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γ-Aminobutyric acid (GABA) content in different varieties of brown rice during germination

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ABSTRACT: Rice grains rich in gamma-aminobutyric acid (GABA) are increasing in popularity, particularly in the health food market. GABA levels in rice are influenced by many factors, including the duration of incubation of seeds, particularly in pre-germinated brown rice grains. In this report, five different incubation durations (0, 12, 24, 36, and 48 h) and 21 rice varieties (11 landraces purple rice and 10 modern white varieties) were tested. Results show that GABA content increased steadily from 3.96 mg/100 g dry matter at 0 h duration (i.e., no incubation period) to 10.04 mg/100 g dry matter after 12 h, reaching the highest levels of 17.87 mg/100 g dry matter at 24 h incubation, and then decreased continuously afterwards to 9.91 and 1.36 mg/100 g dry matter at 36 and 48 h, respectively. A correlation of GABA levels at 0 h and 24 h was detected ($r = 0.48$). Genotypic variation was detected from a minimum of 6.50 to a maximum of 10.10 mg/100 g dry matter, with a mean of 8.03. At 24 h, the white rice variety KDML 105 and the purple rice variety Kum Doi Saket (23.48 and 23.63 mg/100 g dry matter, respectively) exhibited the highest GABA content of all 21 rice varieties. This indicates the importance of the local purple rice cultivars for adding nutritional value to functional food products.

KEYWORDS: incubation duration, modern rice variety, landrace upland rice variety, purple rice varieties

INTRODUCTION

Rice grain quality denotes different properties to different groups in post-harvest systems[1]. Surprisingly, in this small grain, many important nutrients are present that have potential functional food benefits. Carbohydrates represent the majority of the nutrient found in white polished grain, but proteins, vitamins B and C, and many minerals are present in the embryo, bran (pericarp) and husk. Furthermore, fat extracted from the bran contains vitamin E, phytosterol, and γ-oryzanol, an antioxidant used in many alternative herbal therapies, inhibitor of gastric acid secretion, antioxidant, and inhibitor of platelet aggregation preventing heart attacks[2]. In addition, during malting, as biochemical processes take place, carbohydrates are converted to oligosaccharides and reducing sugars. The digestion of grain protein results in amino acids, peptides, and also in an accumulation of many nutrients such as gamma oryzanol, tocopherol, tocotrienol, and γ-aminobutyric acid (GABA)[3–6].

GABA is a four carbon non-protein amino acid, produced primarily by the decarboxylation of L-glutamic acid, catalysed by the enzyme glutamate decarboxylase during the germination process of brown rice (BR)[7]. Soaking BR for 72 h in 30 °C water, at moisture content of 13–15%, increased the GABA content of pre-germinated brown rice from 6.0 mg in ordinary BR before germination up to 69.2 mg and to 149.0 mg at 96 h, 40 times higher than in polished rice (1.7 mg)[4]. The content was higher for 21 h gaseous treatment at 35 °C (24.9 mg/100 g) than for the conventional soaking method (10.1 mg/100 g)[8]. GABA is a neurotransmitter in the brain and spinal cord of mammals, and induces hypotensive, diuretic, and tranquillizing effects[8,9]. Furthermore, GBR extracts containing GABA are also used as medication to ameliorate blood flow in the brain[10], to inhibit cancer-cell proliferation[11–13], and other beneficial health effects. The total content of insoluble phenolic compounds increased from 18.47 mg/100 g of flour in BR to 24.78 mg/100 g of flour in GBR[14].

GABA levels in GBR are influenced by many factors, including the duration of incubation of seeds. Genetic differences among rice variety in their ability to synthesize GABA in grains are also worth evaluating, as genetic diversity is a basic prerequisite for successful exploitation of desirable traits through

Table 1 Rice varieties used in the experiment.

Modern non-glutinous rice varieties	Modern glutinous rice varieties	Landrace upland rice varieties	Landrace purple glutinous rice varieties
KDML 105	RD10	Bue Ki	Kum Nan
(Pathum Thani 1)	Niaw Sanpatong	La Sor Dang	Kum Phayao
Chai Nat 1	Muey Nawng 62 M	Bue pa dao	Kum Doi Saket
Suphan Buri 90	Taichung	Ni Kor	Kum Vietnam
Phitsanulok 2		Ja Nor Na	Kum Doi Musur
RD23			Kum 88 082

Note: Variety names of Landrace and Purple rice varieties are given their local names

breeding[15]. In this report, five different incubation durations (0, 12, 24, 36, and 48 h) and 21 rice varieties (11 landraces and 10 modern varieties; *Oryza sativa* L.) were tested. A factorial design with 4 replicates was applied: brown rice grains of 4 types of rice varieties (Table 1) were main plots, and incubation durations were subplots. The experiment was conducted at the Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University, Thailand.

MATERIALS AND METHODS

Rice grain samples (50 mg) were soaked for 24 h at room temperature and placed in an incubator for the specified durations. The germinated grains were dried in a hot-air oven at 100 °C for 30 min. At 13–14% moisture content[16], the grains were then threshed as germinated brown rice. The germinated brown rice samples were ground before extracting GABA using the method of Kitaoka and Nakano[17]. GABA content was quantified by spectrophotometry.

Quantification of GABA content

Each of the ground rice samples (3 mg) was dissolved with 80% ethanol in a test tube (18 × 120 mm), shaken thoroughly, and then filtered with filter paper (no. 1). The filtered solution was boiled in a water bath (80 °C) to evaporate the ethanol. This was followed by addition of 0.5 ml distilled water, and then centrifugation at 10 000 rpm for 10 min. The floating portion on top was aspirated, and 0.2 ml of 0.2 M borate buffer and 1.0 ml of 6% phenol were added. For the standard GABA 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 solutions were mixed thoroughly and cooled in a cooling bath for 5 min. Next, 0.4 ml of 10–15% NaOCl was added, and the solution was shaken vigorously for 1 min, and again cooled in a cooling bath for 5 min. Finally, the solution was boiled in a water bath (100 °C) for 10 min, and allowed to cool. Optical density was determined by spectrophotometry at a wavelength of 630 nm, with ethanol 2.0 ml as a blank. GABA content was quantified by comparing the optical density reading with the standard GABA content curve ($y = 0.049 + 10.14\,x$).

RESULTS

Quantification of GABA content of samples

The results (Table 2) show that GABA content was detected, even at 0 h duration (i.e., no incubation period), with a mean of 3.96 mg/100 g dry matter for all varieties. Mean GABA content increased steadily to 10.04 mg/100 g dry matter after 12 h, reaching the highest levels of 17.87 mg/100 g dry matter at 24 h incubation, and then decreased continuously afterwards to 9.91 and 1.36 mg/100 g dry matter at 36 and 48 h, respectively. This indicates that 24 h incubation was optimal for producing GABA rice. In six varieties the GABA content exceeded 20.0 mg/100 g dry matter at 24 h; three were modern varieties (KDML 105, Phitsanulok 2, RD10), two were purple rice (Kum Nan and Kum Doi Saket), and the other was a landrace upland rice (Ni Kor) (Fig. 1). Moreover, genetic differences in GABA content among varieties were detected: 6.50 mg/100 g dry matter to 10.10 mg/100 g dry matter (mean value of all incubation times). Modern varieties (KDML 105, RD10 and Niaw Sanpatong) along with purple rice varieties (Kum Doi Saket and Kum 88 082) showed the highest GABA content. However, rice varieties responded differently to the incubation period. For example, at 12 h incubation Chai Nat 1 had GABA content equal to that of KDML 105, however at 24 h they were different (Table 2).

The correlation coefficient $r = 0.48$ ($p \leqslant 0.05$) of GABA levels at 0 h and 24 h was positive indicating a genotype with a high GABA level at 0 h could be expected to be the genotype synthesized higher GABA at 24 h incubation (Fig. 1).

In comparing the four major rice types (Table 3), at 0 h (no incubation period) GABA levels were not significantly different: 3.96–4.29 mg/100 g dry matter. But differences appeared once the grains were incubated. Modern varieties rapidly synthesized the nutrient. At 12 h, GABA levels were higher in modern varieties (10.64 and 11.72 mg/100 g dry matter) than in landrace and purple rice varieties (8.90 and 9.28 mg/100 g dry matter). However, the levels were equal with incubation duration of 24 h, and remained very low at 48 h.

Table 2 GABA content (mg/100 g dry matter) in unpolished rice seeds at five germination durations.

Type of rice	Variety	GABA content at various incubation times (hours)					Mean
		0	12	24	36	48	
Modern	KDML 105	4.04	14.6	23.48	6.2	2.16	10.10^{a*}
non-glutinous	Pathum Thani 1	3.62	7.55	16.22	4.46	0.68	6.51^{i}
varieties	Chai Nat 1	3.35	13.4	15.45	8.13	2.43	8.55^{de}
	Suphan Buri 90	5.51	10.21	19.07	3.75	0.98	7.90^{fg}
	Phitsanulok 2	5.88	10.9	21.16	6.39	0.99	9.06^{cd}
	RD23	3.32	7.17	17.01	5.3	2.51	7.06^{hi}
Modern	RD10	4.56	14.76	20.36	7.15	1.51	9.67^{ab}
glutinous varieties	Niaw Sanpatong	3.75	12.99	19.29	9.7	1.92	9.53^{abc}
	Muey Nawng 62 M	3.96	10.18	19.17	6.62	1.14	8.21^{ef}
	Taichung	3.55	8.95	17.45	3.59	0.81	6.87^{hi}
Landrace	Bue Ki	4.34	7.64	19.88	6.52	1.66	8.00^{ef}
upland varieties	La Sor Dang	4.84	8.36	13.65	6.73	1.83	7.10^{hi}
	Bue Pa Dao	3.31	8.32	19.2	6.66	1.12	7.72^{fg}
	Ni Kor	5.1	9.57	20.27	4.32	1.71	8.19^{ef}
	Ja Nor Na	3.98	10.45	19.88	3.64	1.19	7.82^{fg}
Landrace	Kum Nan	5.43	8.18	21.65	3.51	0.9	7.94^{fg}
purple glutinous	Kum Phayao	3.	11.01	17.4	4.27	1.4	7.41^{gh}
rice varieties	Kum Doi Saket	4.67	10.19	23.63	5.68	1.3	9.09^{bcd}
	Kum Vietnam	3.08	8.12	14.38	5.75	1.4	6.55^{i}
	Kum Doi Musur	3.8	5.83	15.77	6.42	0.68	6.50^{i}
	Kum 88 082	4.12	12.37	18.43	9.25	2.23	8.83^{bc}
Mean		3.96^{D}	10.04^{B}	17.87^{A}	5.91^{C}	1.36^{E}	8.03
		F-test	LSD0.05				
Varieties		**	0.59				
Incubation durations		**	0.36				
Varieties × Incubation durations		**	1.35				
CV (%) 13.37							

* Means followed by different letters differ significantly by $LSD_{0.05}$.

Table 3 Comparison of GABA content (mg/100 g dry matter) between four types of rice varieties.

Type of rice	GABA content at various incubation times (hours)					Mean
	0	12	24	36	48	
Modern non-glutinous	4.29	10.64	18.73	5.71	1.63	8.2
Modern glutinous	3.96	11.72	19.07	6.77	1.35	8.57
Landrace upland	4.31	8.9	18.58	5.57	1.5	7.77
Landrace purple glutinous rice	4.02	9.28	18.54	5.81	1.32	7.72

DISCUSSION

The initial GABA content detected at 0 h duration (i.e., no incubation period) could be gamma amino acid which consists mainly of globulin and a minor portion of albumin[18]. As grains are being soaked, imbibition begins, and within 6–12 h, respiration is accelerated, which further stimulates the metabolism of amino acids, resulting in the formation of enzyme systems. Amino acid such as GABA is also synthesized, and its accelerated increase can be detected at 12 h incubation. The acceleration is still rapid from 12–24 h, when GABA content reaches its highest levels and shows a peak at 24 h incubation. This was slightly different from a study with *japonica* rice[8], in which the highest GABA levels were detected at 72 h. The GABA content decreased once the incubation durations reached 36 and 48 h, implying that 24 h incubation is the optimal duration for processing germinated brown rice (GABA rice or GBR). The significant interaction of genotype and incubation period clearly indicates that, if any duration other than 24 h was used for incubating brown rice in processing for GBR, the specific favourable rice variety should be taken into consideration.

The similarity in GABA levels at 24 h duration in comparing modern rice varieties to landrace and purple rice varieties showed the significant usefulness of the unimproved local rice varieties in processing GBR. In particular, a purple rice variety, Kum Doi Saket, not only accumulated high GABA content but also possessed the purple pigment of anthocyanidin (cyanidin-3-glucoside and peonidin-3-glucoside). The content of the pigment is significantly correlated to the total antioxidant activity of black rice bran, and possess 6–8 times higher activity than white rice

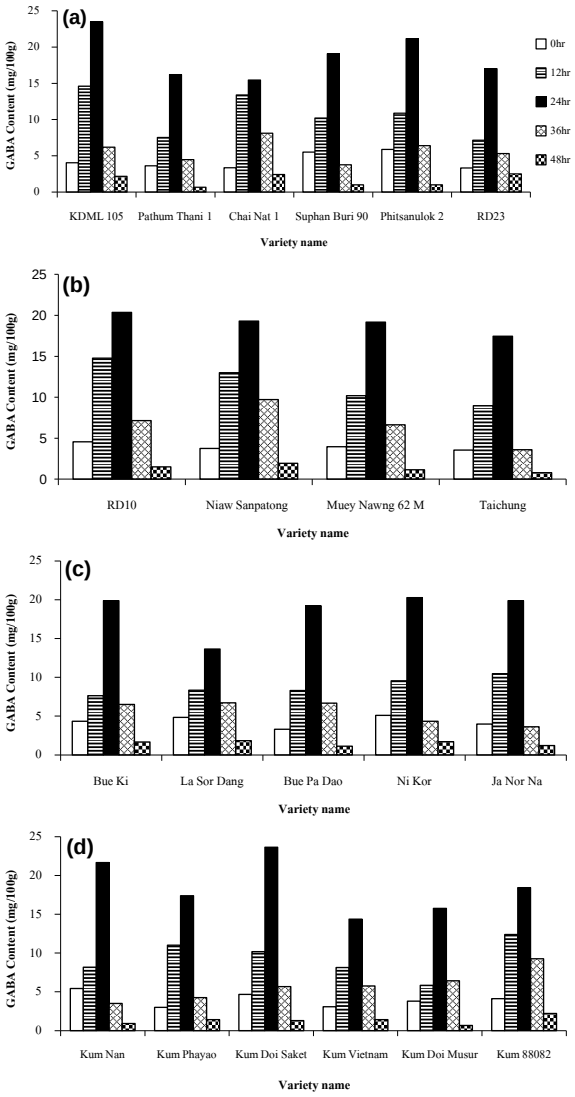

Fig. 1 GABA content (mg/100 g dry matter) in germinated grains of (a) modern non-glutinous rice, (b) glutinous modern rice, (c) landrace upland rice, and (d) purple rice varieties at five incubation durations.

bran[19]. This indicates the importance of the local rice cultivars for adding nutritional value to functional food products.

The different amounts of GABA content found among the rice varieties are mainly caused by their genetic constitution. The variety KDML 105 also has the ability to synthesize GABA, and differences in GABA accumulation do occur among the Thai rice varieties[20]. Inheritance for this trait is rather complex. The variation we found in the landrace rice was within the variation found in the modern rice, signifying that genetic variation existed in nature, as evaluation of genetic diversity is a basic prerequisite for successful exploitation of desirable traits through breeding[15]. The results presented in this paper, therefore, show opportunities in breeding for GABA accumulation and KDML 105, RD10, Niaw Sanpatong and Kum Doi Saket (purple glutinous rice), could be used as parent varieties for introducing the trait to current *indica* cultivars grown in Asia.

The positive correlation found between GABA contents at 0 h and at 24 h incubation durations signified that GABA levels at 0 h could predict the GABA levels at 24 h. This could be profitable for a rice breeding program for enhancing GABA and also be of advantageous for optimizing the selection criteria. As GABA is a compound of bio-functionality, this could become a promising method of developing a GABA-rich food product, while also enhancing GBR consumption in Thailand as well as in all others rice-consuming countries.

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