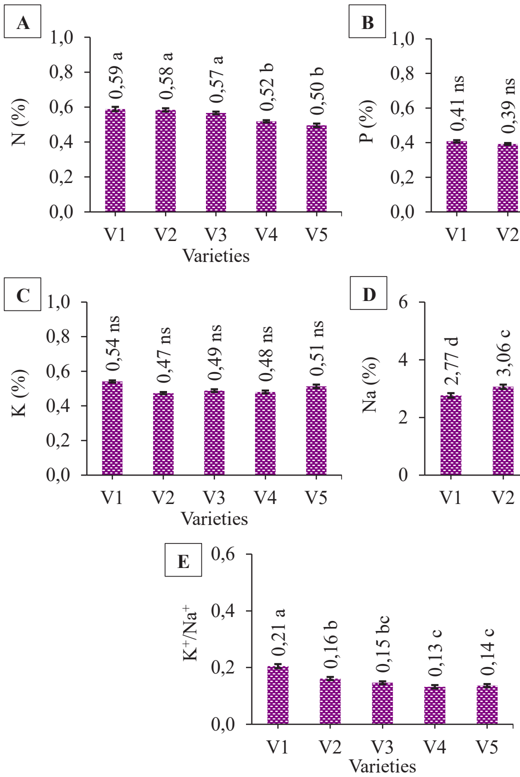
Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at $P < 0.05$. CV = coefficient of variation. MGP = maximum growth potential; GP = germination percentage; VI = vigor index; RL = root length; RV = root volume; N-c = N content; P-c = P content;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



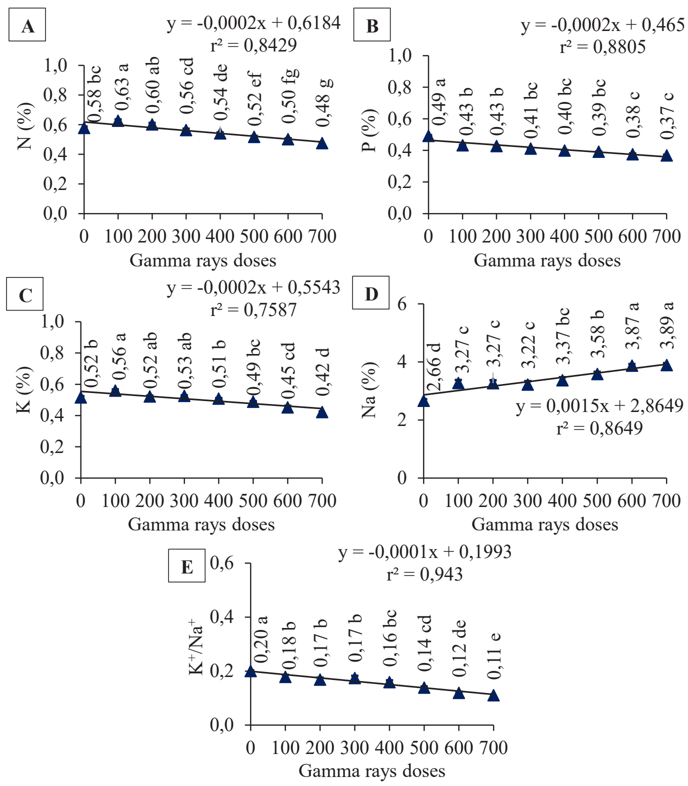
3: The root length and volume of red rice plants due to the varieties (A, C) and gamma irradiation doses (B, D) under salinity conditions. V1 = MSP-17; V2 = Inpari-24 Gabusan; V3 = Pamera; V4 = Pamelen; V5 = Inpara-7.



4: The lethal doses of 20ş50 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.

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, Mn^{2+}, and Fe^{2+} 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 peroxide, 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, Inpari-24 Gabusan, Pamera, 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 µmol/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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ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

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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 = Pamera, V4 = Pamelen; and V5 = Inpara-7) and sub-plot 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 (Suriany, 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 LD_{50} (Oldach, 2011; Ángeles-Espino *et al.*, 2013) and growth reduction 30/50 or GR_{30}/GR_{50} (Khalil *et al.*, 2014). Mutants resulting from gamma-ray irradiation will produce the highest diversity around LD_{20} and LD_{50} (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 mmosh/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 mmosh/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 (Co^{60} 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 (LLDIKTI-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 (LD_{20} and LD_{50}). 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 LD_{20} to LD_{50} (Indrayanti *et al.*, 2011) through probit regression analysis based on the germination percentage.

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

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

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

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).

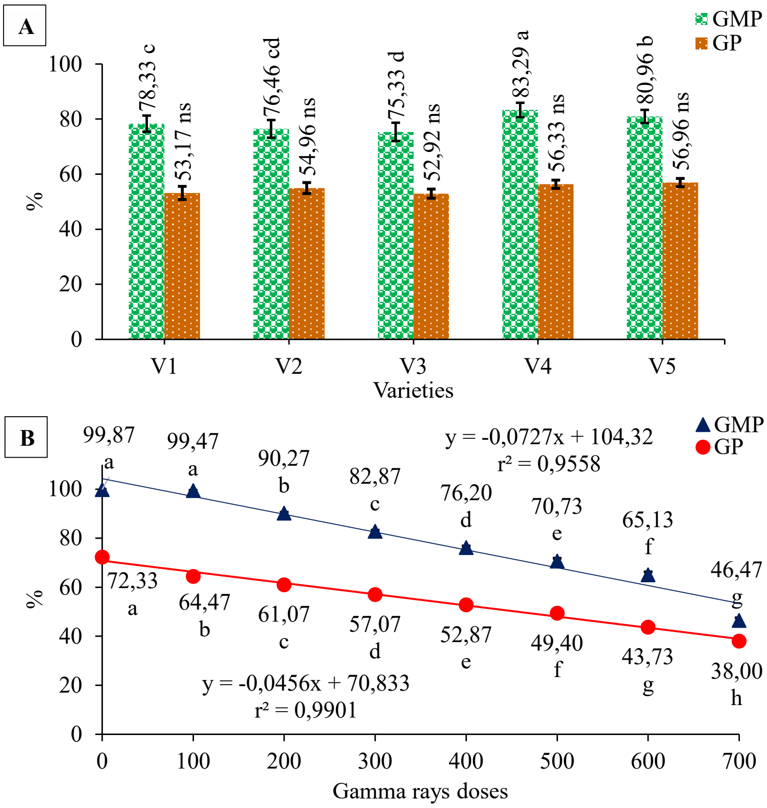
Maximum Growth Potential and Germination Percentage (%)

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).

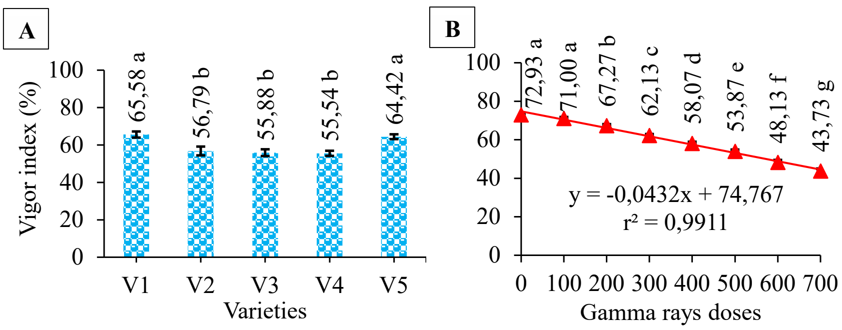
I: *F-value of the gamma-ray doses effect on the seedling characteristics and nutrient absorption in the vegetative stage in five varieties of red rice under salinity conditions*

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)

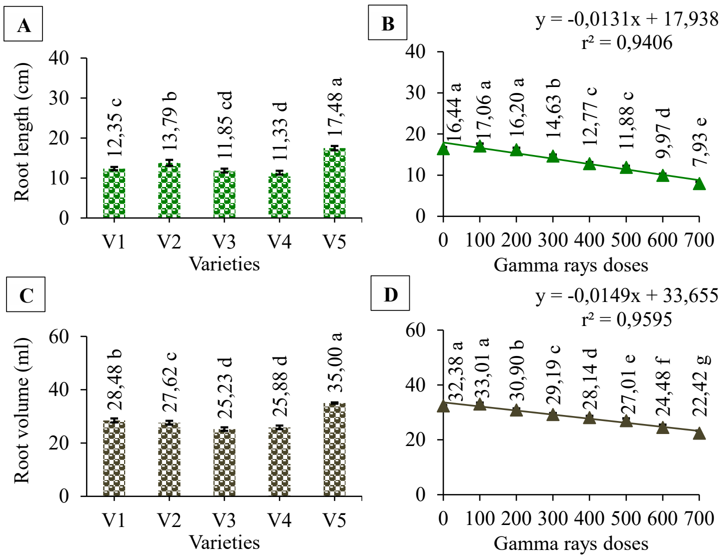
The Inpara-7 variety had the highest root length and volume of 17.48 cm and 35.00 ml compared to other varieties (Fig. 3A, C). The gamma-ray at 100 Gy significantly increased the highest root length and volume were 3.77 and 1.95%, respectively compared to the control and decreased along with the increase 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*

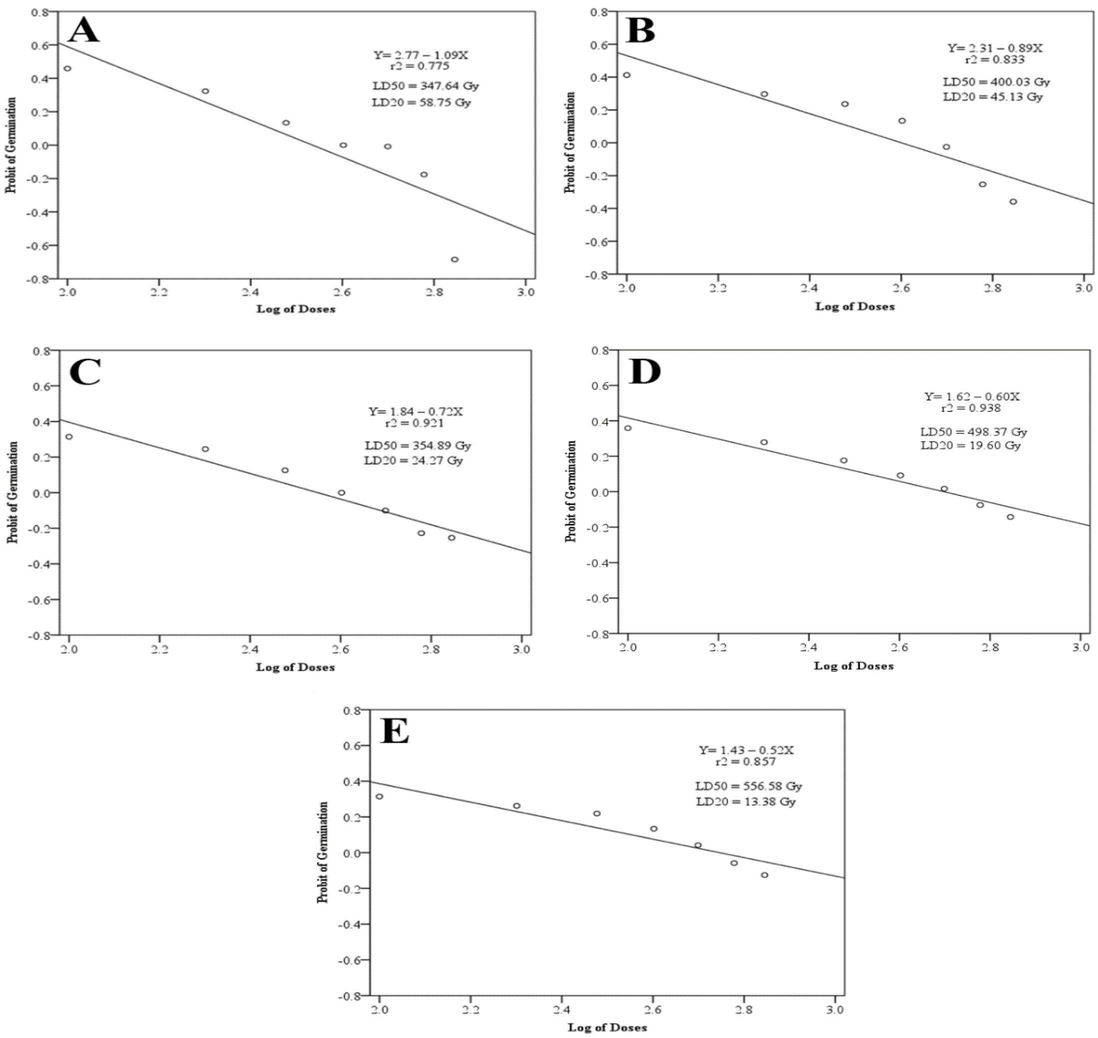
Red rice varieties	Gamma doses	Seedlings stage					Vegetative stage					
		MGP (%)	GP (%)	VI (%)	RL (cm)	RV (ml)	N-c (%)	P-c (%)	K-c (%)	Na-c (%)	K^+/Na^+	Prol (µmol/g)
MSP-17	0	100.00 a	72.33 abc	77.67 a	15.20 efg	34.24 b-e	0.58 d-i	0.49 ns	0.54 ns	3.15 h-k	0.18 c-h	83.09 e-h
	100	99.67 a	67.67 b-e	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
	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
Inpari-24 Gabusan	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 i-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
	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
Pamera	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
	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
Inpara-7	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
	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
CV (%)		4.43	5.54	4.96	10.14	4.71	8.12	13.29	10.97	9.37	20.22	7.38

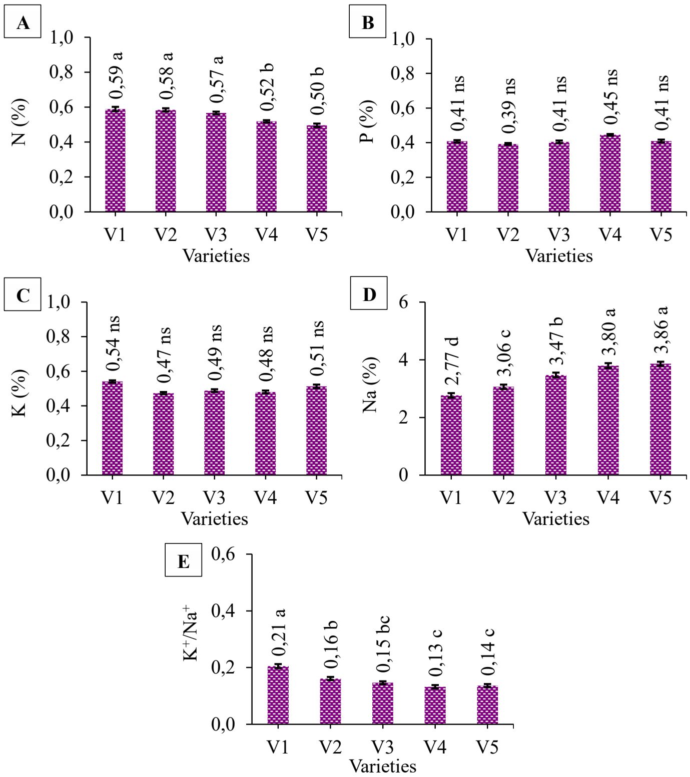
Note: the mean followed by different letters in the same column and row indicate a significant difference by DMRT at $P < 0.05$. CV = coefficient of variation. MGP = maximum growth potential; GP = germination percentage; VI = vigor index; RL = root length; RV = root volume; N-c = N content; P-c = P content;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



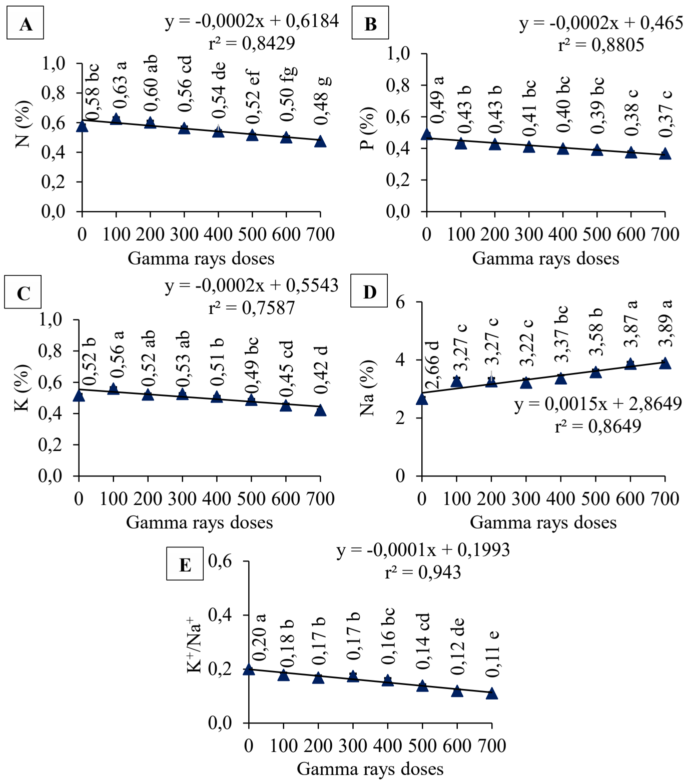
3: *The root length and volume of red rice plants due to the varieties (A, C) and gamma irradiation doses (B, D) under salinity conditions. V1 = MSP-17; V2 = Inpari-24 Gabusan; V3 = Pamera; V4 = Pamelen; V5 = Inpara-7.*



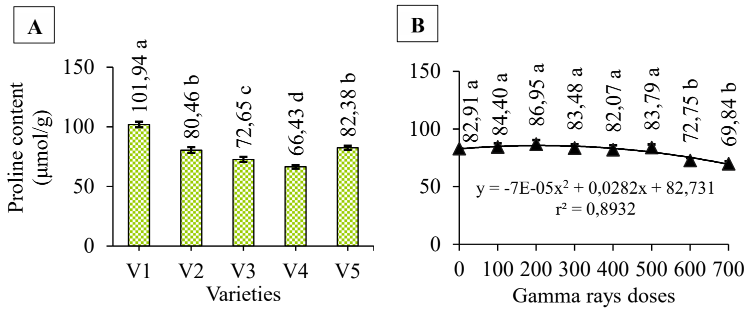
4: *The lethal doses of 20–50 gamma rays from red rice varieties under saline. A = MSP-17; B = Inpari-24 Gabusan; C = Pamera; D = Pamelen; E = Inpara-7.*



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 = Inpari-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, Mn^{2+}, and Fe^{2+} 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 peroxide, 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, Inpari-24 Gabusan, Pamera, 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 µmol/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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