

# Gamma Irradiation Promotes the Growth Rate of Thai Pigmented Rice As Well As Inducing the Accumulation of Bioactive Compounds and Carbohydrate Hydrolyzing Enzymes Inhibitors ( $\alpha$ -Glucosidase and $\alpha$ -Amylase) under Salt Conditions

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**ABSTRACT:** Rice contains many bioactive compounds that perform various biological activities. Some of these compounds have been identified as  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors, including guaiacol, vanillin, methyl vanillate, vanillic acid, syringic acid, and 2-pentyl furan. In this study, we assessed the growth rate, photosynthetic pigment content, phenolic content, and flavonoid content of gamma-irradiated Thai pigmented rice. Bioactive components of gamma-irradiated rice that had been subjected to salt treatment were also investigated. The findings showed that production of photosynthetic pigments, which are associated with plant growth, was induced by low gamma exposure. Phenolic and flavonoid content of rice was increased after gamma irradiation at 5 to 1,000 Gy. Both gamma irradiation and the salt conditions changed the quantity of vanillin, methyl vanillate, and vanillic acid in the rice. However, at a salt concentration of 40 mM, the salt stress had more of an effect than the gamma dosage. However, the high concentrations of methyl vanillate and vanillic acid detected in the rice under salt conditions were ameliorated by gamma irradiation. Guaiacol served as the substrate of guaiacol peroxidase for catalyzed reactive oxygen species, as evidenced by the observation that the guaiacol content of rice decreased between increased gamma dosages. A gamma dose of 40 to 1,000 Gy resulted in the production of syringic acid. Under salt stress, syringic acid buildup was also seen to be ameliorated by gamma irradiation. In comparison to salt conditions, particularly for 20 mM salt, gamma irradiation had less of an impact on the 2-pentyl furan in rice.

**Keywords:** diabetes mellitus, gamma irradiation, phenolic compounds, pigmented rice

## INTRODUCTION

Rice is a substantial source of bioactive compounds (Samyot et al., 2017). The bioactive compounds in rice have a wide range of biological effects, including anticancer, antiallergic, and anti-inflammatory activities (Peanparkdee and Iwamoto, 2019). Some of the bioactive components of rice have been investigated for the treatment of diabetes mellitus (Sansenya et al., 2021). Due to their anti-melanin production properties, such as suppression of tyrosinase activity, several bioactive compounds from rice have also found use in cosmetic products (Miyazawa et al., 2003). Rice is used as a food source and a medical therapy in many countries. The improvement of bioactive compounds in rice through biotic and abiotic stress has been studied previously (Nascimento et al., 2020;

Katiyar et al., 2022). The most common abiotic stresses used to promote the synthesis of bioactive compounds in rice include gamma radiation and salt, flooding, and drought stress (Dash et al., 2018; Chinvongamorn and Sansenya, 2020; Panda and Barik, 2021). Gamma rays generate highly reactive chemicals or reactive oxygen species (ROS) and cause DNA damage to produce plant mutations (Belli et al., 2002). The physiological changes in plants and rice by gamma irradiation are dependent on the gamma dose (Katiyar et al., 2022). The growth rate of rice can be stimulated by low gamma doses, while growth is inhibited by large gamma doses (Archanachai et al., 2021). In jujube (*Ziziphus jujuba* var *vulgaris*), gamma irradiation at a dose of 2.5 kGy stimulated an increase in anthocyanin and phenolic content (Najafabadi et al., 2017). In the well-known Thai rice cultivars, a gamma dosage

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of 60~100 Gy induced an increase in flavonoid and phenolic content (Archanachai et al., 2021). Another previous study reported that gamma irradiation at 4 krad (40 Gy) enhanced amino acid, protein, and total chlorophyll production in the leaf of the M5 mulberry mutant (*morus* sp.) (Ramesh et al., 2021). Thus, the morphological and phytochemical changes in a plant depend on the gamma dose and plant species.

Gamma irradiation has been reported to ameliorate abiotic stress in various plant species (Katiyar et al., 2022). In some plant species, metals have been alleviated by low gamma doses. The development of lead/cadmium-stressed Highland barley seedlings was improved by 50 Gy of gamma radiation treatment (Wang et al., 2017). Other plant stresses, including drought stress, have been demonstrated to be ameliorated by low gamma dosages, while the antioxidant enzyme activities, water balance, and drought tolerance have been reported to be improved (Katiyar et al., 2022). The abiotic stress of salt on plants has also been shown to be ameliorated by gamma radiation; of salt stress, a low gamma dosage was reported to enhance growth patterns and morphology (Qi et al., 2014; Katiyar et al., 2022). Therefore, abiotic stresses can be alleviated by low gamma irradiation.

Phenolic compounds such as guaiacol, vanillin, methyl vanillate, vanillic acid, and syringic acid have been identified in Thai rice cultivars. These compounds were examined for their ability to inhibit  $\alpha$ -glucosidase and  $\alpha$ -amylase activity. The increase in blood glucose level has been functioned by  $\alpha$ -glucosidase and  $\alpha$ -amylase. Therefore, a new inhibitor from the plant for antidiabetes may be a potential molecule for use in the management of diabetes mellitus.

Vanillin and guaiacol showed a stronger potential to inhibit  $\alpha$ -glucosidase than  $\alpha$ -amylase (Sansenya et al., 2021). However, these phenolic compounds inhibited both  $\alpha$ -glucosidase and  $\alpha$ -amylase in a mixed-type manner (Nanok and Sansenya, 2021). A recent study reported that methyl vanillate, vanillic acid, and syringic acid also inhibited  $\alpha$ -glucosidase's higher efficiency compared to  $\alpha$ -

amylase. Another bioactive compound found in Thai rice cultivars is 2-pentyl furan (Nanok and Sansenya, 2021). According to Hinge et al. (2016), this chemical is the main component that contributes to the scent intensities of fragrant rice cultivars. Additionally, 2-pentyl furan demonstrated better  $\alpha$ -glucosidase inhibitory efficacy compared to  $\alpha$ -amylase (Sansenya et al., 2021). Moreover, 2-pentyl furan showed mixed-type inhibition against both  $\alpha$ -glucosidase and  $\alpha$ -amylase.

In the current study, we focus on improving the 2-pentyl furan, guaiacol, vanillin, methyl vanillate, syringic acid, and vanillic acid contents in a pigmented Thai rice cultivar (Khao Riceberry) via gamma irradiation. The effect of salt concentration during bioactive compound accumulation by gamma irradiation was also investigated.

## MATERIALS AND METHODS

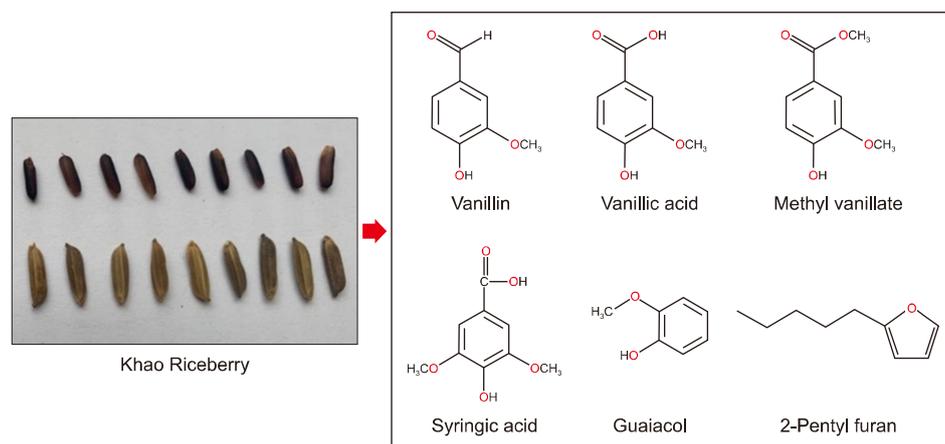
### Chemical reagents

Syringic acid, vanillic acid, methyl vanillate, vanillin, guaiacol, and 2-pentyl furan (Fig. 1) were purchased from Sigma-Aldrich.

### Plant materials

Thai paddy rice, cultivar Khao Riceberry (Fig. 1) was harvested from a rice farm in Pathum Thani, Thailand in 2021). The sterilized seed samples were rinsed with distilled water after being soaked with 0.1% sodium hypochlorite for 30 min. A 40°C oven was used to dry the sterilized rice seed.

The paddy rice was exposed to gamma rays at dosage rates of 0 (control rice), 5, 40, 100, 500, and 1,000 Gy at the Gamma Irradiation Center of Kasetsart University, Thailand, using a  $^{137}\text{Cs}$  gamma-ray source. The samples were soaked with and without salt solution (20 mM and 40 mM NaCl) for 24 h and then germinated at 30°C for 24 h on germinating paper. The germinated rice was harvested after 24 h. Rice samples were kept at  $-20^\circ\text{C}$  until phenolic and flavonoid analysis and gas chromatography-



**Fig. 1.** Rice samples and the structure of the selected bioactive compounds.

mass spectrometry (GC-MS) and liquid chromatography (LC)-MS/MS quantification.

The gamma-irradiated rice and control rice was steeped in distilled water for 24 h. The rice samples were allowed to germinate for 96 h at 30°C on germination paper dampened with distilled water. The germinated rice was harvested after 96 h. The shoot length of the germinated rice was measured, and fresh rice samples were used for photosynthetic pigment determination.

#### Plant extraction

Methanol was used to extract the rice samples at a sample (g) to methanol (mL) ratio of 1:3. The extraction experiment was conducted over three days, and rice residues were discarded. The solvent was then eliminated using a hot air oven at 50°C. The crude sample was stored at -20°C.

#### Phenolic and flavonoid analysis

The procedure described by Archanachai et al. (2021) was used to determine the phenolic and flavonoid contents of the rice extract. The quantity of phenolic compounds was determined at 760 nm using the Folin-Ciocalteu reagent method. The calibration curve for gallic acid was used to estimate the extract's phenolic concentration. The quantity of flavonoid compounds was determined at 415 nm. The flavonoid content of the crude extract was determined by employing the quercetin calibration curve.

#### GC-MS quantification of 2-pentyl furan, guaiacol, vanillin, and methyl vanillate in rice extract

Calibrations curves were prepared for 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 µg/mL. Dimethyl sulfoxide (DMSO) was used to dilute the rice extracts before they were filtered through a 0.22 µm filter. An GC-MS analyzer (Agilent 7890A GC-7000 Mass Triple Quad; Agilent) was used for the GC-MS analysis. The guaiacol, 2-pentyl furan, vanillin, and methyl vanillate contents were determined at the retention times of 13.7, 28.6, 37.8, and 38.4 min, respectively; while ions were determined at the *m/z* of 138, 124, 151, and 182, respectively. Each compound's peak area in the standard solutions was measured to create a calibration curve, and the peak areas for each compound in the samples were measured to calculate each compound's concentration using the appropriate calibration curves. Finally, the concentration of each compound in the original samples was calculated using the compound's weight and sample volume.

#### LC-MS/MS quantification of vanillic acid and syringic acid in rice extract

Calibration curves were prepared at 0.2, 0.5, 1.0, 5.0, 10.0, and 20.0 µg/mL. The extracts were dissolved with DMSO and filtered through a 0.22 µm filter. The Ultimate

3000 UHPLC system (Dionex) in conjunction with the micrOTOF-Q II ESI tandem mass spectrometer (Bruker) were used to conduct the LC-MS/MS quantification. The target compounds vanillic acid and syringic acid were found at the retention times of 15.7 and 16.9 min, respectively, and ions at 152 and 182 *m/z*, respectively, were employed for quantification. The calibration curves, which were produced using peak regions of various concentrations of the standard, were used to develop the equation for linear regression, which was used to calculate the concentration of the targeted chemicals.

#### Photosynthetic pigment determination

The modified method of Gao et al. (2020) was used for the extraction and calculation of photosynthetic pigments, comprising chlorophylls a and b (Chl a, Chl b) and carotenoid. The 0.2-g samples of rice were mixed with 5 mL of 80% acetone and 5 mL of absolute ethanol, and the mixtures were then left to sit for 24 h in the dark. The extract was then centrifuged for 15 min at 1,411 g. For Chl a, Chl b, and carotenoid, the supernatant was measured at 663, 645, and 470 nm, respectively. The formulas of Gao et al. (2020) were used to calculate the photosynthetic pigment contents.

#### Statistical analysis

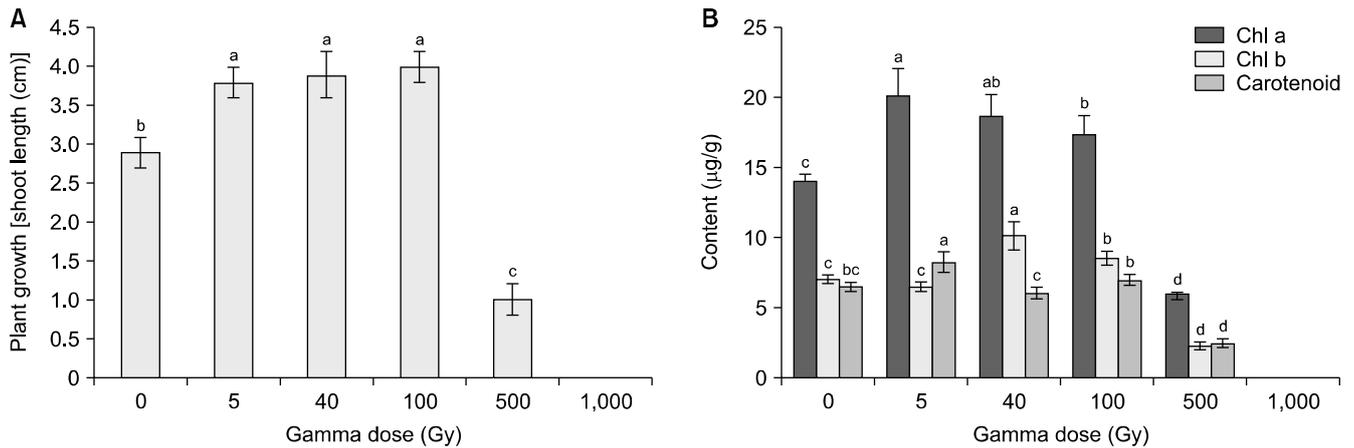
Each set of results, which were recorded in triplicate, was represented by the mean and standard deviation. One-way analysis of variance was used to examine the statistical differences, and differences were deemed significant at *P*-values under 0.05.

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## RESULTS AND DISCUSSION

#### Effect of gamma irradiation on plant growth and Chl a, Chl b, and carotenoid contents of pigmented rice

The growth rate of the gamma-irradiated rice and the photosynthetic pigments, including Chl a, Chl b, and carotenoid, were examined (Fig. 2). Gamma doses of 5 to 100 Gy were found to accelerate plant growth considerably. A significant increase in Chl a and Chl b was also stimulated by gamma irradiation (at the doses of 5~100 Gy for Chl a and the doses of 40~100 Gy for Chl b). The highest carotenoid concentration was achieved with a gamma dosage of 5 Gy, which also enhanced the carotenoid level. Previously researchers reported that a low gamma dosage altered plant development by encouraging plant growth (Singh et al., 2013). According to Mounir et al. (2022), *Helianthus tuberosus*'s photosynthetic pigments (Chl a, Chl b) as well as plant development were stimulated by gamma doses of 2.5 to 10 Gy; this study also revealed that a low gamma dose induced carotenoid accumulation (Mounir et al., 2022). However, the gamma



**Fig. 2.** The influence of gamma irradiation on (A) shoot length and (B) chlorophylls a and b (Chl a, Chl b), and carotenoid levels in pigmented rice. The identical letters above bars (a-d) reveal no statistically significant changes in plant growth, Chl a, Chl b, and carotenoid of pigmented rice ( $P>0.05$ ).

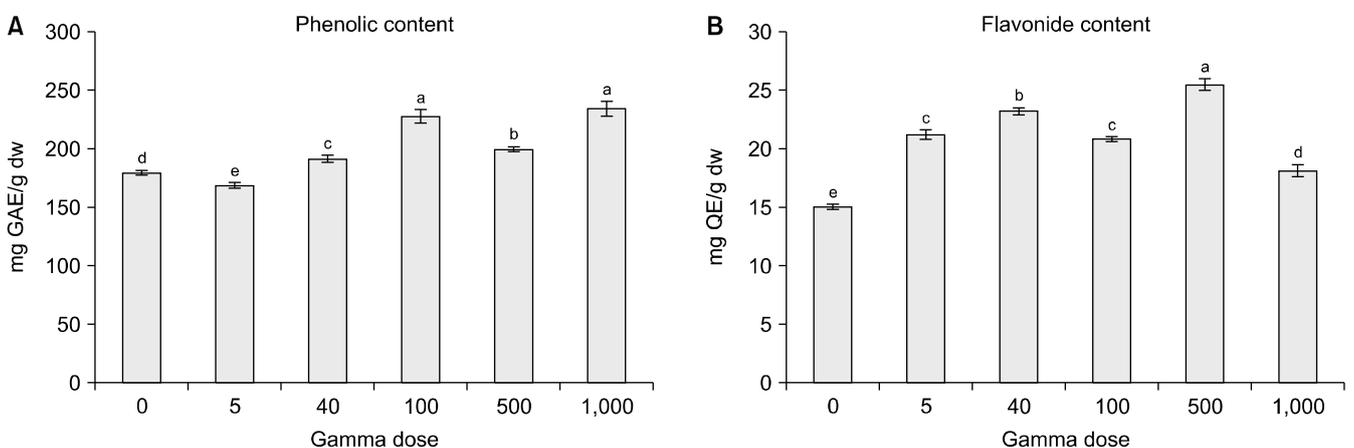
dose at 100 Gy was found to inhibit the growth of *Zea mays* (maize), and exposure to the gamma doses  $>500$  Gy induced plant death (Mounir et al., 2022). Under a high gamma dose (100 Gy), the photosynthetic pigment contents of Chl a, Chl b, and carotenoid have been reported to decrease (Marcu et al., 2013). Our findings demonstrated that gamma doses of more than 100 Gy decreased plant growth and photosynthetic pigment accumulation. Furthermore, rice that had been gamma irradiated did not survive a gamma dose of 500 Gy. Thus, the gamma dose has significance for plant growth, plant survival, and pigment accumulation by stimulating plant growth at low gamma doses and inhibiting it at high radiation doses.

#### Gamma irradiation's effect on the phenolic and flavonoid contents of pigmented rice

Fig. 3 shows the comparison between the phenolic and flavonoid contents of rice after treatment with 5 to 1,000 Gy of gamma irradiation and that of control rice. The phenolic content increased significantly at gamma doses between 40 and 1,000 Gy. Gamma-irradiated rice's flavo-

noid content was also noticeably stimulated at 5 to 1,000 Gy. High gamma doses stimulated the phenolic and flavonoid contents, especially that of phenolic compounds.

It has been reported previously that gamma irradiation stimulates ROS production, and that high doses induce more ROS than low gamma doses (Choi et al., 2021). The ROS production destroys plant cells, where the plant generates antioxidant enzymes and antioxidant metabolites such as phenolic and flavonoid compounds (Gill and Tuteja, 2010; Hong et al., 2018). Our findings indicated that rice that had been gamma-irradiated at 100 Gy had the highest phenolic content. Additionally, rice that had been gamma-irradiated at 500 Gy had the highest flavonoid content (Fig. 3). In colored wheat, high total phenolic and anthocyanin contents were generated by a high gamma dose (300 Gy); the anthocyanin biosynthesis gene profile was found to be related to the anthocyanin content (Hong et al., 2018). According to earlier studies and our findings, high gamma doses generate more ROS than low gamma doses. Therefore, antioxidant metabolites are produced to destroy the ROS in plant cells under high



**Fig. 3.** Gamma irradiation affected the phenolic (A) and flavonoid (B) levels of colored rice. The identical letters above bars (a-e) reveal no statistically significant changes in phenolic and flavonoid content of pigmented rice ( $P>0.05$ ).

gamma dose conditions.

### Gamma irradiation's effect on the content of phenolic compounds of pigmented rice under salt condition

Vanillin, vanillic acid, and methyl vanillate belong to the phenolic group. Methyl vanillate is a derivative of vanillic acid (Kundu, 2017), while vanillic acid is an intermediate of vanillin synthesis. Gamma-irradiated rice (5 Gy) had the highest amount of vanillin ( $32.8 \pm 2.8$   $\mu\text{g/g}$ ; Table 1). The highest vanillin content of gamma-irradiated rice was found at the gamma doses of 40 Gy ( $28.1 \pm 2.1$   $\mu\text{g/g}$ ) and 1,000 Gy ( $27.9 \pm 2.1$   $\mu\text{g/g}$ ) under a 20 mM salt concentration. The vanillin content of the control rice was comparable to that of the gamma-irradiated rice in the 40 mM salt condition. However, the vanillin content of gamma-irradiated rice with 40 mM salt was higher than that of the control rice in the without salt (0 mM) and 20 mM salt conditions. The highest content of methyl vanillate of gamma-irradiated rice with and without salt was obtained at 100 Gy ( $120.7 \pm 11.0$   $\mu\text{g/g}$ ; 0 mM salt), 500 Gy ( $166.3 \pm 13.8$   $\mu\text{g/g}$ ; 20 mM salt), and 5 Gy ( $159.8 \pm 10.1$   $\mu\text{g/g}$ ; 40 mM salt). Furthermore, under salt conditions (20 and 40 mM), the methyl vanillate content of gamma-irradiated rice was higher than that under the 0 mM salt condition. Interestingly, the high gamma dose stimulated vanillic acid production in gamma-irradiated rice in both salt conditions (20 and 40 mM) and without salt conditions (0 mM; Table 1). The highest content of vanillic acid was identified after irradiation at 1,000 Gy both for 0 mM and 20 mM salt ( $665.3 \pm 18.5$  and  $632.5 \pm 25.5$   $\mu\text{g/g}$ ,

respectively), while under 40 mM salt conditions, the gamma dosage of 100 Gy yielded the highest concentration of vanillic acid ( $765.4 \pm 15.4$   $\mu\text{g/g}$ ).

According to previous studies,  $\alpha$ -glucosidase and  $\alpha$ -amylase are inhibited by vanilla, vanillic acid, and methyl vanillate (Sansenya et al., 2021). Both pigmented and nonpigmented rice included these bioactive compounds (Sansenya et al., 2021; Sansenya and Payaka, 2022). The results indicated the content of vanillic acid was higher than that of both methyl vanillate and vanillin (Table 1). High concentrations of vanillin are toxic to living organisms; thus, plants or other organisms might store vanillin in the form of nontoxic compounds such as compounds that are conjugated with glucose (Gallage et al., 2018). In many organisms, vanillin is then catabolized to other products, such as vanillic acid (Gallage and Møller, 2015). Our results were consistent with this theory; the rice showed a lower accumulation of vanillin compared to that of vanillic acid and methyl vanillate.

The shikimate pathway describes aromatic amino synthesis in plants and it is affected by many abiotic stress conditions, including ultraviolet (UV) irradiation (Tzin and Galili, 2010). Aromatic amino acids are the precursors of vanillin biosynthesis in plants. In tobacco, salt stress influences aromatic amino acid synthesis, total polyphenol content, and the expression of genes related to the shikimate pathway (Oliva et al., 2021). This process might explain the observed accumulation of vanillin, vanillic acid, and methyl vanillate content in the rice under salt conditions and gamma irradiation in the current study.

**Table 1.** Effect of gamma irradiation on the quantity of methyl vanillate, vanillin, vanillic acid, guaiacol, and syringic acid in pigmented rice under salt conditions

NaCl	Gamma dose (Gy)	Bioactive compounds content ( $\mu\text{g/g}$ )				
		Vanillin	Methyl vanillate	Vanillic acid	Guaiacol	Syringic acid
0 mM	0	$27.2 \pm 2.0^b$	$101.4 \pm 9.0^{bc}$	$530.7 \pm 19.8^c$	$560.2 \pm 8.3^a$	$43.6 \pm 4.4^d$
	5	$32.8 \pm 2.8^a$	$106.7 \pm 5.1^{ab}$	$522.2 \pm 14.4^c$	$500.1 \pm 11.2^b$	$42.3 \pm 4.2^d$
	40	$32.0 \pm 3.0^{ab}$	$110.3 \pm 9.8^{ab}$	$515.3 \pm 16.8^c$	$323.8 \pm 15.5^c$	$65.8 \pm 5.2^c$
	100	$31.0 \pm 2.2^{ab}$	$120.7 \pm 11.0^a$	$572.6 \pm 16.5^b$	$249.0 \pm 6.0^d$	$78.6 \pm 1.8^b$
	500	$27.3 \pm 2.8^b$	$100.2 \pm 9.1^{bc}$	$578.3 \pm 22.5^b$	$214.8 \pm 14.5^e$	$93.2 \pm 8.1^a$
20 mM	1,000	$31.3 \pm 2.6^{ab}$	$90.3 \pm 3.4^c$	$665.3 \pm 18.5^a$	$194.8 \pm 5.3^f$	$70.4 \pm 2.9^{bc}$
	0	$25.3 \pm 1.7^{ab}$	$142.2 \pm 3.5^c$	$492.0 \pm 18.9^c$	$231.6 \pm 19.1^c$	$23.0 \pm 2.3^e$
	5	$24.4 \pm 1.6^b$	$144.3 \pm 5.1^{bc}$	$492.3 \pm 7.2^c$	$252.0 \pm 23.0^{bc}$	$36.1 \pm 3.0^{ab}$
	40	$28.1 \pm 2.1^a$	$160.0 \pm 9.0^{ab}$	$568.0 \pm 19.1^b$	$272.7 \pm 17.5^{ab}$	$40.1 \pm 2.4^a$
	100	$26.8 \pm 1.9^{ab}$	$159.5 \pm 6.3^{ab}$	$558.4 \pm 19.4^b$	$296.1 \pm 16.4^a$	$34.4 \pm 1.6^{bc}$
40 mM	500	$24.2 \pm 1.2^b$	$166.3 \pm 13.8^a$	$506.4 \pm 17.8^c$	$275.1 \pm 15.8^{ab}$	$30.4 \pm 2.0^d$
	1,000	$27.9 \pm 2.1^a$	$148.4 \pm 11.7^{bc}$	$632.5 \pm 25.5^a$	$235.6 \pm 23.1^c$	$30.1 \pm 2.1^d$
	0	$29.7 \pm 3.0^a$	$154.1 \pm 5.4^{ab}$	$655.1 \pm 17.0^d$	$201.7 \pm 12.6^c$	$48.6 \pm 3.6^c$
	5	$31.7 \pm 4.0^a$	$159.8 \pm 10.1^a$	$663.3 \pm 31.5^{cd}$	$215.0 \pm 13.7^b$	$37.2 \pm 3.1^d$
	40	$30.2 \pm 2.8^a$	$151.3 \pm 6.7^{ab}$	$712.0 \pm 16.0^b$	$243.9 \pm 17.2^a$	$56.3 \pm 2.7^b$
40 mM	100	$33.1 \pm 2.7^a$	$140.0 \pm 10.0^b$	$765.4 \pm 15.4^a$	$269.3 \pm 10.3^a$	$61.2 \pm 2.3^a$
	500	$29.7 \pm 2.8^a$	$138.7 \pm 8.2^b$	$695.5 \pm 10.5^{bc}$	$212.7 \pm 13.9^b$	$41.2 \pm 1.8^d$
	1,000	$31.0 \pm 2.0^a$	$148.2 \pm 12.3^{ab}$	$722.2 \pm 22.7^b$	$254.4 \pm 18.3^a$	$58.3 \pm 1.5^{ab}$

Values are presented as mean  $\pm$  SD.

The identical superscript letters (a-f) within the same columns under same salt condition reveal no statistically significant variations in the bioactive compounds of rice ( $P > 0.05$ ).

Guaiacol is a phenolic compound that accumulates in many plants. This compound was also identified in the rice in our study and displayed the  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities reported previously (Sansenya et al., 2021). The guaiacol of the control rice (0 Gy) had the highest content of guaiacol ( $560.2 \pm 8.3 \mu\text{g/g}$ ) compared to the gamma-irradiated rice ( $194.8 \pm 5.3$  to  $500.1 \pm 11.2 \mu\text{g/g}$ ), and the lowest content of guaiacol was obtained at 1,000 Gy. Under irradiation conditions, plants generate ROS, resulting in cell damage. To reduce the ROS in plant cells, scavenging antioxidant enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase, and guaiacol peroxidase (GPX) have been synthesized (Gill and Tuteja, 2010). The ROS ( $\text{H}_2\text{O}_2$ ) catalyzed by GPX used guaiacol as the substrate (Mika and Luthje, 2003). The increase in gamma dose resulted in an increase in ROS. Our results showed that gamma doses of 5 to 1,000 Gy caused a reduction in guaiacol synthesis in rice. This result supports the idea that GPX is the major enzyme for catalyzing ROS in rice cells under gamma irradiation conditions. To further illustrate, Table 1 shows that under the salt condition, gamma-irradiated rice's guaiacol content decreased as salt concentration rose (20 to 40 mM salt). However, in the condition of 20 mM salt, the gamma dose of 40 to 500 Gy affected the increase in the guaiacol content. Similar results were observed in the condition of 40 mM salt by an increase of guaiacol content under 5 to 1,000 Gy of gamma irradiation. The results indicated that the reduction in guaiacol accumulation in rice was affected by the salt condition. Conversely, the amelioration by gamma irradiation stimulated guaiacol accumulation under salt conditions.

Syringic acid, a phenolic compound, has been shown to have biological impacts on human health, such as antioxidant qualities and the capacity to inhibit the enzymes  $\alpha$ -glucosidase and  $\alpha$ -amylase (Srivastava et al., 2014; Sansenya et al., 2021). Table 1 shows that gamma irradiation at 40 to 1,000 Gy significantly stimulated the syringic acid accumulation in the rice in this study. In the salt condition (20 mM), control rice had a lower syringic acid content compared to the rice with no salt treatment, while in the 40 mM salt condition, the syringic acid content of the rice was higher compared to the rice under 0 mM salt. However, the gamma-irradiated rice (5 to 1,000 Gy) in 20 mM salt had significantly higher syringic acid contents compared to the control rice. The gamma-irradiated rice (40 to 100 Gy and 1,000 Gy) in the condition of 40 mM salt also had significantly higher syringic acid content than the control rice (0 Gy).

Tryptophan, tyrosine, and phenylalanine are among the aromatic amino acids produced by the shikimic acid pathway. These amino acids act as precursors for several phenolic compounds, including syringic acid (Srinivasulu et al., 2018). The aromatic amino acids products of the shi-

kimic pathway can be stimulated by abiotic stress such as UV irradiation and salt concentration (Tzin and Galili, 2010). This might explain why the gamma irradiation induced syringic acid accumulation in gamma-irradiated rice (40 to 1,000 Gy; 0 mM salt). In the case of salt stress conditions (20 and 40 mM salt), increasing the dose of gamma irradiation induced the accumulation of syringic acid in the rice. This result suggests that the salt stress might have been ameliorated by gamma irradiation, which induced the accumulation of syringic acid.

### Gamma irradiation's effect on the volatile compounds of pigmented rice under salt conditions

2-Pentyl furan is rice's major volatile compound and contributes to the aromatic intensities of certain rice varieties (Hinge et al., 2016). This compound has demonstrated biological activity in the form of inhibiting enzymes  $\alpha$ -glucosidase and  $\alpha$ -amylase. The 2-pentyl furan content of the control rice was  $4.4 \pm 0.2 \mu\text{g/g}$ , and gamma irradiation did not significantly affect the 2-pentyl furan content of the rice (Table 2). In the saline treatment (20 mM), the control rice ( $6.1 \pm 0.5 \mu\text{g/g}$ ) had the significantly highest 2-pentyl furan content compared to gamma-irradiated rice ( $3.4 \pm 0.3$  to  $4.5 \pm 0.3 \mu\text{g/g}$ ). In the condition of 40 mM salt, only the 40 Gy treated rice had a higher 2-pentyl furan content ( $4.5 \pm 0.4 \mu\text{g/g}$ ) compared to the control rice ( $3.9 \pm 0.2 \mu\text{g/g}$ ) and rice exposed to other gamma doses ( $3.3 \pm 0.3$  to  $3.7 \pm 0.2 \mu\text{g/g}$ ). The results indicate that the 2-pentyl furan accumulation in rice is minimally affected by gamma irradiation. Interestingly, the salt condition

**Table 2.** The quantity of 2-pentyl furan in gamma-irradiated rice with and without salt

NaCl	Gamma dose (Gy)	2-Pentyl furan ( $\mu\text{g/g}$ )
0 mM	0	$4.4 \pm 0.2^{\text{ab}}$
	5	$4.0 \pm 0.3^{\text{b}}$
	40	$4.3 \pm 0.4^{\text{b}}$
	100	$5.1 \pm 0.3^{\text{a}}$
	500	$4.7 \pm 0.4^{\text{ab}}$
	1,000	$4.2 \pm 0.4^{\text{b}}$
20 mM	0	$6.1 \pm 0.5^{\text{a}}$
	5	$4.3 \pm 0.3^{\text{b}}$
	40	$4.2 \pm 0.2^{\text{b}}$
	100	$3.8 \pm 0.4^{\text{bc}}$
	500	$3.4 \pm 0.3^{\text{c}}$
	1,000	$4.5 \pm 0.3^{\text{b}}$
40 mM	0	$3.9 \pm 0.2^{\text{b}}$
	5	$3.4 \pm 0.3^{\text{b}}$
	40	$4.5 \pm 0.4^{\text{a}}$
	100	$3.7 \pm 0.2^{\text{b}}$
	500	$3.5 \pm 0.3^{\text{b}}$
	1,000	$3.3 \pm 0.3^{\text{b}}$

Values are presented as mean  $\pm$  SD.

The identical superscript letters (a-c) within same columns under same salt concentration reveal no statistically significant variations in the 2-pentyl furan of rice ( $P > 0.05$ ).

seems to have affected the 2-pentyl furan accumulation in the rice (20 mM salt:  $6.1 \pm 0.5$   $\mu\text{g/g}$  vs. 0 mM:  $4.4 \pm 0.2$   $\mu\text{g/g}$  and 40 mM salt:  $3.9 \pm 0.2$   $\mu\text{g/g}$ ), having a positive effect on the 2-pentyl furan accumulation. According to previous studies (Yang et al., 2008; Grimm et al., 2011), fragrant rice contains a higher concentration of the heterocyclic compound 2-pentyl furan than nonfragrant rice. In addition, the rice aroma quality is positively affected by salt conditions (Hu et al., 2020). The previous reports support our findings regarding the positive effect of the 20 mM salt concentration on 2-pentyl furan accumulation.

In this study, the impact of gamma radiation on pigmented rice growth, photosynthetic pigment, and phenolic and flavonoid content was investigated. The findings showed that gamma irradiation at 5 to 100 Gy could encourage plant development, while a dose of 500 to 1,000 Gy could hinder it. Gamma irradiation was also used to stimulate the production of photosynthetic pigments (Chl a, Chl b, and carotenoid), which are associated with plant growth. The phenolic and flavonoid content of rice was induced by gamma irradiation, and the gamma dose seemed to increase the phenolic and flavonoid content. This research also evaluated the effect of gamma irradiation on the amount of selected bioactive compounds, including vanillin, methyl vanillate, vanillic acid, guaiacol, syringic acid, and 2-pentyl furan under salt conditions. The findings demonstrated that, depending on the gamma dose, gamma irradiation altered the amount of vanillin, methyl vanillate, and vanillic acid in gamma-irradiated rice. However, the vanillin concentration of gamma-irradiated rice was adversely impacted by the salt conditions (20 and 40 mM), while the methyl vanillate content was improved by the salt concentrations of 20 and 40 mM and the gamma dose ameliorated the content of this compound in the gamma-irradiated rice under both salt concentrations. Similar to methyl vanillate, the vanillic acid concentration of gamma-irradiated rice exhibited an accumulation pattern. Gamma irradiation had a negatively effect on the guaiacol content of rice. However, the gamma dose ameliorated guaiacol accumulation in the salt stress condition. The syringic acid content of gamma-irradiated rice was stimulated by increasing the gamma dose. The positive effect of salt condition was observed at a high concentration of salt (40 mM). However, the amelioration by gamma irradiation of syringic acid accumulation in gamma-irradiated rice was observed in both salt concentrations (20 and 40 mM). For the heterocyclic compound, 2-pentyl furan, gamma irradiation was less effective when compared to the salt condition. The findings of this study suggest that gamma irradiation can promote the accumulation of phenolic compounds in rice, particularly those connected to the inhibition of enzymes related to diabetes. Moreover, gamma irradiation can reduce the salt stress via the accumula-

tion of phenolic compounds in rice.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare no potential conflict of interest.

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## AUTHOR CONTRIBUTIONS

Concept and design: MK, SC, SS. Analysis and interpretation: MK, SC, SS. Data collection: MK, SC, SS. Writing the article: MK, SC, SS. Critical revision of the article: MK, SS. Final approval of the article: all authors. Statistical analysis: SC. Obtained funding: SS. Overall responsibility: SS.

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