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The γ-oryzanol Content of Thai Rice Cultivars and the Effects of Gamma Irradiation on the γ-oryzanol Content of Germinated Thai Market Rice

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ABSTRACT

Thirty-nine Thai rice cultivars, including 17 non-colored rice cultivars and 22 colored rice cultivars (black rice cultivars and red rice cultivars), were investigated for γ-oryzanol content. The γ-oryzanol content of non-colored rice cultivars and colored rice cultivars was found to be 226.40 ± 17.50 µg/g to 411.80 ± 19.80 µg/g and 295.80 ± 15.40 µg/g to 459.80 ± 6.60 µg/g, respectively. Black rice cultivars seem to have higher γ-oryzanol content than do red rice and non-colored rice cultivars. The highest γ-oryzanol levels were identified in Khao Jao Dam Sa-Nit, a black rice cultivar, which contained 1.55 and 2.03 times more γ-oryzanol than Khao Man Bpoo, a red rice cultivar with lowest γ-oryzanol levels, and Khao Gor Kor 57, a non-colored rice cultivar with lowest γ-oryzanol levels, respectively. For further investigation, the effects of gamma irradiation on the γ-oryzanol content of four germinated Thai market rice cultivars, Khao Pathum Thani 1, Khao Gor Kor 31, Khao Gor Kor 41, and Khao Gor Kor 57, was investigated. The highest increase in γ-oryzanol content among the gamma irradiated rice cultivars was identified in Khao Pathum Thani 1 and Khao Gor Kor 31, which had γ-oryzanol levels approximately 1.27 times higher than that of the control rice (non-gamma irradiated rice). Our report contains basic knowledge about creating new rice lines that are rich in highly beneficial nutrients.

Keyword: Thai rice cultivars, Thai market rice cultivars, γ-Oryzanol, Gamma irradiation.

INTRODUCTION

γ-Oryzanol is a mixture of ferulated plant sterol compounds first isolated from rice bran[1-3]. γ-Oryzanol's components include ferulic acid esters such as cycloartenyl ferulate, campesteryl ferulate, and 24-methylenecycloartanyl ferulate[2,4,5]. In recent years, γ-oryzanol has been investigated due to its biological activities, such as high anti-oxidant activity[6] and decreased cholesterol levels and cholesterol absorption have been associated with γ-oryzanol[7,8]. γ-Oryzanol has also been reported to decrease hyperglycemia[9,10] and has been investigated for anti-cancer and anti-inflammatory purposes[11,12].

Gamma irradiation is a widely used plant mutation improvement technique. Gamma rays can generate Reactive oxygen species (ROS) and induce DNA damage, base deletion, and base substitution[13,14]. They can also affect plant growth and plant biochemical compounds[15,16]. In rice, Hwang *et al.,*[17] reported that gamma irradiation induced increased tocopherol in rice mutants. Moreover, gamma irradiation effected the expression level of the tocopherol biosynthesis gene (*OsVTE2*) and enzyme (*OsVTE2*). The improvement of characteristic compounds in fragrant rice such as 2-acetyl-1-pyrroline content (2AP) is caused by gamma irradiation[18], and gamma irradiation can alter the growth rate of rice by increasing rice shoot and root length[19,20].

Rice is the most staple food in the world, especially in developing countries. Thailand was the sixth largest rice producing country worldwide in 2018 (Food and Agriculture Organization of the United Nations). In addition, Thailand has many rice varieties, including local rice and market rice, and Thai people consume both non-colored rice and colored rice. However, Priya *et al.,*[21] reported that colored rice seems to have more bioactive compounds that contribute to human health benefits than non-colored rice. In this study, we investigated the γ-oryzanol content of 39 Thai rice cultivars and compared the γ-oryzanol content of colored rice and non-colored rice. Moreover, this study is the first to report the effects of gamma irradiation on the γ-oryzanol content of four Thai market rice cultivars.

MATERIALS AND METHODS

Plant material

Seeds from 39 Thai rice cultivars were obtained from rice fields in four Thai provinces, including Roi-Et, Maha Sarakham, Khon Kaen, and Phetchabun provinces (Fig. 1 and 2). All the rice seeds were harvested during the 2017 and 2018 rice seasons. The rice seeds were sterilized by soaking them in 0.1% NaClO and then in distilled water. The sterilized rice seeds were dried using a hot air oven (60°C) until their moisture content was less than 13%.

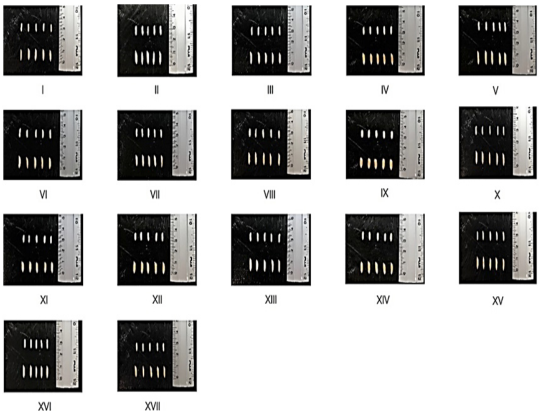

Fig. 1. Photographs of 17 non-colored rice cultivars. Nos. IV, V, VI, IX, X, XII, and XIV are local rice cultivars and include Khao Nieow E-Dtla, Khao Nieow Laao, Khao Mali Doi, Khao Rag Pai, Khao Jek Choie, Khao Nieow Kieow-Ngoo, and Khao Hang Yee. Nos. II, III, VII, VIII, XI, XIII, XV, XVI, and XVII are development rice lines and include Khao Pathum Thani 1, Khao Phitsanulok 2, Khao Chai Nat, Khao Hom Pathum, Khao Gor Kor 57, Khao Gor Kor 49, Khao Gor Kor 41, Khao Gor Kor 31, and Khao Gor Kor 6

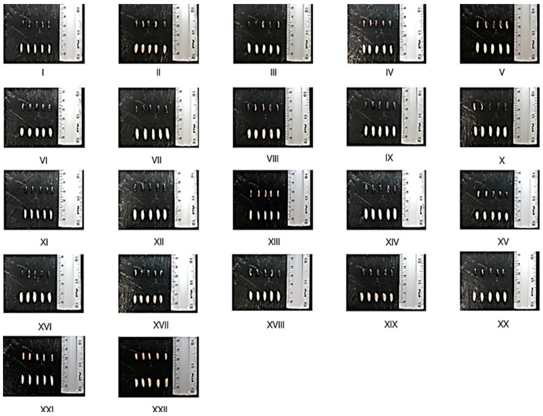

Fig. 2. Photographs of 22 colored rice cultivars. Nos. I through XX are black rice cultivars (Khao Jao Dam Sa-Nit, Khao Gam, Khao Nieow Dam Peun Baan, Khao Dam, Khao Leum Pua, Khao Leum Pua I, Khao Koie Laa, Khao Gam Bpom, Khao Gam Rai, Khao Muang Dam, Khao Nieow Dam, Khao Nieow Dam Ga-Sayt, Khao Gam Gliang, Khao Leum Mia, Khao Dam Nin, Khao Jao Dam, Khao Gam Hmong, and Khao Jao Dam Jeen). Nos. XXI and XXII are red rice cultivars (Khao Man Bpoo and Khao Plae Daeng). Nos. XI and XIII are development rice lines (Khao Rice Berry and Khao Hom Nin)

Gamma irradiation

Thai market rice cultivars, including Khao Pathum Thani 1, Khao Gor Kor 31, Khao Gor Kor 41, and Khao Gor Kor 57, were used to study the effects of gamma irradiation on the γ-oryzanol content of germinated rice. Approximately 50 g of the grains were subjected to gamma rays at doses of 0 (control; non-gamma irradiation), 5, 10, 20, 40, 60, 80, 100, 200 and 300 Gy using 137Cs as the gamma source. The gamma irradiation was carried out by the Gamma Irradiation Service and Nuclear Technology Research Center, Faculty of Science, Kasetsart University, Thailand.

Plant germination

The gamma irradiated rice seeds and non-gamma irradiated rice seeds (0 Gy) were soaked with distilled water for 24 h prior to seed germination. The soaked rice seeds were germinated on germinating paper by moistening them with deionized water at the ambient temperature. The germination experiments were sprayed with deionized water every 12 h until harvest time. The gamma irradiated rice and non-gamma irradiated rice were harvested at the of end germination at 48 hours. The rice samples were kept at -20°C until γ-oryzanol determination.

γ-Oryzanol determination

The rice samples, including seeds of 39 Thai rice cultivars and germinated rice (gamma irradiated rice and non-gamma irradiated rice), were homogenized using a CryoMill (Retsch, Germany) with liquid nitrogen cooling. All homogenized samples were used for γ-oryzanol determination. The γ-oryzanol content was determined using a modified spectrophotometry technique based on the method of Xu and Godber[22] and Bucci *et al.,*[23]. One gram of homogenized rice samples with 0.20 g of ascorbic acid was mixed with 5 mL of water, and the reaction mixture was placed at the room temperature for 30 minutes. Five milliliters of 1:1 hexane: isopropanol was added to the extract, then incubated at room temperature and stirred for 30 minutes. The solvent and rice piece residue were separated using a syringe filter (0.45 µm). The rice piece residue was then extracted again a total of four times using this process. The four extracted solutions were pooled and centrifuged at 6,000 rpm for 15 minutes. The organic solvent layer was separated, and the solvent was then evaporated in rotary evaporator at 60°C to obtain crude samples. Approximately 0.10 g of crude samples was dissolved using 2 mL of isopropanol and measured at 326 nm using a spectrophotometer. The amount of γ-oryzanol was calculated using the pure γ-oryzanol calibration curve, $y = 36.045x + 0.005$ ($R2 = 0.9992$).

Statistical analysis

All experiments were performed in triplicate. One-way analysis of variance (ANOVA) with a least significant difference post hoc test was reported for the statistical significance of differences among experimental groups. The significance level was set at $P < 0.05$.

RESULTS

γ-Oryzanol content of 61 Thai rice cultivars

Table 1 and Fig. 1 show the γ-oryzanol content of 17 non-colored rice cultivars, which ranged from 226.40 ± 17.50 µg/g to 411.80 ± 19.80 µg/g. The highest γ-oryzanol content was obtained from KDML105, while the lowest content was identified in Khao Gor Kor 57. Among 17 non-colored rice cultivars, KDML105 is the standard rice. The 7 local rice cultivars were Khao Nieow E-Dtla, Khao Nieow Laao, Khao Mali Doi, Khao Rag Pai, Khao Jek Choie, Khao Nieow Kieow-Ngoo and Khao Hang Yee, which had γ-oryzanol levels that ranged from 291.70 ± 26.20 µg/g to 372.70 ± 6.70 µg/g. The highest content was found in Khao Nieow E-Dtla, while the lowest content was found in Khao Hang Yee. The 9 non-colored rice cultivars are a line of development rice and include Khao Pathum Thani 1, Khao Phitsanulok 2, Khao Chai Nat, Khao Hom Pathum, Khao Gor Kor 57, Khao Gor Kor 49, Khao Gor Kor 41, Khao Gor Kor 31, and Khao Gor Kor 6. The γ-oryzanol of these rice cultivars ranged from 226.40 ± 17.50 µg/g to 407.40 ± 2.00 µg/g. The highest content was obtained from Khao Pathum Thani 1, and the lowest was obtained from Khao Gor Kor 57.

Table. 1. γ-Oryzanol content of 17 non-colored rice cultivars

No.	Rice cultivars	γ-Oryzanol content (µg/g)
I	KDML105	411.80 ± 19.80
II	Khao Pathum Thani 1	407.40 ± 2.00
III	Khao Phitsanulok 2	392.90 ± 40.00
IV	Khao Nieow E-Dtla	372.70 ± 6.70
V	Khao Nieow Laao	358.20 ± 9.40
VI	Khao Mali Doi	353.70 ± 7.20
VII	Khao Chai Nat	319.90 ± 17.90
VIII	Khao Hom Pathum	318.00 ± 9.80
IX	Khao Rag Pai	315.50 ± 16.00
X	Khao Jek Choie	307.50 ± 11.90
XI	Khao Gor Kor 6	307.30 ± 16.40
XII	Khao Nieow Kieow-Ngoo	303.50 ± 4.30
XIII	Khao Gor Kor 49	293.00 ± 14.20
XIV	Khao Hang Yee	291.70 ± 26.20
XV	Khao Gor Kor 41	264.90 ± 27.20
XVI	Khao Gor Kor 31	258.90 ± 19.30
XVII	Khao Gor Kor 57	226.40 ± 17.50

± indicates the standard deviation of mean (SD) (n = 3).

Table 2 and Fig. 2 show the γ-oryzanol content of 22 colored rice cultivars, which ranged from 295.80 ± 15.40 μg/g to 459.80 ± 6.60 μg/g. The highest and the lowest contents were obtained from Khao Jao Dam Sa-Nit and Khao Plae Daeng, respectively. Khao Rice Berry and Khao Hom Nin are development rice lines, and their γ-oryzanol contents were 424.60 ± 14.90 μg/g and 420.00 ± 13.80 μg/g, respectively. These two rice cultivars are also black rice cultivars. The other 20 rice cultivars are local rice cultivars and were divided into two groups: red rice (two cultivars) and black rice (18 cultivars). Khao Man Bpoo and Khao Plae Daeng are red rice cultivars with γ-oryzanol contents of 297.50 ± 3.10 μg/g and 226.40 ± 17.50 μg/g, respectively. The black rice cultivars were Khao Jao Dam Sa-Nit, Khao Gam, Khao Nieow Dam Peun Baan, Khao Dam, Khao Leum Pua, Khao Leum Pua I, Khao Koie Laa, Khao Gam Bpom, Khao Gam Rai, Khao Muang Dam, Khao Nieow Dam, Khao Nieow Dam Ga-Sayt, Khao Gam Gliang, Khao Leum Mia, Khao Dam Nin, Khao Jao Dam, Khao Gam Hmong, and Khao Jao Dam Jeen. These had γ-oryzanol contents that ranged from 369.30 ± 3.40 μg/g to 459.80 ± 6.60 μg/g.

Table 2: γ-Oryzanol content of 22 colored rice cultivars

No.	Rice cultivars	γ-Oryzanol content (μg/g)
I	Khao Jao Dam Sa-Nit	459.80 ± 6.60
II	Khao Gam	455.70 ± 21.10
III	Khao Nieow Dam Peun Baan	447.10 ± 20.40
IV	Khao Dam	446.20 ± 14.10
V	Khao Leum Pua I	445.90 ± 8.50
VI	Khao Koie Laa	436.40 ± 13.40
VII	Khao Leum Pua	435.30 ± 21.00
VIII	Khao Gam Bpom	431.00 ± 17.30
IX	Khao Gam Rai	429.70 ± 22.40
X	Khao Muang Dam	428.10 ± 1.70
XI	Khao Rice Berry	424.60 ± 14.90
XII	Khao Nieow Dam	421.50 ± 14.50
XIII	Khao Hom Nin	420.00 ± 13.80
XIV	Khao Nieow Dam Ga-Sayt	416.20 ± 17.10
XV	Khao Gam Gliang	412.70 ± 13.20
XVI	Khao Leum Mia	411.50 ± 3.20
XVII	Khao Dam Nin	409.40 ± 4.20
XVIII	Khao Jao Dam	407.40 ± 6.80
XIX	Khao Gam Hmong	401.90 ± 17.30
XX	Khao Jao Dam Jeen	369.30 ± 3.40
XXI	Khao Man Bpoo	297.50 ± 3.10
XXII	Khao Plae Daeng	295.80 ± 15.40

± indicates the standard deviation of mean (SD) (n = 3).

Effects of gamma irradiation on the γ-oryzanol content of Thai market rice cultivars

Four Thai market rice cultivars were subjected to gamma irradiation, and their γ-oryzanol content was evaluated and compared with that of non-gamma irradiated rice. The results indicated that gamma irradiation stimulated the γ-oryzanol content of Khao Pathum Thani 1 at all gamma doses except 300 Gy compared with the control rice (non-gamma irradiated rice) (Fig. 3A). The γ-oryzanol content of gamma irradiated rice (Khao Gor Kor 31) was higher than that of non-gamma irradiated rice at gamma doses of 5 to 60 Gy. However, at gamma doses of 100 to 300 Gy, the γ-oryzanol content of gamma irradiated rice decreased compared to that of the control rice (Fig. 3B). A significantly increase *($P > 0.05$)* in γ-oryzanol content in gamma irradiated rice (Khao Gor Kor 41) was identified only at the 20-Gy gamma dose, whereas gammas dose of 80 to 300 Gy decreased the γ-oryzanol content of gamma irradiated rice (Fig. 3C). At gamma doses of 5 Gy, the γ-oryzanol content of gamma irradiated rice (Khao Gor Kor 57) increased, but at gamma doses of 10 to 300 Gy, the γ-oryzanol content of gamma irradiated rice continuously decreased when compared with that of the control rice (Fig. 3D).

DISCUSSION

γ-Oryzanol content of non-colored and colored rice

The accumulation of γ-oryzanol content in rice is affected by several factors, such as environmental factors, germination time, and rice cultivar[24,25]. The γ-oryzanol content accumulation varied between non-colored rice and colored rice. However, colored rice cultivars seem to have higher γ-oryzanol levels than non-colored rice cultivars[26-28]. Our results indicate that the γ-oryzanol content of colored rice cultivars seems to be higher than that of non-colored rice cultivars. Among colored rice cultivars, the γ-oryzanol content of black rice cultivars (Table 2, nos. I through XX) had higher γ-oryzanol levels than red rice cultivars (Table 2, nos. XXI and XXII). Among black rice cultivars, the highest γ-oryzanol content was obtained from Khao Jao Dam Sa-Nit, which had approximately 1.54 times more γ-oryzanol content than the red rice cultivar with the highest γ-oryzanol content (Khao Man Bpoo). Huang and Ng[27] reported that a black rice cultivar (Kuroo-Mochi) had higher γ-oryzanol content than did a red rice cultivar (Ai-Chueh-Shien-Hung-No). However, these researchers also reported that one red rice cultivar (Hung-No) had higher γ-oryzanol content than did a black rice cultivar (Ch'o-Tzu). Tsuzuki *et al.,*[29] also indicated that the γ-oryzanol content of back-purple rice cultivars was higher than that of red rice cultivars and brown rice cultivars.

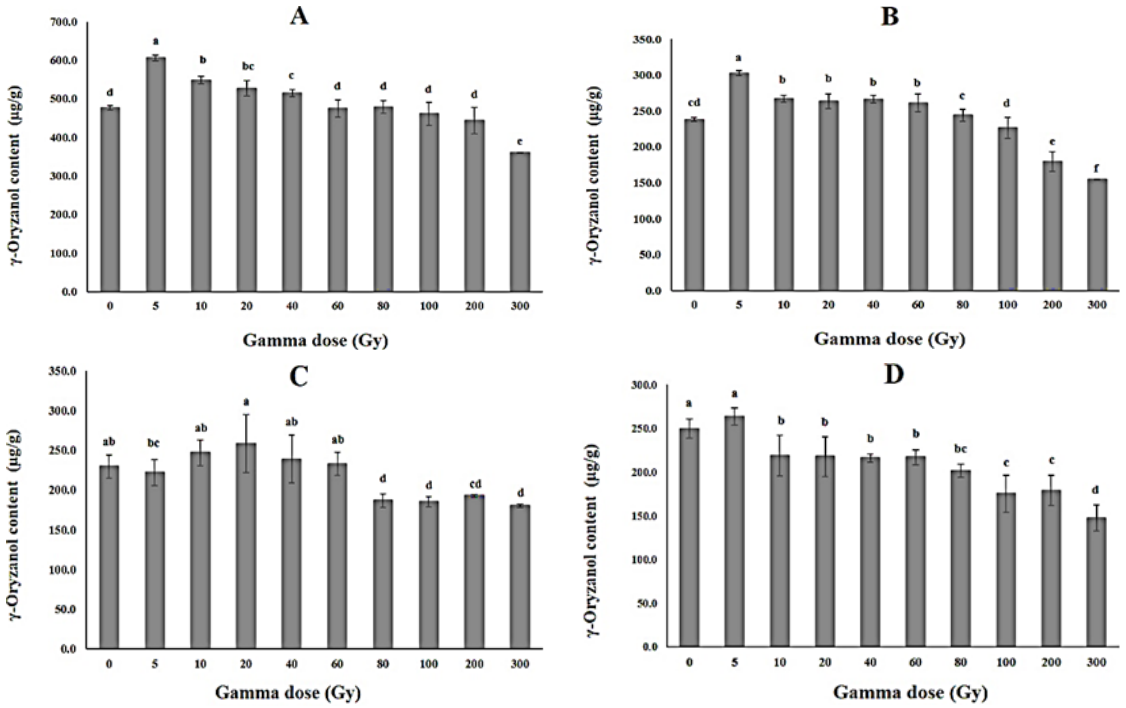

Fig. 3. Effects of gamma irradiation on the γ-oryzanol content of four Thai market rice cultivars. A: Khao Pathum Thani 1; B: Khao Gor Kor 31; C: Khao Gor Kor 41; D: Khao Gor Kor 57. ± indicates the standard deviation of mean (SD) (n = 3). The use of the same superscript letter in the same letter indicates that the results were not significantly different (*P* > 0.05)

Black rice seems to have higher γ-oryzanol content than non-colored rice. Black rice cultivars I to XV (Table 2) had higher γ-oryzanol content than did all non-colored rice cultivars (Tables 1 and 2). Four colored rice cultivars, including Khao Dam Nin, Khao Jao Dam, Khao Gam Hmong, and Khao Jao Dam, had lower γ-oryzanol content than some non-colored rice cultivars, such as KDML 105, Khao Pathum Thani 1, Khao Phitsanulok 2, and Khao Nieow E-Dtla. The lowest γ-oryzanol content among colored rice cultivars was obtained from two red rice cultivars, Khao Man Bpoo and Khao Plae Daeng. Their γ-oryzanol content was also lower than that of 12 non-colored rice cultivars (Fig. 2, nos. I to XII; Table 1). Tsuzuki *et al.,*[29] reported the highest γ-oryzanol content in back-purple rice cultivars, which also had higher γ-oryzanol content than red rice cultivars and brown rice cultivars (non-colored rice cultivars). However, the γ-oryzanol content of some back-purple rice cultivars (Kitanomurasaki) had lower γ-oryzanol content than red rice cultivars and non-colored rice cultivars. Boonsit *et al.,*[26] also indicated that all colored rice cultivars (purple rice) in their report had higher γ-oryzanol content higher than the non-colored rice cultivars KDML 105 and RD6 (Khao Gor Kor 6).

Effects of gamma irradiation on the γ-oryzanol content of rice

Our study investigated the extent to which γ-oryzanol content changed after gamma irradiation. The γ-oryzanol content of three market rice cultivars increased at low gamma irradiation doses. Levels increased significantly ($P < 0.05$) in Khao Pathum Thani 1 at 5 to 40 Gy, in Khao Gor Kor 31 at 5 to 60 Gy, and in Khao Gor Kor 41 at 20 Gy. A small increase in γ-oryzanol content was observed at 5 Gy in Khao Gor Kor 57, but this increase was not significant ($P > 0.05$) when compared with the control rice (Fig. 3). Gamma irradiation tolerance varied in the four market rice cultivars, but high gamma doses seemed to have more effect than low gamma doses. A significant ($P < 0.05$) decrease in γ-oryzanol content was observed at 300 Gy for Khao Pathum

Thani 1, while the γ-oryzanol content of gamma-irradiated rice was similar to that of the control rice at gamma doses of 60 to 200 Gy. A similar trend was observed in Khao Gor Kor 31, as the γ-oryzanol content of this rice decreased at 100 to 300 Gy. Low gamma irradiation tolerance was observed in Khao Gor Kor 41 and Khao Gor Kor 57 based on the significant ($P < 0.05$) decrease in γ-oryzanol content in Khao Gor Kor 41 at 80 to 300 Gy and the decrease of γ-oryzanol content of Khao Gor Kor 57 at 10 to 300 Gy. Furthermore, the lowest γ-oryzanol content of the four market rice cultivars was obtained at 300 Gy. Low gamma doses can induce plant growth and tolerance, whereas decreased plant survival and decreased plant biochemical compounds result from high gamma doses[30]. In rice, decreased shoot and root lengths were observed at gamma doses of more than 250 Gy[19]. Increases in biochemical compounds such as GABA and chlorophyll (a, b) were stimulated in rice by low gamma doses, as was seed yield, but these characteristics were reduced by high gamma doses[20,31,32]. Marcu *et al.,*[33] also reported similar results in maize at low gamma doses, which induced plant growth and biochemical compounds such as chlorophyll (a, b) and carotenoids. However, high gamma doses inhibited these characteristics, and the plants did not survive more than 10 days at 0.5 kGy. Our results and those of previous reports indicate that low gamma irradiation can create new rice mutants that contain suitable physiological characteristics.

CONCLUSION

In this study, we investigated a variety of γ-oryzanol contents in colored and non-colored Thai rice cultivars. The results indicate that black rice cultivars had γ-oryzanol contents higher those of than red rice cultivars and non-colored rice cultivars. The highest γ-oryzanol content among the studied Thai rice cultivars was obtained from Khao Jao Dam Sa-Nit (black rice cultivar), Khao Man Bpoo (red rice cultivar), and KDML105 (non-colored rice cultivar) respectively. We also investigated the effects of gamma irradiation on the γ-oryzanol content of four Thai market rice cultivars: Khao Pathum Thani 1, Khao Gor Kor 31, Khao Gor Kor 41, and Khao Gor Kor 57. The results indicate that at low gamma irradiation stimulated γ-oryzanol content, but high gamma irradiation inhibited γ-oryzanol synthesis. Thus, our results establish basic knowledge regarding the creation of new rice mutants with high γ-oryzanol content.

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Conflict of interest

The authors declare no conflict of interest.

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