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Germinated Millets and Legumes as a Source of Gamma-Aminobutyric Acid

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Abstract: Gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter is known for its beneficial action with respect to brain and nerve functions. The enrichment of food grains with respect to GABA content is possible by simple process of controlled germination. Hence, an effort has been made to evaluate a few Indian millets and legumes for their potential to enhance their GABA content by soaking and germination. Identification of GABA content in the seeds were carried out by TLC method and further confirmed by HPTLC and quantified by rapid spectrophotometric method. It was observed that the GABA content increased significantly during soaking as well as germination in all the millets and legumes studied, but extent of increase varied considerably. Among the millets, it is attained to peak level in 96 h of germination in finger millet (361.8 nanomole/100 mg) from 21.4 nanomole/100 mg in its native seeds. Whereas, among the legumes studied, moth bean exhibited highest GABA content (1401.7 nanomole/100 mg) and horse gram showed the least values (241.0 nanomole/100 mg) on 96 h of germination. These results indicate that the germinated food grains could be useful for the development of functional foods especially health foods for children and geriatric population.

Key words: Gamma-aminobutyric acid (GABA) · Millets · Legumes · TLC · HPTLC

INTRODUCTION

Gamma-aminobutyric acid (GABA) is an important ubiquitous four-carbon non-protein amino acid widely distributed in plants which acts as a major inhibitory neurotransmitter in the mammalian central nervous system [1]. It is produced primarily by the decarboxylation of L-glutamic acid, catalysed by the enzyme α-glutamate decarboxylase (GAD) [2]. It plays a crucial role in human by regulating neuronal excitability throughout the nervous system and also directly responsible for the regulation of muscle tone [3]. It is also effective in lowering stress, blood pressure and hypertension [4].

In plants, GABA content increases by stress, such as hypoxia, cytosolic acidification, cold shock, mechanical stimulation, water stress and darkness [5]. Besides these, GABA content also increases during soaking and germination of food grains [6]. Kayahara and Tsukahara [7] showed that germinated brown rice (GBR) contained higher levels of GABA, besides other nutrients. Recently, it has been shown that the extracts from brown rice containing GABA had an inhibitory action on leukemia cell proliferation and also stimulatory action on the cancer cell apoptosis [8]. Hence GABA-enriched food serves as a source of nutraceuticals besides carbohydrates, lipids and proteins. Such a food may reduce the complications of sleeplessness, depression and autonomic disorder and may stimulate immune cells [4]. Therefore food grains that are rich source of GABA, have high potential to use as a base material for development of geriatric food formulations. Realizing the health benefits of GABA, nowadays methods for production of GABA-enriched foods are gaining importance.

Considerable information on GABA content of germinated brown rice are available. But the information on the GABA content in Indian millets and legumes in their native form and also during processing is scanty. Millets and legumes form staple food for the population of low income groups in India and also worldwide. Hence, enriching them with GABA will offer health benefits to the consumers. The food industries are more and more opting for development of functional foods specially designed for the children and geriatric population. Since, the geriatric population is increasing substantially due to increase in life expectancy worldwide, so it is necessary to develop food enriched with GABA content. Therefore, the

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objective of the present work was to evaluate the GABA content in few millets and legumes during soaking and germination, so as to identify their suitability for GABA-enriched functional food formulations for the children and geriatric population.

MATERIALS AND METHODS

Plant Material: Sorghum (*Sorghum vulgare*), finger millet (*Eleusine coracana*), little millet (*Panicum sumatrense*), bengal gram (*Cicer arietinum*), green gram (*Phaseolus aureus Roxb*), black gram (*Phaseolus mungo Roxb*), horse gram (*Dolichos biflorus*), lentil (*Lens esulenta*), moth bean (*Phaseolus aconitifolius, Jacq*) and green peas (*Pisum sativum*) were procured from the local market, cleaned and tested for viability and were used for the studies.

Chemicals: Gamma-aminobutyric acid (GABA) and GABase (a mixture of GABA transaminase [EC 2.6.1.19] and succinic semialdehyde dehydrogenease [EC 1.2.1.24] enzyme) from *Pseudomonas fluorescence* were purchased from Sigma Chemical Co. St. Louis, MO, USA. Nicotinamide adenine dinucleotide phosphate ($NADP^+$), α-ketoglutarate and 2-mercaptoethanol were procured from Sisco Research Laboratories, Mumbai, India. Potassium pyrophosphate and lanthanum chloride were obtained from HiMedia Laboratories Pvt. Ltd, Mumbai, India. All other chemicals and reagents used were of analytical grade. Triple distilled water was used for all the analysis.

Methods

Sample Preparation: The seeds were soaked in distilled water at ambient temperature (~30°C) for 24 h and germinated for different intervals of time upto 96 h in a BOD incubator (M/s Scientronic Instruments, New Delhi, India). The germinated seeds were sorted out and lyophilized (M/s Heto-Holten A/S, Type-FD3, Denmark). After lyophilization germinated grains rootlets were separated and powdered using a laboratory pulvarizer (M/s IKA-Werke Gmbh & Co. KG, Germany) to obtain whole meals of 250 μm particle size. The whole meals were packed and stored in an airtight container in a deep freezer (-20 °C) until analysis.

Spectrophotometric Determination: Gamma-aminobutyric acid was determined according to the spectrophotometric method [9]. The brief description of the process followed is as follows.

Extraction: About 0.1 g of flour was taken in preweighed 1.5 ml capacity eppendorf tube containing 400μl of methanol at 25°C and the precise weight of the sample was noted. The tube was shaken for 30 min in vortex shaker, vacuum dried and 1 ml of 70 mM lanthanum chloride was added, again vortexed for 30 min and centrifuged at 13,000 x g for 5 min. An aliquot of 0.8 ml supernatant was transferred into a fresh eppendorf tube, to that 160μl of 1 M KOH was added, shaken for 5 min and centrifuged and the supernatant was used for GABA determination by spectrophotometric method.

Analysis: To 550μl of the supernatant obtained, 150μl of 4 mM $NADP^+$, 200μl of 0.5 M potassium pyrophosphate buffer (pH 8.6), 50μl of GABase per ml and 50μl of 20 mM α-ketoglutarate were added, mixed well and the absorbance was measured. The initial absorbance was read at 340 nm before adding α-ketoglutarate and final absorbance was read after 60 min. The GABA content in the sample was calculated based on the difference in absorbance. Standard GABA was prepared in nanomole concentration and used for constructing the calibration graph. For this purpose the commercially available GABase enzyme was prepared by dissolving required quantity in 0.1 M potassium phosphate (pH 7.2) containing 12.5% glycerol and 5 mM 2-mercaptoethanol. The resultant solution was stored in frozen condition until used.

Identification of GABA by Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC): GABA content from the millets and legumes was identified by TLC and HPTLC (CAMAG TLC Scanner 3, M/s CAMAG, Sonnenmattstr.11, 4132 Muttenz, Switzerland) on activated (20 × 20 cm) high performance silica gel plates (Silica gel 60 F_{254}, Merck Co. Darmstadt, Germany) using n-butanol:acetic acid:water (5:2:2, v/v) as solvent system, by ascending mode (multiple development). Standard GABA solution (1 mg/ml) and the sample extracts were applied to the plate by micropipette. The volume applied for each analysis was 10μl. The chromatogram was viewed after spraying with 2% ninhydrin in acetone and developing at 105 °C for 5 min. Scanned area of samples were matched with the scan area of the standard GABA. The GABA spots on HPTLC plates were viewed at 480 nm [10, 11].

Statistical Analysis: Data were analysed using GraphPad Instat statistical software (GraphPad Software, Inc. La Jolla, CA, USA). Each experiment was performed in triplicate and the results were expressed as the mean values ± standard deviation. Results were analysed and significance level was calculated using Tukey-Kramer multiple comparison test by means of one way ANOVA. Values with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

GABA Content in Millets and Legumes: The GABA content in the native, soaked and germinated millets and legumes are shown in the Table 1. It is clear that the GABA content increased during both soaking and germination. Similar results have been reported earlier also by many authors [12, 13, 14]. In general, millets contained more GABA compared to legumes in their native form (Table 1), but the germinated legumes contained higher GABA than germinated millets. Among the millets studied, finger millet showed maximum increase of GABA content (~17-times) during germination, but in the case of sorghum, GABA content increased considerably (88.7 nanomole/100 mg) during soaking, but decreased afterwards upto 96 h of germination. On the other hand, GABA content was maximum in little millet at 24 h of germination and it decreased on further germination. Similar results are also reported by other authors [14, 15] during germination of rice and barley, respectively. However, finger millet exhibited a constant increase of GABA content during germination upto 96 h with a highest value of 361.8 nanomole/100 mg. The GABA content also increased significantly ($p < 0.05$) in legumes during soaking as well as germination (Table 1). Among the legumes, moth bean showed the highest value for GABA content (1401.7 nanomole/100 mg) during germination, whereas it was least (241.0 nanomole/100 mg) in horse gram during germination. Similar results are reported by Kayahara and Tsukahara [8] who showed that GABA content in germinated brown rice (GBR) was 10 times higher than native milled rice.

The results indicated that soaking and germination process influences the GABA production in millets and legumes significantly. Increase in GABA content during soaking and germination of grains is mainly due to decrease in glutamate content [7,15,16]. The increase in GABA content during soaking may be due to the activation of α-glutamate decarboxylase (GAD), which converts glutamate to GABA [17]. Soaking also leads to hypoxia due to the limited availability of oxygen to the seeds [18] which probably triggers the synthesis of GABA during germination [19].

Identification of GABA by TLC and HPTLC: In the present study, GABA content was identified by thin layer chromatography (TLC) followed by high performance thin layer chromatography (HPTLC) using silica plates. Native millets and legumes extracts were spotted on silica plates with standard on both ends for easy identification. Figure 1 shows that, the TLC chromatogram of colored spots of GABA from standard and the extracts of native millets and legumes on TLC plate. The identification of GABA was done on the basis of retention factor (Rf = 0.54). The chromatogram shows the presence of GABA in all the samples.

The presence of GABA in native millets and legumes was further confirmed by HPTLC that shows the chromatogram in all 12 tracks (Figure 2). The identification

Table 1: Gamma-aminobutyric acid content in native, soaked and germinated millets and legumes

		Germination period (h)				
Samples	Native	0	24	48	72	96
Sorghum	24.9^{a} ± 1.5	88.7^{b} ± 2.0	68.9^{e} ± 4.1	59.3^{dc} ± 4.5	51.6^{ed} ± 5.1	49.0^{fde} ± 4.3
Finger millet	21.4^{a} ± 1.1	83.8^{b} ± 2.5	256.5^{c} ± 3.2	311.2^{d} ± 6.3	345.0^{e} ± 3.9	361.8^{f} ± 6.7
Little millet	38.2^{a} ± 0.8	77.2^{b} ± 3.2	84.6^{eb} ± 5.7	65.6^{d} ± 5.9	59.0^{ed} ± 1.2	55.4^{fde} ± 3.0
Bengal gram	10.9^{a} ± 2.7	312.4^{b} ± 5.5	238.1^{c} ± 2.9	290.0^{d} ± 3.0	323.1^{e} ± 3.8	367.7^{f} ± 4.4
Green gram	20.6^{a} ± 2.8	385.0^{b} ± 3.4	610.4^{c} ± 6.9	644.5^{d} ± 4.7	699.6^{e} ± 3.7	781.0^{f} ± 5.6
Black gram	11.1^{a} ± 1.6	349.4^{b} ± 5.7	208.9^{e} ± 1.1	312.2^{d} ± 3.1	352.8^{eb} ± 3.9	385.5^{f} ± 3.3
Horse gram	16.9^{a} ± 2.1	63.5^{b} ± 3.6	104.2^{e} ± 1.6	162.7^{d} ± 1.9	197.7^{e} ± 5.1	241.0^{f} ± 5.7
Lentil	12.6^{a} ± 3.2	139.5^{b} ± 3.3	207.9^{e} ± 4.7	301.4^{d} ± 5.6	347.4^{e} ± 1.1	384.6^{f} ± 6.8
Moth bean	20.7^{a} ± 2.8	531.6^{b} ± 3.0	947.6^{c} ± 4.9	1242.5^{d} ± 5.3	1313.4^{e} ± 5.8	1401.7^{f} ± 7.7
Green pea	14.2^{a} ± 0.9	89.0^{b} ± 1.8	305.6^{c} ± 2.3	447.6^{d} ± 3.5	500.0^{e} ± 3.9	573.1^{f} ± 5.8

Results are mean of three determinations ± SD. Mean values in the same row followed by different superscript are significantly different ($p < 0.05$). Values expressed as nanomole/100 mg, dry basis.

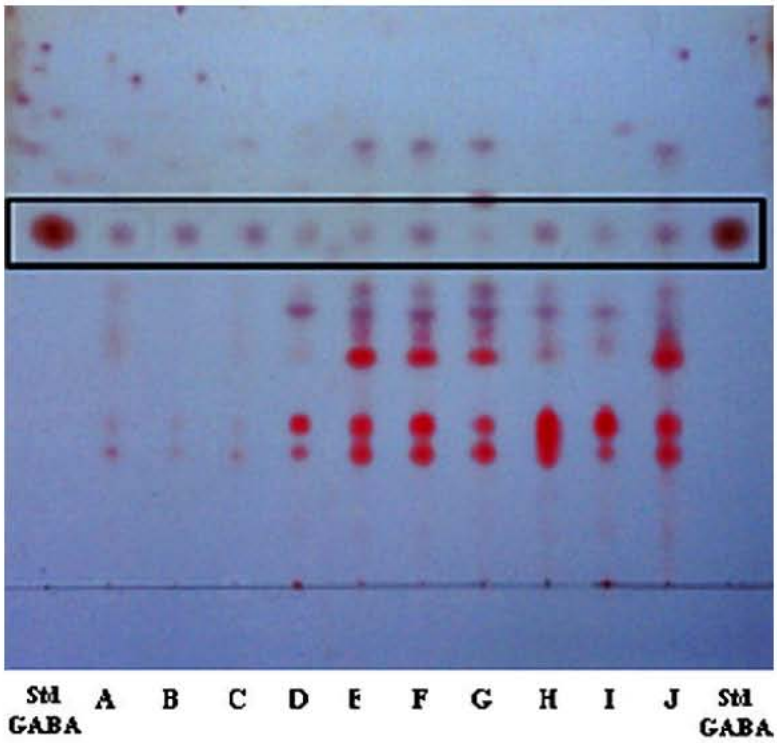

Fig.1: Thin layer chromatogram of standard GABA and extracts of millets and legumes. A: Sorghum, B: Finger millet, C: Little millet, D: Bengal gram, E: Green gram, F: Black gram, G: Horse gram, H: Lentil, I: Moth bean, J: Green pea.

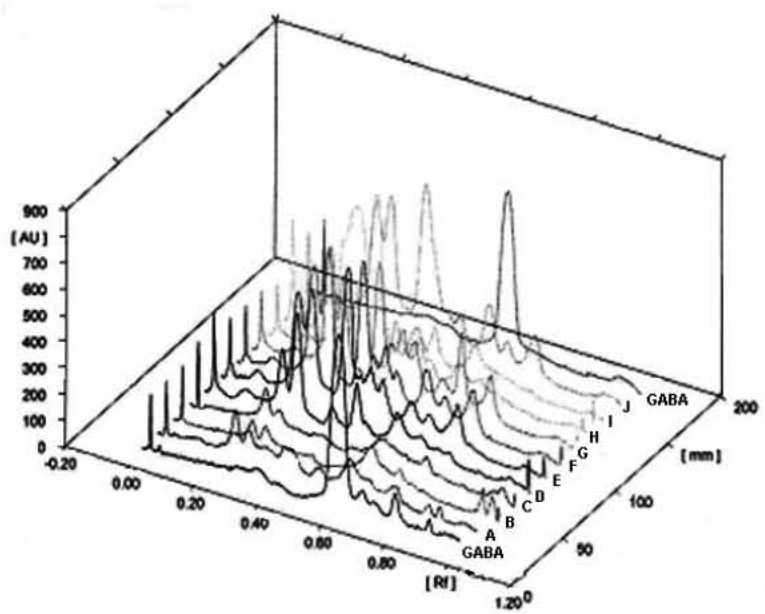

Fig.2: High performance thin layer chromatogram of standard GABA and extracts of millets and legumes. A: Sorghum, B: Finger millet, C: Little millet, D: Bengal gram, E: Green gram, F: Black gram, G: Horse gram, H: Lentil, I: Moth bean, J: Green pea.

of GABA was done on the basis of retention factor (Rf = 0.59) which automatically calculated by the system. T he present finding indicates that GABA is present in all millets and legumes and TLC, HPTLC methods could be easily used for identification of GABA. Since, it is well known that GABA plays an important beneficial role in the central nervous system as an inhibitory neurotransmitter [3]. It also accelerates the metabolism of brain in growing children, prevents or minimizes the risk of headache, colon cancer, heart disease, Alzheimer's disease, constipation, regulates blood sugar level, lowers blood pressure, hypertension and promotes sleep and rejuvenates the memory [4].

CONCLUSIONS

The present study confirmed that GABA content increased during soaking and germination of few numbers of Indian millets and legumes. It was observed that in general increase in GABA content during germination of legumes was higher compared to millets. Among the millets and legumes studied, finger millet and moth bean at 96 h of germination responded favorably by increasing the GABA content by ~ 17 and 68 folds respectively. The study showed that it is possible to enrich millets and legumes with GABA content just by soaking and germination. Hence, the GABA-enriched food formulations could serve as functional foods and may provide health benefits of this natural biomolecules to targeted populations, especially for the children and geriatrics.

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