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Influence of Seed Quality Stimulation in "Khao Dawk Mali 105" Rough Rice during the Deterioration Period Using an Automatic Soaking and Germination Accelerator Unit and Infrared Radiation Treatment

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Abstract: This study aimed to improve the seed quality during the deterioration period of rough rice (*Oryza sativa* L.), cultivar 'Khoa Dawk Mali 105' (KDML 105), using an automatic soaking and germination accelerator unit (ASGA) together with stimulation via infrared radiation treatment (IRT) to stimulate seed quality (germination rate and γ-aminobutyric acid (GABA) content). This study used a general full factorial design, and the independent variables were the storage period (10, 11 and 12 months), methods of germinated rough rice preparation (conventional method (CM) and an automatic soaking and germination accelerator unit (ASGA)), and stimulation with IRT. The initial grain moisture content did not exceed 14% (wet basis (wb)). The germination rate of the rough rice by CM and ASGA with stimulation with IRT was significantly higher than non-stimulated rice, by 6.56 and 8.11%, respectively, in each storage period. The GABA contents of the germinated rough rice using CM and ASGA stimulated with IRT were significantly higher than ungerminated rough rice, by 19.52 and 21.24% (10 months), respectively; 16.36 and 23.58% (11 months), respectively; and 69.88 and 67.69% (12 months), respectively.

Keywords: germination rate; γ-aminobutyric acid; infrared radiation; deterioration period; germinated rough rice

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1. Introduction

Rice is a staple food that has been studied and developed continuously to increase its nutrition value [1] (Fresco, 1947). This includes techniques for rice processing that are currently gaining research interest, such as the use of germinated rough rice (GRR). Several studies have found that GRR has a higher concentration of nutrients and bioactive compounds compared with germinated brown rice (GBR), in addition to its simple germination process, since GRR has a high germination rate [2–4], as well as its lack of odor defects [5]. In addition, the important bioactive compounds in GRR show significant improvements after germination, especially regarding the concentration of γ-aminobutyric acid (GABA), which accumulates during this process [6,7]. GABA has several physiological functions, such as neurotransmission, induction of hypotensive effects, and a tranquilizer effect. However, when considering the GRR production process, there are still limitations in terms of the difficulty and duration of the process, unevenness of the germination rate, and odor defection of the product. Moreover, when considering the deterioration of the seeds due to the storage period, which directly affects the seed quality, the germination rate and activity of various enzymes are reduced. This directly affects the synthesis of nutrients and bioactive compounds that accumulate in the grains.

The objective of this research was to examine the influence of the seed germination method and stimulation conditions on the germination rate and GABA content of 'Khao

Dawk Mali 105' to improve the seed quality in the deterioration period using an automatic soaking and germination unit (ASGA), together with stimulation via infrared radiation treatment (IRT). Therefore, this study will provide the agricultural industry with the opportunity to increase the production capacity and quality of products and provide knowledge for the development of innovative rice products that will be recognized as a valuable food with high nutritional benefits for human health.

2. Materials and Methods

2.1. Rice Samples

Rough rice of *Oryza sativa* L., cultivar Khao Dawk Mali 105 (the most popular jasmine rice cultivar for planting in Northeast Thailand), was selected for this study, which was obtained from a single planting field at Mahasarakham University, Thailand. To prepare the GRR samples, after harvesting the paddy was threshed, the moisture content was reduced to ≤14% (wb), and impurities and invalid grains were removed, while maintaining the condition of the paddy seeds with the husks. The samples were packed in plastic bags and stored in an air-tight storeroom for ten months [8–10]. The preparation of ungerminated rice (UGR) or brown rice samples was prepared using a laboratory de-husker to remove the husk, following the methods studied and reported by Moongngarm and Saetung [2]. These grains were used as the control samples in the experiment.

2.2. Automatic Soaking and Germination Accelerator Unit (ASGA)

The details of the laboratory test unit used to accelerate the soaking and germination process in this research consisted of an ASGA made of stainless steel 304 with a 2 mm thickness (Figure 1). A spiral spray nozzle was installed on the inside of the canister lid to ensure an even distribution of water (Figure 2a). A 0.5 hp centrifugal pump was used to pump water from the reservoir to the cycle through the top of the rice container (Figure 2b), and an automatic electrical control was used to control and stop the water spraying (Figure 2c).

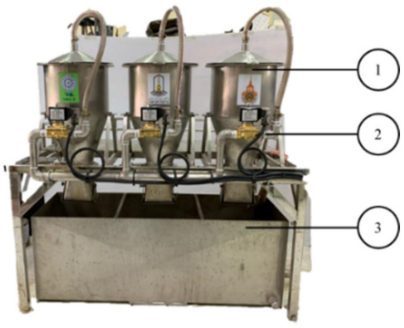

Figure 1. Component details of an automatic soaking and germination accelerator unit: (1) the paddy container has a tight lid, and the paddy capacity size is 1–1.5 kg; (2) solenoid valve for controlling the water spray; (3) reservoir for receiving water from the soaking and germination process for recycling in the system.



(**a**) (**b**) (**c**)

Figure 2. Spiral spray nozzle (**a**), centrifugal pump (**b**), and automatic control system unit (**c**).

The machine was operated to spray water continuously for 4 h to ensure the paddy absorbed sufficient water. The water spray cycle lasted for 60 min and spraying was stopped for 90 min; this cycle was repeated for 24 h to allow soaking and to prevent germination loss at the surface of the paddy from the germinated paddy production process [11] and cell damage due to an abundance of water. Moreover, the soaking and germination process occurred in a sealed container to induce stress conditions (oxygen depletion) and to stimulate root and endosperm growth [12,13].

2.3. Infrared Radiation Unit

The infrared radiation unit had a rectangular trough measuring 0.15 × 1 m, a horizontal angle of 11 degrees with a level of 0.01 m in every 0.1 m step, and a 0.5 HP vibrating motor was installed to ensure the paddy received consistent infrared radiation while being turned over. A near-infrared radiation generator equipped with tungsten incandescent lamps of 1000 W (Figure 3a) was installed at a distance of 0.2 m from the rails to allow effective radiation and heat transmission to the surface [14] (Figure 3b). The wavelength could be adjusted with a Stendal voltage controller, as shown in Figure 3c.



(**a**) (**b**) (**c**)

Figure 3. Infrared radiation unit (**a**), near-infrared radiation generator with tungsten (**b**), and voltage controller (**c**).

The infrared radiation treatment was investigated by following the method used by Vipattanaporn et al. [15]. The paddy that was stored in accordance with the quality inspection standard at different storage periods. The feed rate was constant at 20 kg·h^{-1}. The paddy was stimulated by infrared radiation. Three levels of infrared wavelengths were used in the experiment: 4.00, 3.57, and 3.52 µm. The temperatures at the surface of the infrared generator were 450, 500, and 550 °C, respectively. The infrared stimulation used in the study varied between the first, second, and third cycles, and each experiment was repeated three times. It was found that the storage period, infrared wavelength, and duty cycle of the radiation significantly affected the seed quality in terms of the germination rate at $p \leq 0.05$. For good performance, we recommend an infrared wavelength of 3.57 µm and a duty cycle of 2 cycles.

2.4. Measurements of Hydration and Physical Characteristics of GRR after Soaking Using the Conventional Method (CM) and Automatic Soaking and Germination Accelerator Unit (ASGA)

Each of the paddy samples was soaked using the CM with tap water at room temperature (30 ± 2 °C) and with soaking in the ASGA unit for 24 h. After soaking, the paddy samples were analyzed for 1000 grain weight, density, and moisture content [16].

2.5. Preparation of GRR by the CM and Stimulation with IRT

The experimental preparation of GRR samples, according to each storage period, occurred for 10, 11, or 12 months. Rough rice was divided into two samples measuring 1 kg each. Samples were stimulated with IRT at a wavelength of 3.57 µm for 2 cycles, with a grain surface temperature after stimulation of 49.43 ± 3 °C [15]. The control was not stimulated. Each sample was soaked in tap water at a ratio of 1:10 at room temperature for 24 h, and the water was changed every 4 h and drained at the end of soaking. The soaked rice grains were wrapped with cotton cloth to maintain moisture and left in a plastic box

(30 cm × 15 cm × 15 cm) with a lid. The germination took place in the chamber for 24 h at 30 ± 2 °C with 90–95% relative humidity, with grains totally soaked and germinated for 48 h. Soaking and germination by the CM could lead to hypoxia due to the limited oxygen availability [12] and could stimulate root and endosperm growth [13]. The germinated grains were steamed for 20 min to eliminate microorganisms that multiplied during soaking and germination. The samples were sun-dried to a ≤14% (wb) moisture content and stored at −20 °C for later analysis, following the method used by Moongngarm and Saetung [2] and Komatsuzaki et al. [17] with some modifications.

2.6. Preparation of GRR via the ASGA and Stimulation with IRT

The experimental preparation of GRR samples according to each storage period occurred for 10, 11, and 12 months. Rough rice was divided into two samples measuring 1 kg each. Samples were stimulated with IRT at a wavelength of 3.57 µm for 2 cycles, with a grain surface temperature after stimulation of 49.43 ± 3 °C [15]. The control was not stimulated. Each sample was soaked and germinated in the ASGA unit using tap water at a ratio of 1:10 at room temperature for 24 h. The soaked rice grains were germinated for 24 h. Soaking and germination by ASGA could lead to hypoxia during the stop-spraying phase. The germinated grains were steamed and were sun-dried to decrease the moisture content. Samples were stored for further analysis as indicated in the preparation using CM.

The germination rate was investigated by following the method used by Moongngarm and Saetung [2] and Jiamyangyuen and Ooraikul [18] with some modifications. The paddy was considered a germinated seed when the primary root or young radicle (white root emerging from the germ of the rice seed) was visible. Approximately 300 seeds from those that germinated were sampled, and these germinated seeds were counted. The results of three replicates were calculated as the percentage of germinated seeds to total paddies used.

2.7. Determination of γ-Aminobutyric Acid (GABA) in GRR

The GABA content was determined via the method used by Moongngarm and Saetung [2] and Varanyanond et al. [19] with slight modifications. Finely ground GRR samples (2–5 g) were weighed in a plastic tube and 1.8–2.0 mL deionized water was added. The slurries were shaken at room temperature for 1.5 h. Subsequently, 200 mL 3% (by volume) sulfosalicylic acid was added and the mixtures were centrifuged at 4500 rpm for 10 min. To the supernatant (50 mL), 50 mL 100 mM $NaHCO_3$ and 50 mL 4 mM 4-dimethylaminoazobenzene-4-sulfonyl chloride acetonitrile solutions were added. The mixtures were heated to 70 °C for 10 min to cause derivatization. Then, 250 mL absolute ethanol and 250 mL 25 mM phosphate buffer were added (pH 6.8). The samples were filtered and 5 mL of the filtrate was injected into the Agilent HPLC instrument (1200 Series, Osaka, Japan). For the standard γ-aminobutyric acid (Sigma-Aldrich, purchased from Union science trading co., ltd., Muang, Khonkaen), solution (0.1–0.3 mL) was added to test tubes (18 × 120 mm) together with 0.2 mL of borate buffer and 1.0 mL of phenol reagent. The GABA content was quantified by comparing the optical density reading with the standard GABA content curve ($y = 0.049 + 10.14x$), and the results were expressed in mg GABA/100 g d.w.

2.8. Experimental Design and Statical Analyses

All experiments used a general full factorial design, and the independent variables studied were the storage period (10, 11, and 12 months), method of GRR preparation (CM and ASGA), and stimulation with IRT (N-S and S). Three freshly prepared germinated samples and three replicates of each sample for each storage period were analyzed, for a total of 36 samples. The results were statistically analyzed using analysis of variance (ANOVA). Means were compared using least significant difference tests (LSD) with the mean square error at 5% probability. Differences between the control samples and GRR

were assessed by paired *t*-test with a significance level of 0.05. The following second-order polynomial equation was used to express responses as a function of independent variables:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{2} \beta_{ij} X_i X_j$$

where Y represents the response variable to be modelled, β_0 is a constant, β_i is the linear term coefficient, β_{ii} is the quadratic term coefficient, β_{ij} is the interaction term coefficient, and X_i and X_j are the independent variables.

The goodness-of-fit of the methods obtained was evaluated by calculating the multiple determination coefficients (R^2) and using ANOVA of the regression coefficient of the fitted polynomial equations for each response variable, where the *p* values indicated whether the terms were significant.

3. Results

3.1. Hydration and Physical Characteristics of GRR after Soaking via CM and ASGA

Rough rice of KDML 105 exhibited different water uptake behaviors during imbibition. At the early stage of soaking, the water uptake rapidly increased due to absorption into the embryo [11,20]. Subsequently, rice grains absorbed water slowly and came to equilibrium. During this stage, water diffused slowly into the endosperm of the grain. After soaking using the CM and ASGA methods for 24 h, the 1000 grain weights were 27.60 and 25.06%, respectively; the density values were 6.14 and 4.03%, respectively; and the moisture contents were 66.98 and 66.01%, respectively, higher than the non-soaked rough rice paddy (control) (Table 1). Normally, GRR containing 30–35% moisture promotes mold and microbial growth [17,21]. These results agree with research results that reported the moisture content of Indica rice var. Khao Dawk Mali 105 after 24 h.

Table 1. Comparison of hydration and physical characteristics of non-soaked 'Khao Dawk Mali 105' in raw rough rice (control) and soaking using the conventional method (CM) and an automatic soaking and germination accelerator unit (ASGA).

Parameter	Control	CM	ASGA
1000 grain weight (g)	24.82 ± 0.1 [b]	34.28 ± 0.5 [a]	33.12 ± 0.7 [a]
Density (kg/m^3)	537.66 ± 7.9 [b]	572.83 ± 10.4 [a]	560.22 ± 12.0 [a]
Moisture content (%wb)	12.42 ± 0.2 [b]	37.61 ± 0.5 [a]	36.54 ± 0.1 [a]

Means within rows followed by the same letter are not significantly different at $p < 0.05$.

3.2. Germination Rate

The germination conditions of the rough rice with the CM and ASGA unit followed the stimulation with IRT, as reported by Vipattanaporn, Laohavanich, Chiawchanwattana, Khaengkan, and Yangyuen [15]. The germination rate of the rough rice germinated by CM and ASGA with stimulation with IRT at each storage period was significantly higher than not-stimulated rice, by 6.56 and 8.11%, respectively (Table 2).

From Table 3, the regression model obtained for the germination rate showed that the storage period, method, and IRT had a highly significant effect on the germination rate at a confidence level of 0.01. This result is consistent with other studies by Maisont et al. [22], Roohinejad et al. [23], and Prakhethanang et al. [24] explaining that the increased storage time had an effect on seed quality in terms of reduced germination rates due to seed deterioration. A correlation graph was constructed to show the storage period, method, and IRT that affected the levels of germination (Figure 4). As the storage period increased, the germination rate decreased.

Table 2. Comparison of the germination rates for the conventional method (CM) and an automatic soaking and germination accelerator unit (ASGA) with no stimulation and stimulation via infrared radiation treatment (IRT) at each storage period.

Method	IRT	Germination Rate (%)					
		10 Months		11 Months		12 Months	
		24 h	48 h	24 h	48 h	24 h	48 h
CM	N-S	n/a	77.67 ± 2.1 ^{B}	n/a	81.00 ± 2.0 ^{B}	n/a	65.33 ± 3.1 ^{CD}
	S	n/a	86.67 ± 2.5 ^{A}	n/a	87.33 ± 3.2 ^{A}	n/a	69.67 ± 4.7 ^{C}
ASGA	N-S	53.33 ± 1.5 ^{E}	n/a	44.00 ± 2.7 ^{F}	n/a	39.00 ± 2.0 ^{G}	n/a
	S	62.00 ± 2.7 ^{D}	n/a	52.00 ± 2.0 ^{E}	n/a	46.67 ± 2.1 ^{F}	n/a

Data are the means ± standard deviations of three replicates; within columns, values followed by the same letter are not significantly different at $p < 0.05$; CM = conventional method; ASGA = automatic soaking and germination accelerator unit; IRT = infrared radiation treatment; N-S = no stimulation; S = stimulation; n/a = not available.

Table 3. Statistical analysis with ANOVA for the effects of the storage period (months), soaking method, and infrared radiation treatment (IRT) on the germination rate.

Indicators	df	MS	F-Value
Storage period	2	702.86	98.84 **
Method	1	7281.78	1024.00 **
IRT	1	484.00	68.06 **

** significant at $p \leq 0.01$.

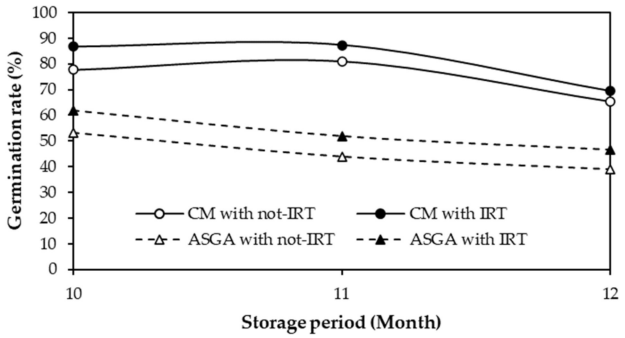

Figure 4. Correlation between storage period and germination rate in germinated rough rice (GRR) using the conventional method (CM) with stimulation and with no stimulation via infrared radiation treatment (IRT).

3.3. The Influence of Germination Conditions on GABA Content of GRR

The GABA contents of GRR germinated using CM and ASGA and stimulated with IRT were significantly higher than UGR in each storage period (19.52 and 21.24% higher at 10 months, respectively; 16.36 and 23.58% higher at 11 months, respectively; 69.88 and 67.69% higher at 12 months, respectively; Table 4).

The regression model obtained for GABA contents in GRR was not significant for the studied methods. The storage period and IRT had significant effects on the GABA content at confidence levels of 0.01 and 0.05, respectively (Table 5).

Table 4. Comparison of GABA contents of germinated rough rice samples soaked using the conventional method (CM) and an automatic soaking and germination accelerator unit (ASGA) with and without stimulation via infrared radiation treatment (IRT) and ungerminated rice (control) in each storage period.

Method	IRT	GABA Contents (mg GABA/100 g d.w)		
		10 Months	11 Months	12 Months
CM	N-S	12.85 ± 2.3^{ABCD}	4.63 ± 2.0^{G}	8.35 ± 1.8^{EF}
	S	15.11 ± 1.6^{AB}	12.59 ± 0.6^{ABCD}	14.71 ± 1.1^{AB}
ASGA	N-S	11.59 ± 2.4^{CD}	6.88 ± 0.2^{FG}	8.61 ± 1.5^{EF}
	S	15.44 ± 2.2^{A}	13.78 ± 1.6^{ABC}	13.71 ± 3.0^{ABC}
Control		12.24 ± 1.8^{BCD}	10.53 ± 2.0^{DE}	4.43 ± 0.2^{G}

Data are the means ± standard deviations of three replicates; within columns, values followed by the same letter are not significantly different at $p < 0.05$; CM = conventional method; ASGA = automatic soaking and germination accelerator unit; IRT = infrared radiation treatment; N-S = no stimulation; S = stimulation; n/a = not available.

Table 5. ANOVA of the effects of the storage period (months), method, and infrared radiation treatment (IRT) on GABA content.

Indicators	df	MS	F-Value
Storage period	2	80.58	19.83 **
Method	1	1.57	0.39^{ns}
IRT	1	7.20	1.77 *

* Significant at $p \leq 0.05$; ** highly significant at $p \leq 0.01$; ns = non-significant.

This result is consistent with other research by Cho and Lim [6], Sasathorn and Gi-Hyung [25], who explained that the increase in GABA content occurs when the cereal germinates. GABA has multiple health benefits, such as blood pressure regulation, stress control [26], plasma cholesterol level reduction [27], and carcinogenesis inhibition [28] effects. Therefore, the production of GABA-enriched cereals has been actively pursued. However, as far as we know, no research has been performed on increasing the nutritional value, especially the GABA content, in GRR (var. Khao Dawk Mali 105) using the CM and ASGA together with stimulation with IRT. A change graph was constructed to show the storage period, method, and IRT changes that affected the GABA content (Figure 5). As the storage period increased, the germination rate decreased.

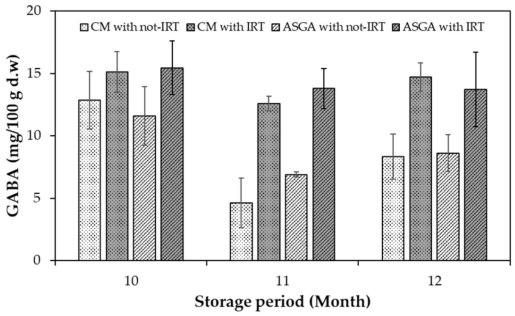

Figure 5. Changes in storage period and GABA content in germinated rough rice (GRR) using the conventional method (CM) and an automatic soaking and germination accelerator unit (ASGA) with and without stimulation via infrared radiation treatment (IRT).

4. Discussion

Correlations of the germination rates between the factors and the responses were highly negative. When the storage period increased, the germination rate decreased accordingly. Although longer storage periods had a greater impact on germination rate, IRT

also improved the germination rates. These results agree with research results reporting that the germination rate of KDML 105 was increased by IRT [15]. IRT has been used in other studies to increase the temperature, to cause germination, and to generate stress. Heat stress has been shown to activate heat shock proteins to improve the quality of various aspects of broccoli sprouts and wheat seedlings [29]. These studies agreed with the generated quadratic polynomial predictive models for high germination rates in GRR for each germination condition stimulated with IRT, as seen below in Equations (1) and (2). An R^2 of 0.95 was obtained, and in general only values of R^2 higher than 0.75 indicate a good fit [30].

$$\text{Germination rate } (\text{CM} - \text{IRT}) = -251 + 69.4\text{SP} - 3.54\text{SP} * \text{SP} \quad (1)$$

$$\text{Germination rate } (\text{ASGA} - \text{IRT}) = -288 + 70.2\text{SP} - 3.54\text{SP} * \text{SP} \quad (2)$$

During soaking and germination process using both methods (CM and ASGA), the soaking loss process indicates the volume of particles leaching out of the rice grains. Soaking loss was mainly due to three factors: displacement of residual dust, leaching of soluble materials, and the metabolic activity of the grain releasing CO_2 and small amounts of ethanol [11]. In addition, the microorganisms greatly multiplied [31], affecting the quality of the GRR and leading to an odor defect. From the preliminary observations in this research, the odor defect of GRR was found when processing using the CM more than processing when using the ASGA method, which involved automatic soaking and germination loss management and spraying cycles every 60 min to wash away microorganisms from the surfaces of the paddies.

Similar results were reported for Indica rice var. KDML 105 [32,33]. The GABA content shows a high correlation with the germination rate in cereals such as wheat, barley, and rice [6,25,34,35], due to the activation of enzymes involved in GABA (e.g., glutamate decarboxylase) and glutamate synthesis [6,36]. However, knowledge on the effects of new techniques on the germination and GABA levels of rice var. Khao Dawk Mali 105 is still limited. The differences in GABA content between both preparation methods can be attributed to the different concentrations in different germination conditions.

From the results regarding the changes between the factors and responses, stimulation using IRT improved the germination rates; as a result, the GABA content increased during germination due to the activation of enzymes involved in GABA synthesis. This result is consistent with the results found by Cho and Lim [6]. The generated quadratic polynomial predictive models showed a high germination rate in GRR for each germination condition stimulated with IRT, as seen in Equations (3) and (4). The R^2 was 0.78.

$$\text{GABA content } (\text{CM} - \text{IRT}) = -386.4 + 67.9\text{SP} - 3.076\text{SP} * \text{SP} \quad (3)$$

$$\text{GABA content } (\text{ASGA} - \text{IRT}) = -393.9 + 68.5\text{SP} - 3.076\text{SP} * \text{SP} \quad (4)$$

There is no information in the literature on the effects of both methods using appropriate stimulation with IRT on the GABA content in rice (cultivar KDML 105). To our knowledge, this is the first study showing a detailed description of the germination rate and GABA content profiles of GRR, demonstrating that the ASGA method and stimulation with IRT are feasible options to be further developed for use to increase the nutritional value of cereals.

5. Conclusions

GRR is a good source of GABA, and perhaps other nutrients and bioactive compounds that were not determined in this study. It is already well recognized that the germination rate and GABA content in GRR are inversely proportional to the storage period due to deterioration. The germination method for GRR did not have a significant effect on the GABA content. ASGA did not cause an unpleasant smell in GRR and could reduce the germination process time in the paddy to only 24 h compared to germination using the CM. Stimulation with IRT improved the germination rate and GABA content, even after

deterioration resulting from the storage period. The results suggest that ASGA–IRT could be used to maintain both the quantity and quality during rice processing. This is a useful opportunity for the food and seed industry, and further development of this knowledge would benefit the development of other cereals.

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