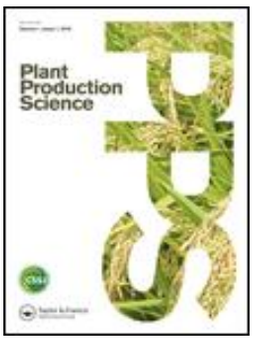

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Distribution of γ-oryzanol in the outer layers of brown rice and its variation among cultivars

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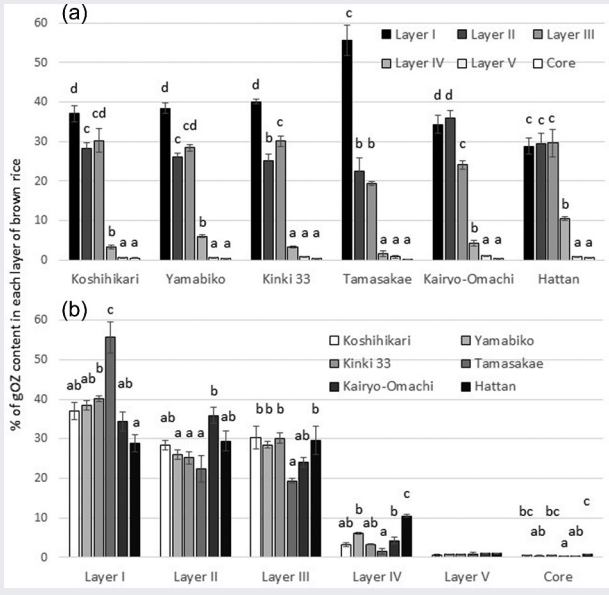
ABSTRACT

Rice (*Oryza sativa* L.) bran accumulates many compounds that are associated with the promotion and maintenance of human health, such as γ-oryzanol (gOZ) and γ-aminobutyric acid (GABA). These beneficial compounds in the bran fraction are mostly removed in polished rice. The present study sought rice cultivars with high concentrations of gOZ, and also of GABA, in polished rice, and examined the distribution of gOZ content in the outer layers of brown rice. The effects of germination treatment were also examined as a means to enrich gOZ and GABA in polished rice. GABA concentration showed no significant variation among 19 cultivars in polished rice, whereas a wide variation was detected in brown rice, for which cultivar Kinuhikari had the highest amount. Germination treatment for 5 h significantly enhanced GABA concentration. gOZ concentrations also showed a wide variation among cultivars in both brown rice and polished rice, with no clear correlation. Hattan, a sake-brewing cultivar, exhibited the highest gOZ concentration in polished rice. This cultivar involved higher gOZ in the outermost layer of polished rice, whereas it had a lower content in the bran fraction. This permeation of gOZ into polished rice might be related to the endosperm properties characteristic to sake-brewing cultivars. In contrast to GABA, gOZ concentration did not increase with germination treatment in brown rice or polished rice. Because the gOZ concentration in polished rice was insufficient even in the Hattan cultivar, breeding and/or other processing techniques are needed to obtain gOZ-enriched polished rice.



Percentages of γ-oryzanol (gOZ) content in different layers and the core of brown rice. (a) Comparison among layers and the core in each of six rice cultivars, and (b) comparison among six cultivars in each of the layers and the core. Means with the same letter did not differ significantly at the 0.01 probability level, within each cultivar (a) and within each layer or the core (b). See text for layers and core.

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Introduction

In rice (*Oryza sativa* L.) bran, including the pericarp, integument, aleurone layer, and germ, many compounds with pronounced benefits for the promotion and maintenance of human health are accumulated (Cho & Lim, 2016; Kato et al., 2017; Zarei et al., 2017; Zhou et al., 2020). These compounds show antioxidation (Watanabe et al., 2016), cholesterol-lowering, anti-tumor (Qureshi et al., 2000) and anti-inflammatory activities (Bramley et al., 2000), and other effects (Bergman & Xu, 2003; Watanabe et al., 2016). They include vitamin E derivatives (tocotrienols and tocopherols) (Sookwong et al., 2007), γ-aminobutyric acid (GABA) (Ng et al., 2013), γ-oryzanol (gOZ) (Heineman et al., 2008; Ng et al., 2013), and various forms of dietary fiber (Abdul-Hamid & Luan, 2000). In addition, there are apparent variations in the contents of these compounds among rice cultivars (Bergman & Xu, 2003; Heineman et al., 2008; Huang & Ng, 2011; Kato et al., 2017; Ng et al., 2013). Several quantitative trait loci have been identified for the contents of gOZ (Kato et al., 2017; Nakano et al., 2018) and vitamin E derivatives (Zhang et al., 2019).

Many studies that examined these contents predominantly did so in brown rice. However, the bran is usually removed by polishing or milling to be acceptable for usual consumption. Consequently, these beneficial compounds are lost in this polishing step. Lloyd et al. (2000) reported that gOZ content in processed rice decreased dramatically with polishing of brown rice from 100% of total lipid basis (brown rice) to 10.8%. In addition, gOZ in the most polished rice in their experiment retained about 23% of unpolished brown rice in long-grain cultivars, whereas it was not detected in a medium-grain cultivar. Schramm et al. (2007) also examined gOZ contents in different fractions after polishing brown rice for different periods (from about 97% to 87% of the weight of unpolished brown rice), using two cultivars. They showed that gOZ contents decreased gradually with increasing polishing period, although vitamin E content did not show a significant change. These results showed that gOZ mostly concentrates on the outer layers of brown rice. In wheat (*Triticum aestivum* L.) grains, gOZ is also rich in bran fraction and rare in flour (Tsuzuki et al., 2017).

Sufficient quantitative research has not yet been conducted on the contents of gOZ and other compounds, such as GABA, in polished rice. If genotypes with higher contents of these beneficial compounds, and/or some processing techniques to retain their higher contents in polished rice are developed, these beneficial compounds might be obtained through a usual daily diet of polished rice. For example, GABA is already known to be enriched by germination processing in both brown rice and polished rice (Cho & Lim, 2016; Komatsuzaki et al., 2007). In addition to direct intake as a food, if processed materials made from rice, such as rice flour and rice confectionary, include higher amounts of these beneficial compounds, additional value can be attributed to these products.

The present study examined the distribution of gOZ, a water-insoluble compound, in the outer layers of brown rice, particularly in polished rice, and evaluated variation among rice cultivars. The effect of germination (pre-germination) treatment especially on gOZ concentrations in brown rice and polished rice was also examined to explore new techniques to retain or enrich beneficial compounds in polished rice. GABA, a water-soluble compound, was also examined as a reference for the case of gOZ.

Materials and methods

Plant materials

First, the gOZ and GABA contents (concentrations) in brown rice (B) and polished rice (P) without germination treatment (N), and with germination treatment (G) were evaluated using 19 *japonica*-type rice cultivars, Akihikari, Akinishiki, Gohyakumangoku, Hattan (Hattan 35), Kairyo-Omachi, Kinki 33, Kinuhikari, Koganemasari, Koshihikari, Manryo, Norin 1, Norin 6, Norin 8, Norin 22, Rikuu 132, Tamasakae, Todorokiwase, Yamabiko, and Yamadanishiki in the 2017 growing season. Cultivars of *indica*-type were not used, because they are generally easy to be cracked during polishing. In 2018, six of these cultivars, Hattan, Kairyo-Omachi, Kinki 33, Koshihikari, Tamasakae, and Yamabiko, were evaluated in more detail on the distribution of gOZ in the outer layers of brown rice in N condition only. Of the 19 cultivars, Gohyakumangoku, Hattan, Kairyo-Omachi, Tamasakae, and Yamadanishiki are cultivars for sake brewing, which have several different endosperm properties, compared with the other ordinary cultivars.

The tested cultivars were seeded in nursery boxes in the middle of May, transplanted in the middle of June into a paddy field of the Faculty of Biology-Oriented Science and Technology, Kindai University, Kinokawa, Wakayama, Japan (34° 17′ N, 135° 20′ E, 97 m above sea level). Each field plot for a cultivar consisted of three rows (30 cm inter-row) and 12 hills (15 cm inter-hill) per row, with single plant per hill. Fertilizers were applied as basal dressing at the rate of 6:6:6 g m^{-2} for $N:P_2O_5:K_2O$. Ordinary cultivation practices were conducted for pest control, irrigation, etc., as those of this region.

Sample preparation

These materials were harvested at 40–50 days after heading, dried, threshed, and hulled. Part of the obtained brown rice was subjected to germination treatment using a milling machine 'Magic Mill' (RSKM3D, Satake Corporation, Higashi-Hiroshima, Japan), in which brown rice was incubated in humid condition for 5 h. In 2017, brown rice, from N and G conditions, were polished to obtain standard polished rice using a milling machine 'Twinbird' (MR-E520W, Twinbird Co. Ltd., Tsubame, Niigata, Japan). In 2018, the milling processes in various polishing periods were conducted using a compact grain polisher 'Pearlest' (TP-3000, Kett Electric Laboratory, Tokyo, Japan) with a 'Pearlest timer' (TZ-910, Kett Electric Laboratory, Tokyo, Japan). The polishing periods were set at 0 (brown rice), 7, 15, 30 (standard polished rice), 60, and 120 sec. These materials were weighed before and after polishing to obtain the polishing rate (%, the ratio of material weight after polishing to that before polishing). After polishing, the materials were immediately rinsed very briefly with diethyl ether to remove any residue on the surface of the polished rice. These polished and brown rice were crushed with a mixer mill (MM 400, Verder Scientific Co., Ltd., Tokyo, Japan), at the frequency of 25 for 2.0 min to make fine powder.

Extraction and determination of gOZ and GABA concentrations

After weighed precisely around 250 mg, the powder for gOZ determination was immersed in a 1.5 mL test tube with 1.0 mL methanol per sample, mixed vigorously, and stored under 4°C for 24 h. This procedure was repeated three times as replicates. After centrifugation at 850 × *g*, for 10 min at room temperature, the supernatant was filtered through a 0.45 µm filter. Total gOZ concentration was determined using the HPLC system described in Kato et al. (2017). This gOZ concentration was expressed as ng cycloartenyl ferulate (CA, Wako Pure Chemical Industries, Osaka, Japan) equivalent mg DW^{-1}.

GABA extraction was done by immersing the samples, precisely weighed around 500 mg, in 10 mL of 0.1% (v/v) phosphate in a 15 mL tube, mixed thoroughly, dispersed with an ultrasonic incubator for 1 h, and stored overnight at room temperature. This procedure was repeated three times as replicates. After centrifugation (9300 × *g*, for 20 min at room temperature) of an aliquot of liquid fraction, the supernatant was mixed with equal volume of borate buffer (pH 10.0), and stored in 4°C until use (Maruyama & Tsushida, 2009). The determination of GABA concentration was conducted basically in accordance with the method of Ng et al. (2013). An extracted sample (55 µL) was mixed in a 100 µL cell with 50 mM (final concentration) Tris-HCl buffer (pH 8.6), 0.6 mM $NADP^+$, and 0.1 U mL^{-1} GABase (G7509, Sigma-Aldrich Co., Ltd., Tokyo, Japan) to make a mixture of 95 µL. The GABase was solved in a 100 mM phosphate buffer (pH 7.2), 12.5% (v/v) glycerol, and 5 mM 2-mercaptoethanol, to make 2 U mL^{-1}. This mixed sample was incubated at 30°C for one h. After measuring the absorbance at 340 nm using a UV-spectrophotometer (UV-1700, Shimazu Co., Ltd., Kyoto, Japan), the sample was mixed in the same cell with 1 mM (final concentration) α-ketoglutarate to make a mixture of 100 µL and again incubated at 30°C for 20 min. A set of standard samples with known GABA concentrations were also treated as above. After the second incubation, the sample was measured its absorbance at 340 nm. From the difference between before and after the addition of α-ketoglutarate, the GABA concentration (ng GABA mg DW^{-1}) was determined from the responses of standard samples.

GABase, used in the present GABA determination, involves two kinds of enzyme: GABA-α-ketoglutarate aminotransferase (EC 2. 6. 1. 19) and succinic semialdehyde-dehydrogenase (NAD^+) (EC 1. 2. 1. 24). The former catalyzes the reaction from GABA and α-ketoglutarate to succinic semialdehyde and glutamate, and the latter catalyzes from succinic semialdehyde, $NADP^+$ and H_2O to succinic acid, NADPH, and a proton. In the present assay system, background evolution of NADPH in the first incubation was recorded; then, NADPH derived from the α-ketoglutarate in the second incubation was determined with the absorbance at 340 nm, as an equivalent molar to GABA (Tsukatani et al., 2005).

gOZ content in the *n*th layer from the brown rice surface (L_n) was estimated from

$$L_n = C_{n-1}W_{n-1} - C_nW_n \quad (1)$$

where C_n and W_n are the concentration and dry weight of the *n*th polished grain {brown rice (polishing period of 0 sec) corresponds to $n = 0$}, respectively. The content of core part (regarded as 6th layer) was estimated as $L_{n+1} = C_n W_n$ ($n = 5$). The dry weight of polished rice was estimated from its polishing rate, and the average weight of brown rice in five (7, 15, 30, 60 and 120 sec) polishing periods. Percentage of gOZ in the *n*th layer was calculated as $100 \times L_n/\Sigma L_n$.

Results

Polishing of brown rice

The mean polishing rates of 19 cultivars in the 2017 experiment were 91.4% (SE = 0.71) in N and 91.1%

(SE = 0.85) in G. No significant correlation coefficients (df = 17) were obtained between polishing rate and gOZ or between polishing rate and GABA concentrations (Table 2). Figure 1 shows the polishing rates of various polishing periods in the 2018 experiment, indicating that there were no major differences in polishing profiles among cultivars. In addition, no significant correlations (df = 4) were obtained between polishing rate and gOZ concentration among six cultivars in any of the polishing periods (from r= –0.308, P= 0.553 to r= 0.414, P= 0.415). These results indicated that differences in polishing rates among the present cultivars were not great enough to affect their variations in gOZ and GABA concentrations. In addition, no hard cracking was found after any polishing.

gOZ and GABA concentrations in brown rice and polished rice without and with germination treatment

Analyses of variance in a three-way classification (cultivar, B vs. P, and N vs. G, as fixed-effect factors) were conducted for gOZ and GABA concentrations (Table 1). The most notable result for gOZ was that no significant differences were detected between N and G, and also interactions relating this factor (Table 1, Figure 2(a)), whereas highly significant variations were found in all factors and interactions for GABA (Table 1, Figure 2(b)). Therefore, gOZ concentration did not increase or change clearly in both BG and PG with the present germination treatment for 5 h. gOZ concentration in PN was confirmed to be extremely lower than that in BN (Figure 2(a)). In contrast, GABA concentration was increased much more with germination treatment in both BG and PG (Figure 2(b)). In particular for PG, GABA concentrations exceeded those in BN in many cultivars. The degree of enhancing effect with germination treatment was lower in PG than in BG, resulting in a significant interaction of (B vs. P) × (N vs. G) for GABA concentration (Table 1). On average, BN showed significantly higher GABA concentrations compared with PN. However, several cultivars showed nearly equal or higher GABA concentrations in PN compared with in BN, resulting in a significant interaction of C × (B vs. P) in GABA concentration (Table 1 and Figure 4).

As other reports have already demonstrated (Heineman et al., 2008; Huang & Ng, 2011; Kim et al., 2015; Miller & Engel, 2006; Roohinejad et al., 2011), this experiment also showed very wide and significant variations in gOZ and GABA concentrations in BN, BG, and PG among cultivars (Figures 3 and 4). Only for GABA concentration in PN, no significant variation was detected. For gOZ concentration in PN, Hattan showed the highest value. The second, fourth, and sixth highest cultivars were Yamadanishiki, Tamasakae, and Kairyo-Omachi, respectively (Figure 3(b)). These three cultivars, as well as Hattan, are cultivars for sake brewing. Gohyakumangoku, also a sake-brewing cultivar among

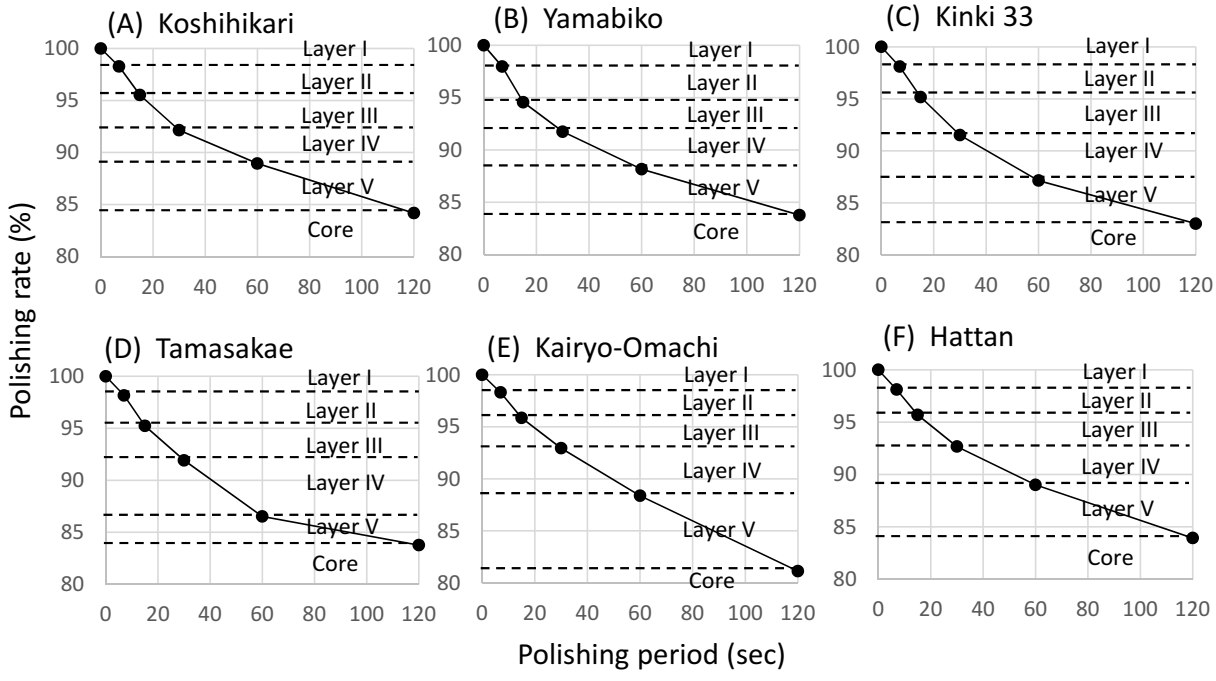

Figure 1. Relationships between polishing rates and polishing periods for (a) Koshihikari, (b) Yamabiko, (c) Kinki 33, (d) Tamasakae, (e) Kairyo-Omachi and (f) Hattan in the 2018 experiment. Proportions of the respective layers and core are also shown in the same figures. See text for layers and core.

Table 1. Analyses of variance for γ-oryzanol and γ-aminobutyric acid concentrations in BN, BG, PN, and PG.

		gOZ		GABA	
Factor	df	*F*-value	*P*-value	*F*-value	*P*-value
Cultivar (C)	18	15.756	<0.001	16.270	<0.001
Brown vs Polished (B v P)	1	12,717.738	<0.001	637.040	<0.001
Without vs with germination (N v G)	1	0.872	0.352	3513.896	<0.001
C × (B v P)	18	15.083	<0.001	5.108	<0.001
C × (N v G)	18	1.431	0.124	7.653	<0.001
(B v P) × (N v G)	1	1.753	0.187	217.084	<0.001
C × (B v P) × (N v G)	18	1.049	0.410	4.774	<0.001

gOZ and GABA indicate γ-oryzanol and γ-aminobutyric acid, respectively. B, P, N, and G indicate brown rice, polished rice, without germination treatment, and with germination treatment, respectively.

the 19 cultivars, did not show a higher gOZ concentration in PN. For GABA concentration in BN, the highest value was observed in Kinuhikari, which showed a significantly higher value among all cultivars (Figure 4(a)). Kinuhikari also kept this very high GABA concentrations in germination treatment of both B and P (Figure 4(a,b)). The highest three cultivars in PG, Kinuhikari, Todorokiwase, and Rikuu 132, were also included within the cultivars showing higher values in BG.

Table 2 shows correlation coefficients for all combinations among measured traits, and also polishing rates in N and G (df = 17). Three significant coefficients ($P< 0.01$) were obtained for gOZ concentrations in BN and BG, gOZ in PN and PG, and GABA concentrations in BG and PG. For gOZ, the coefficient between the concentrations in BG and PG was not significant ($r= -0.015$, $P= 0.951$). Moreover, no significant correlations were obtained among gOZ and GABA concentrations in all cases. In addition, single polished-rice weight was not significantly correlated with gOZ and GABA concentrations in any cases (data not shown).

Table 2. Correlation coefficients among γ-oryzanol and γ-aminobutyric acid concentrations in BN, BG, PN, and PG, and polishing rates in N and G.

Trait	A.	B.	C.	D.	E.	F.	G.	H.	I.
A. gOZ in BN									
B. gOZ in BG	0.858**								
C. gOZ in PN	0.173	0.228							
D. gOZ in PG	−0.207	−0.015	0.671**						
E. GABA in BN	−0.103	−0.063	0.037	0.026					
F. GABA in BG	−0.176	−0.183	−0.422	−0.468*	0.358				
G. GABA in PN	0.209	0.245	0.061	−0.116	0.222	−0.042			
H. GABA in PG	−0.461*	−0.347	−0.404	−0.136	0.388	0.713**	0.000		
I. %P in N	−0.398	−0.355	0.319	0.507*	0.395	0.126	−0.128	0.449*	
J. %P in G	−0.165	−0.237	0.245	0.361	0.024	−0.216	0.253	0.178	0.560*

*, **; significant at the 0.05 and 0.01 probability levels, respectively. gOZ and GABA indicate γ-oryzanol and γ-aminobutyric acid, respectively. B, P, N, and G indicate brown rice, polished rice, without germination treatment, and with germination treatment, respectively. %P indicates polishing rate.

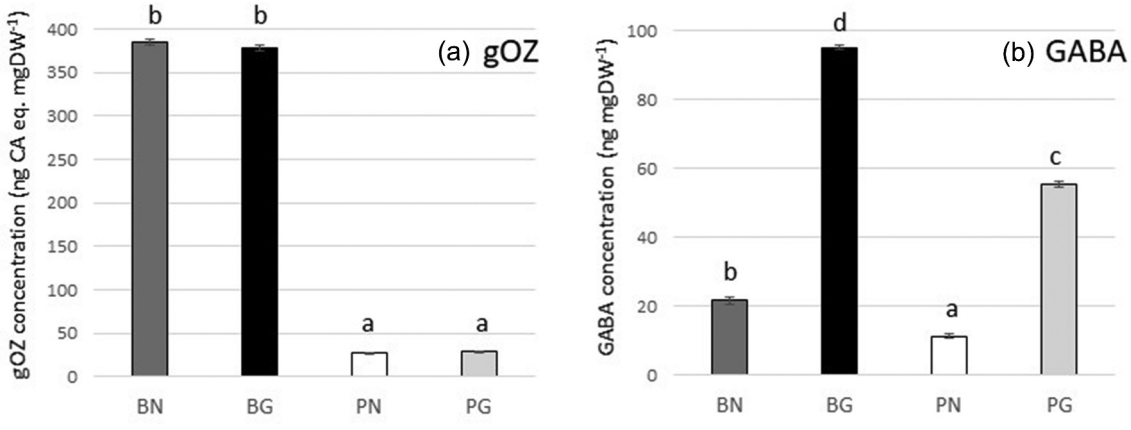

Figure 2. Effect of germination treatment on (a) γ-oryzanol (gOZ) and (b) γ-aminobutyric acid (GABA) concentrations in brown rice and polished rice. B, P, N, and G indicate brown rice, polished rice, without germination treatment, and with germination treatment, respectively. Means with the same letter did not differ significantly at the 0.01 probability level.

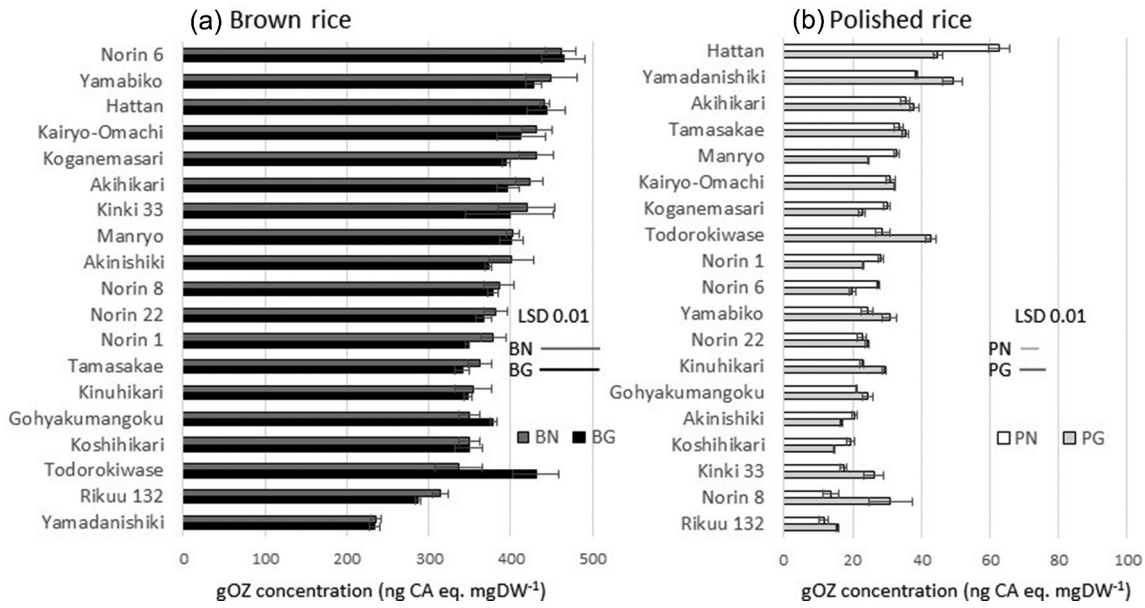

Figure 3. γ-oryzanol (gOZ) concentrations in (a) brown rice and (b) polished rice of 19 rice cultivars. LSD0.01 indicates the least significant difference at the 0.01 probability level. B, P, N, and G indicate brown rice, polished rice, without germination treatment, and with germination treatment, respectively. CA eq. indicates cycloartenol ferulate equivalent.

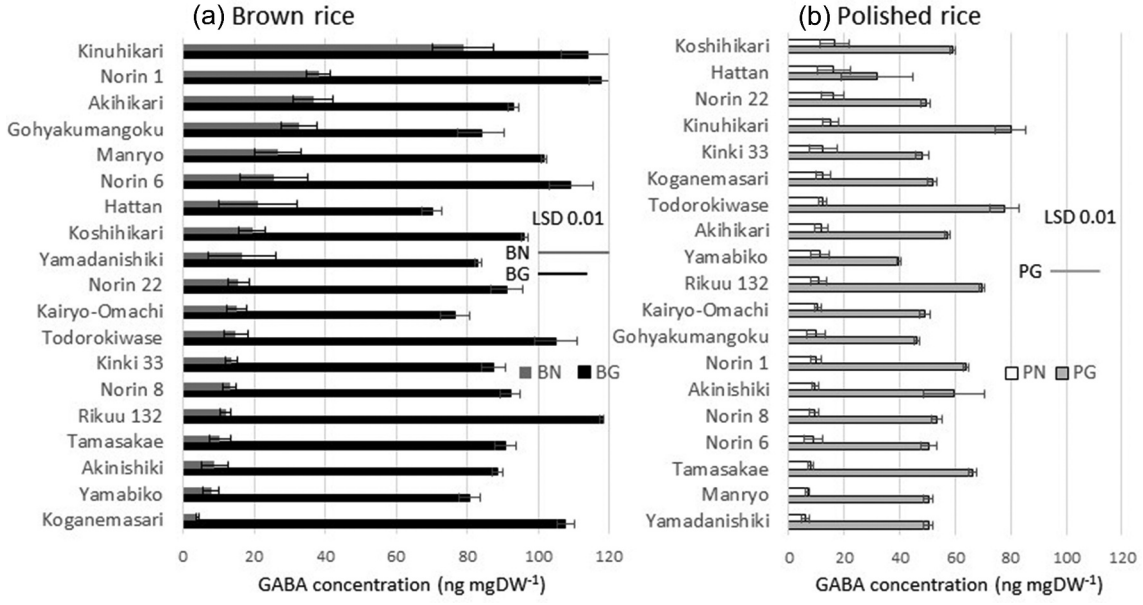

Figure 4. γ-aminobutyric acid (GABA) concentrations in (a) brown rice and (b) polished rice of 19 rice cultivars. LSD0.01 indicates the least significant difference at the 0.01 probability level. B, P, N, and G indicate brown rice, polished rice, without germination treatment, and with germination treatment, respectively.

Distribution of gOZ content in outer layers of brown rice

More detailed distribution of gOZ in the outer layers of BN was examined for six cultivars in 2018. Of these six cultivars, the significant highest concentration in PN was found in Hattan as in 2017, although significant difference between two growing years and significant interaction of cultivar × growing year were found (Figure 5). Figure 6(a) represents percentages of gOZ in each layer within a cultivar. Figure 6(b) arranges the same data in each cultivar within a layer, to facilitate the evaluation of cultivar differences. It should be noted that the bran fraction of brown rice mostly corresponded to the total of layers I, II, and III, whereas layers IV and V, and core, comprised of

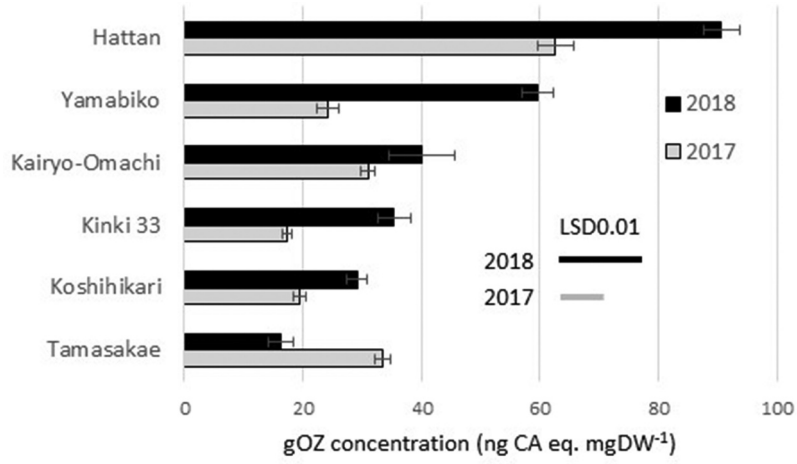

Figure 5. γ-oryzanol (gOZ) concentration in polished rice of 6 rice cultivars in 2017 and 2018. LSD0.01 indicates the least significant difference at the 0.01 probability level.

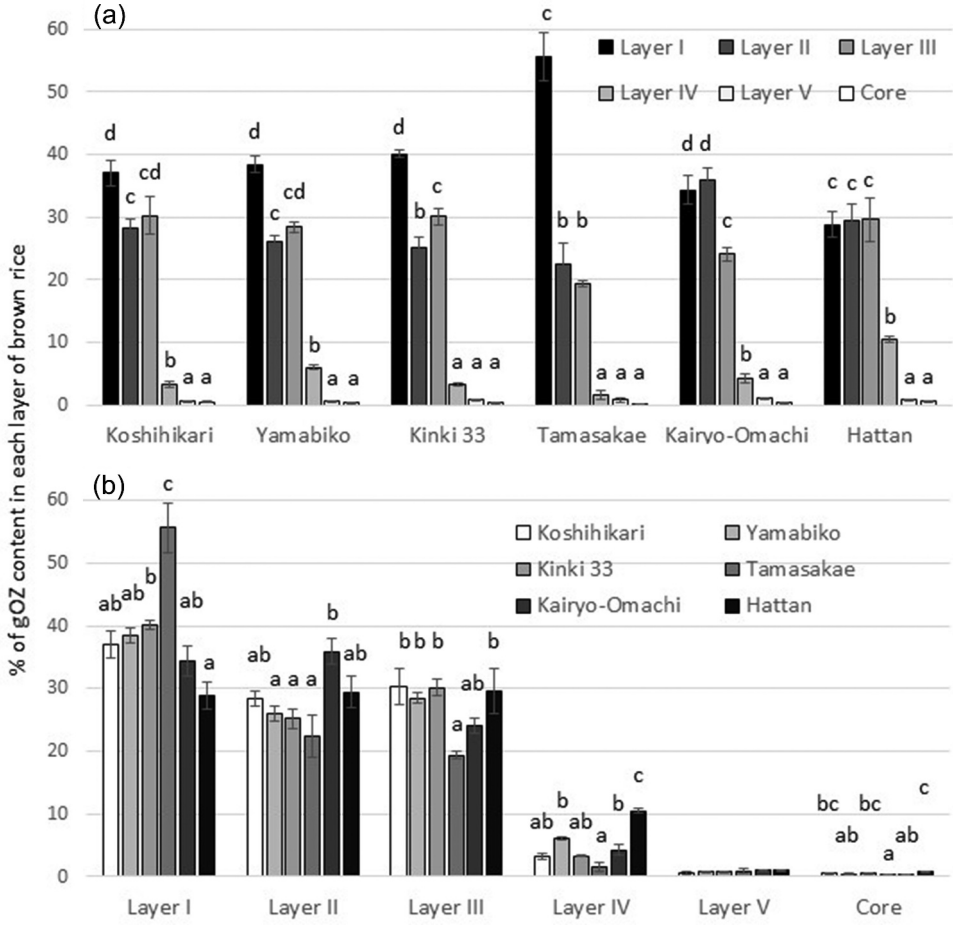

Figure 6. Percentages of γ-oryzanol (gOZ) content in different layers and the core of brown rice. (a) Comparison among layers and the core in each of six rice cultivars, and (b) comparison among six cultivars in each of the layers and the core. Means with the same letter did not differ significantly at the 0.01 probability level, within each cultivar (a) and within each layer or the core (b). See text for layers and core.

polished rice. In Figure 6(a), Koshihikari, Yamabiko, and Kinki 33, which showed lower gOZ concentrations in PN and PG (Figure 3), exhibited a similar gOZ distribution within brown rice. In these three cultivars, gOZ was predominantly concentrated in layers I, II, and III, i.e., the bran fraction, particularly in the

outermost layer I. On the other hand, the inner layers of polished rice, layers IV, V, and core, barely contained any gOZ (Figure 6(a)). No significant differences among these three cultivars were detected in any layers (Figure 6(b)).

In contrast to these cultivars, Hattan, which showed the highest gOZ concentration in PN, demonstrated relatively lower amounts of gOZ distributed evenly in the bran fraction (layers I, II, and III), resulting in the lowest percentage of gOZ in the outer layers of brown rice, compared with other cultivars (Figure 6(a,b)). Kairyo-Omachi also showed a lower percentage of gOZ in layer I (Figure 6(a)). Hattan, on the other hand, had significantly higher gOZ in layer IV, the most outer layer of polished rice (Figure 6(b)). Conversely, Tamasakae, also a cultivar for sake brewing as Hattan and Kairyo-Omachi, had the highest gOZ distribution in layer I and very low percentages in polished rice (Figure 6(a)). All three sake-brewing cultivars showed very low percentages of gOZ in layer V and core portion, as other three cultivars.

Discussion

The present results clearly showed that Hattan had the highest gOZ concentration in polished rice without germination treatment, resulting in a significant difference ($P<0.01$) from the second highest cultivar, Yamadanishiki (Figure 3(b)). A detailed analysis of the distribution of gOZ in Hattan showed higher gOZ content in the outermost layer of polished rice (layer IV) and lower content in the bran fraction of brown rice than other cultivars. If the polishing period was set at 15 sec, instead of the standard 30 sec, the polished rice of Hattan can retain up to about 40% of gOZ content in brown rice, whereas other cultivars retain approximately 25–30% after the same polishing period (Figure 6(a)). These results were also found, in part, in Kairyo-Omachi.

Hattan, Kairyo-Omachi, and Yamadanishiki have common features: they are sake-brewing cultivars and all had higher gOZ contents in PN. In general, sake-brewing cultivars are required to show larger grain sizes, more frequent appearance of white-core and lower nitrogen contents in grains, more rapid water absorption in the sake-brewing process (Tamaki et al., 2005). These preferable characteristics for sake brewing may be related to some physicochemical properties of the endosperm organ, that is, the packing of endosperm cells might be looser compared with non-sake-brewing cultivars, presumably leading to the white-core appearance in grains (Ebata & Nagato, 1960). Such properties of endosperm organ may facilitate the permeation of gOZ from the bran fraction of brown rice into polished rice, even though gOZ is a water-insoluble compound. The correlation coefficient between gOZ concentrations in BN and PN was very low and not significant ($r= 0.173$, $P= 0.479$, Table 2). In addition, in the case of water-soluble GABA, the correlation coefficient between GABA concentrations in BN and PN was not significant ($r= 0.222$, $P= 0.361$, Table 2). These results meant that the permeability of gOZ and GABA from outer to inner layers cannot depend on the original gOZ and GABA concentrations in bran, suggesting that some properties of endosperm, such as the packing status of endosperm cells, may be attributable to the permeability of these compounds, particularly for gOZ, depending on the genotypes. Tamaki et al. (2008) demonstrated that Hattan (Hattan 35, derived from a cross between Hattan 10 and Shuhou) showed some different characteristics of white-core structure from other 'Hattan-type' cultivars, derived from a common ancestral cultivar, Hattan-so.

The results also demonstrated that the present germination treatment of 5 h did not increase gOZ concentration, whereas the treatment clearly increased GABA concentration, in both brown rice and polished rice (Figure 2). This indicated that GABA synthesis may be promoted de novo in the bran fraction by germination treatment with mild heating and humidity. Part of this synthesis may be driven by the activity of glutamate decarboxylase (Komatsuzaki et al., 2007; Shimajiri et al., 2013). The product can permeate into inner portions of the grain in a constant manner irrespective of genotype, indicating a significant positive correlation between GABA concentrations in BG and PG ($r= 0.713$, $P< 0.001$), whereas no correlation between BN and PN was found. This was in clear contrast to gOZ, implying that de novo synthesis of gOZ in bran hardly induced with germination treatment, compared with GABA.

To increase the GABA concentration in polished rice, and also in brown rice, several novel rice cultivars with large embryo (Satoh & Omura, 1981) have already developed as GABA-enriched rice (Ohtsubo, 2018), as well as germination treatment. In contrast to GABA, the present results demonstrated that even in Hattan gOZ concentration in its polished rice would be insufficient. This compound seems to be blocked at the outermost layer of polished rice. Therefore, we should enrich gOZ in polished rice through pyramiding of genetic factors facilitating gOZ permeation into the inner part of polished rice as well as by stimulation of synthesis or accumulation of gOZ in bran. The present results strongly suggested that some sake-brewing cultivars with appropriate endosperm characteristics may be used as candidates, although not all sake-brewing cultivars showed high gOZ concentrations in polished rice.

Alternatively, some other procedures, including the techniques to improve the taste of cooked brown rice to sufficient for direct consumption (Ohtsubo, 2018), should be developed.

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Disclosure statement

No potential conflicts of interest were reported by the authors.

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