The Pharma Innovation Journal 2021; SP-10(11): 2993-2996

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(11): 2993-2996

www.thepharmajournal.com
Received: 03-09-2021
Accepted: 09-10-2021

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Germinated brown rice as a potential source of Gaba: An inhibitory neurotransmitter

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Abstract
Explicit nutrient deficiencies, along with diet-related chronic disorders, contribute to malnutrition that affects the health of individuals. Breeding rice cultivars with high nutrient concentrations in the grains has been proposed as an efficient and cost-effective method of ensuring nutritional security among rice consumers. Gamma Amino Butyric Acid (GABA), a non-protein amino acid with four carbons, is an inhibitory neurotransmitter that possess numerous health benefits. We conducted a study at Plant Molecular Laboratory, University of Agricultural Sciences Raichur to estimate GABA content in 24 rice genotypes under differential soaking periods. GABA content was estimated in germinated brown rice (GBR) pre-soaked in water for different durations (24, 48 and 72 h) and incubated at room temperature. The results showed that the GABA concentration varied with the genotypes and showed no specific trend for an increase or decrease in GABA with the soaking duration.

Keywords: Gamma amino butyric acid, germinated brown rice, soaking duration, variability

Introduction
Rice being the most consumed cereal grain represents the major pillar of food security among rice eating population of the globe. Although rice is a valuable source of carbohydrates; it's grain has significant pile of proteins, vitamins, fibre, iron, and minerals. (Patil and Khan, 2011) [9]. Milled rice, which is extensively consumed, loses its nutrient-rich bran layer during processing, reducing it to a simply starchy grain. Brown rice grains provide more nutritious components than typical milled rice grains, in terms of phytic acids, dietary fibres, vitamins E and B, and GABA content. The germination process triggers the activity of Hydrolytic enzymes in cereal grains that degrade proteins, starch, and non-starch polysaccharides, resulting in an increase in oligosaccharides as well as amino acids (Ohtsubo *et al.*, 2005) [8].
GABA is the predominant inhibitory neurotransmitter in the central nervous system of the mammals. It is a four-carbon non-protein amino acid whose primary role is to reduce neuronal excitability throughout the neurological system. It improves fat reduction by stimulating the synthesis of human growth hormone; it lengthens the sleep cycle, allowing for deeper sleep; it strengthens the immune system; it reduces blood pressure; it prevents the formation of cancer cells; and it aids in treating some anxiety disorders (Patil and Khan, 2011) [9]. As a result, developing rice varieties with high nutritional concentrations in the grains has been proposed as an efficient and cost-effective method of alleviating malnutrition (Xiongsiyee *et al.*, 2018) [13]. Hence a shift from quantity centered breeding programmes towards "quantity plus quality" oriented approaches is of prime importance. It has been reported previously by several researchers that, Germinated Brown Rice (GBR) is a rich source of GABA (Komatsuzaki *et al.*, 2007; Patil and Khan, 2011; Lin *et al.*, 2015) [6, 9, 7]. GBR is produced by soaking brown rice grains in water to induce germination. This procedure helps in increased GABA accumulation in rice grains. Together with GABA, the GBR also contains fibres, vitamins, minerals, and active ingredients like phytic acid and ferulic acid. Other advantages of GBR over regular brown rice include easiness in cooking and a softer texture than brown rice. As a result, GBR may become a popular healthy meal in near future. (Komatsuzaki *et al.*, 2007) [6].
Thitinunsomboon *et al.* (2013) [12] tested the effect of repeated soaking (in tap water at 35 °C, 3 h) and incubation (at 37 °C, 21 h) during germination on GABA content. They reported highest amount of GABA (116.88 ±9.24 mg/100 g GBR on dry basis). However, the researchers also reported that, certain microbes produced a disagreeable odour during prolonged hours of germination. Lin *et al.* (2015) [7] reported higher GABA content under prolonged soaking (72h) and high temperature (36°) conditions.

However, Ding *et al.* (2016) [1] observed that the accumulation of GABA exhibited genotype-specific modes in both normoxic and hypoxic treatments. In the present study, 24 rice genotypes (*Oryza sativa* L.) were tested in 3 different soaking durations (24, 48 and 72 h). The experiment was conducted at the Plant Molecular Laboratory, Department of Genetics and Plant Breeding, University of Agricultural Sciences Raichur, Karnataka, India.

Material and Methods

The experiment was conducted with 24 rice genotypes which include four popular varieties as checks *viz.*, Gangavati Sona, BPT 5204, GNV 10-89, and MTU-1010. The crop was raised at ARS Gangavathi, University of Agricultural Sciences Raichur during the season *Kharif* 2018. The trial was set in a randomized complete block design (RCBD) with three replications. Standard agronomic practices were followed during the crop growth period. Grains of the randomly selected plants of all the 24 genotypes were taken to Plant Molecular Laboratory, Department of Genetics and Plant Breeding, UAS Raichur to estimate the GABA content. The GABA content of germinated brown rice of all the 24 selected genotypes was estimated by using spectro-photometrical techniques as described in the modified method of Kitaoka and Nakano (1969) [5].

Preparation of germinated brown rice powder

1. 50 mg of rice grain samples were soaked for three different durations (24, 48, 72 hours) at room temperature and placed in an incubator for specified durations.
2. The germinated grains were dried in a hot air oven at 100 ^{0}C for 30 minutes.
3. At 13-14% moisture content the grains were threshed as germinated brown rice.
4. The germinated brown rice was ground into a fine powder before extracting

Quantification of GABA content

1. 3 mg of each of the ground samples was dissolved in 80% alcohol in a test tube, shaken thoroughly, and filtered with a filter paper.
2. Filtered solution was kept in a boiling water bath at 80 ^{0}C for 15 minutes to evaporate the ethanol present in the samples.
3. 0.5 ml distilled water was added to the samples and centrifuged at 10000 rpm for 10 minutes. The floating portion on the top was aspirated.
4. 0.2 ml of 0.2 M borate buffer and 1.0 ml of 6% phenol were added.
5. For the standard, GABA solution (0.1-0.3 ml) was added to test tubes together with 0.2 ml of borate buffer and 1.0 ml of phenol reagent.
6. The solutions were mixed thoroughly and kept in a cooling bath for 5 minutes.
7. 0.4 ml of 10-15% NaOCl was added to all the test tubes and shaken vigorously for 1 minute.
8. Again the test tubes were kept in a cooling bath for 5 minutes followed by a hot water bath (100^{0}C) treatment for 10 minutes. The samples were allowed to cool at room temperature.
9. The optical density was measured at a wavelength of 630 nm in a spectrophotometer with ethanol 2.0 ml as a blank.
10. Quantification of GABA content was done by comparing the optical density reading of samples with that of standard GABA using graphical approach.

Estimation of genetic variability parameters was done using the following formulae

- Genotypic Coefficient of Variation (GCV)

$$GCV\ \% = \frac{\text{Genotypic standard deviation } (\sigma g)}{\text{General mean } (\bar{X})} \times 100$$

- Phenotypic Coefficient of Variation (PCV)

$$PCV\ \% = \frac{\text{Phenotypic standard deviation } (\sigma p)}{\text{General mean } (\bar{X})} \times 100$$

GCV and PCV values were categorized as low, moderate, and high as indicated by Sivasubramanian and Madhavamenon (1973) [11].

- Heritability (Broad sense) denoted as h^2

$$\text{Heritability } (h^2) = \frac{\text{Genotypic variance } (\sigma^2 g)}{\text{Phenotypic variance } (\sigma^2 p)} \times 100$$

The heritability was categorized as low, moderate, and high as given by Robinson *et al.* (1949) [10].

- Genetic Advance (GA) and Genetic Advance as a percent of Mean (GAM)

Genetic advance (GA) = $ih^2\sigma p$; and GAM = $\frac{GA}{\bar{X}} \times 100$

Where *i* stand for selection intensity and was taken as 2.06 assuming 5% selection intensity. The Genetic advance as percent mean (GAM) was categorized as low, moderate, and high as given by Johnson *et al.* (1955) [2].

Results and Discussion

The GABA content estimated in germinated brown rice of 24 selected genotypes which were pre-soaked for differential durations (24, 48, and 72h) has been presented in Table 1 and the different genetic parameters worked out for GABA at different pre-soaking durations has been presented in Table 2. The results are discussed hereunder.

Table 1: Mean data of GABA estimated in Germinated Brown rice of 24 genotypes pre-soaked for 24h, 48h, and 72h in water

Sl. No.	Genotype name	GABA (24 h) (mg/100g)	GABA (48 h) (mg/100g)	GABA (72 h) (mg/100g)
1	BPT-Mutant 1801	25.27	36.51	39.87
2	BPT-Mutant 1802	19.15	28.62	35.53
3	Gontra Bidhan	22.07	31.41	39.71
4	Rp-Bio-226 Mutant 614	32.55	35.56	26.99
5	IET-25451	34.09	45.86	40.14
6	IET-27162	31.18	40.91	19.56

7	IET-25520	31.72	39.20	45.71
8	SMW-09-32	29.72	41.66	24.85
9	Rp-Bio-226	33.49	43.38	35.10
10	BPT-Mutant 1808	17.99	26.42	20.36
11	Gangavati sona (Check)	31.33	41.10	44.12
12	BPT-5204 (Check)	16.86	24.79	35.86
13	IABT-17	33.76	46.85	44.95
14	Gangavati Sanna	18.65	35.70	42.47
15	IET-22066	29.78	37.53	41.23
16	IET-24767	20.17	25.50	30.62
17	GNV-1108	22.07	42.70	49.06
18	GNV-1602	22.37	28.83	35.35
19	RNR-15048	19.71	31.15	40.21
20	IET-25497	30.22	33.80	31.07
21	IET-26241	32.82	43.20	48.05
22	GNV 1109	29.96	27.92	35.80
23	GNV 10-89 (Check)	26.49	31.49	40.73
24	MTU-1010 (Check)	19.05	33.10	49.98

Table 2: Genetic variability parameters for GABA Content estimated in germinated brown rice 24h, 48, and 72 h after soaking

Sl. No.	Characters	Max	Min.	Mean	Vg	Vp	GCV	PCV	h^2 bs (%)	GA @ 5%	GAM
1	GABA (24h)	34.09	16.86	26.27	35.43	37.13	22.66	23.20	95	11.98	45.59
2	GABA (48h)	46.85	24.79	35.55	42.40	47.47	18.32	19.38	89	12.68	35.66
3	GABA (72h)	49.98	19.56	37.39	67.63	74.59	22.00	23.10	91	16.13	43.15

Gamma Amino Butyric Acid (24 h after soaking)

At 24h, the range of GABA varied from 16.86 mg (BPT-5204) to 34.09 mg (IET-25451) per 100g of GBR and the GABA content of germinated grains of all the genotypes averaged up to 26.27 mg per 100 g. Karladee and Suriyong (2012) [4] have earlier reported GABA content of 13.65 to 23.48 mg per 100g GBR in 21 purple and modern white rice varieties soaked for 24 h and incubated at room temperature for germination.

At 24h, the genotype IET-25451 showed highest GABA content (34.09 mg/100g GBR) followed by IABT-17 (33.76 mg/100g GBR) and Rp-Bio-226 (33.49 mg/100g GBR). However, the GABA content of IET-25451 increased after 48 hours of soaking (66.86 mg/100g) but decreased again after 72 hours of soaking (42.10 mg/100g). IABT-17 also showed the same trend as IET-25451. In contrast to this trend, the genotype GNV-1109 showed higher GABA at 24 h (29.96) which declined after 48 h (27.92) and again increased after 72 h of soaking period (35.80). Karladee and Suriyong (2012) [4] also reported high GABA content at 24 h soaking and incubation and continuous decrease afterwards in *indica* cultivars; in contrast, they also observed higher GABA content in *japonica* cultivars soaked for 72 h.

The GCV (22.66%) and PCV (23.20%) were high indicating the presence of greater variability among the selected genotypes. The heritability (95%) and GAM (45.59) were also higher indicating the presence of additive gene actions and selection for the genotype showing higher trait performance might be useful in crop improvement programs. (Table 1 and 2 should be inserted here)

Gamma amino butyric acid (48h after soaking)

GBR of IABT-17 pre-soaked for 48 h showed highest GABA content of 46.85 mg/100g followed by IET-25451 (45.86 mg/100g) and Rp-Bio-226 (43.38 mg/100g). An increase in GABA content with an increase in soaking period till 48 hours was observed in all the genotypes studied but it declined thereafter in a few genotypes. Zhang *et al.,* 2014 reported higher GABA concentration at 36 h and its decline thereafter, showing least concentration at 48 h treatment. They also reported higher GABA content in *indica* cultivars compared to *japonica.*

GABA estimated in GBR pre-soaked for 48 hours showed moderate GCV and PCV values (18.32 and 19.38 respectively) indicating the presence of a considerable amount of variation for this trait among the selected genotypes. The higher heritability (89%) and GAM (35.66) exhibited by the trait provide a good scope for selection and thus help in breeding rice varieties with improved GABA content.

Gamma Amino Butyric Acid (72 h after soaking)

The highest GABA content recorded in this treatment group was 49.98 mg/100g (MTU-1010) followed by 49.06 mg/100g (GNV-1108). The GABA content of 15 out of 24 genotypes studied including popular variety BPT-5204 showed an increasing trend with an increasing soaking period. However, BPT-5204 showed the lowest GABA content among all the genotypes studied at 24 and 48 h of soaking treatment (16.86 and 24.79 mg/100g respectively) but showed moderate GABA content after 72 hours of soaking (35.86 mg/100g) compared to other varieties investigated. Kaosa-Ard and Songsermpong (2012) [3] and Lin *et al.,* 2015 [7] also reported a drastic increase in GABA content consistent with the soaking period.

The GCV and PCV values for the trait (GABA estimated in GBR pre-soaked for 72 hours) were 22.00 and 23.10. A higher magnitude of PCV than the GCV indicated the influence of environment on trait expression. The heritability (91%) and GAM (43.15) were higher indicating the scope for selection for the trait.

Summary and Conclusions

Out of 24 genotypes studied, 23 genotypes showed increasing GABA content with an increased soaking duration from 24h to 48 h. However, 8 genotypes showed a decline in GABA content with an increase in the soaking period from 48 h to 72 h. These observations taken from GBR soaked at differential soaking period suggests that GABA content of GBR varies with varying soaking durations. There is no specific trend for increase or decrease in GABA with the soaking duration and

it purely depends on the genotype under question similar to the report of Ding *et al.,* 2016 [1]. So selecting genotypes possessing high GABA content and including them in the breeding program would be the better choice for increasing the GABA content in rice.

Conflicts of Interest: Auntors declare no conflicts of interest exist.

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