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Effect of vitamin B6 on GABA accumulation and growth stimulation in germinated mung beans

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Abstract

γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter in animals and humans. It has several health advantages involving relief of anxiety and depression, as well as memory and immunity enhancement. GABA is also found in seeds as a reserve protein for plant growth. It is synthesized from glutamic acid by the glutamic acid decarboxylase (GAD) enzyme using vitamin B6 as a co-enzyme. To enhance the GABA content in bean sprouts, the effect of vitamin B6, in the form of pyridoxine hydrochloride (PN), on GABA accumulation in mung beans was examined. Supplementation of PN at various concentrations (1, 10, and 50 mg/L) was carried out in soaked and germinating mung beans. The results demonstrated a negative effect of PN on GABA accumulation. GABA was dramatically reduced with increased PN concentrations during soaking and germination. The highest GABA content was found in the control sample with no PN addition. Interestingly, PN showed an unexpected effect on promoting the growth of mung bean sprouts. The stem lengths of mung bean sprouts were significantly extended by increased PN concentrations. At 50 mg/L, PN induced a 35.6% elongation of stem length over that of a control. GABA content was negatively affected by PN in soaked seeds and germinated bean sprouts. However, it promotes mung bean growth during germination. PN is an effective growth stimulant for mung beans during germination.

Keywords: Mung bean**,** Germination, Gamma aminobutyric acid (GABA), Vitamin B6, Pyridoxine hydrochloride (PN)

1. Introduction

γ-aminobutyric acid (GABA), a non-proteinogenic amino acid, serves as an inhibitory neurotransmitter in animals and humans, regulating cardiovascular activities. It has several health advantages such as decreased hypertension [1], anti-diabetic effects [2], neurological disorder prevention [3], regulation of blood glucose [4], modulation of blood cholesterol levels [5], relief of anxiety and depression [6], and enhancement of memory and immunity [7]. Unfortunately, the GABA level in the human brain decreases with age and can also be reduced by some chronic illnesses [8], eventually leading to neurological deterioration and associated ailments. However, these effects can be reduced by exogenous consumption of GABA [9]. GABA is naturally found in many kinds of seeds such as beans, rice, and sesame. GABA accumulation in plant seeds can be promoted by controlling temperature [10], oxygen concentration [11], microorganisms present [12], additives [13], heat shock [10], and cold shock [14] during soaking, germination, and fermentation processes. GABA is synthesized from glutamic acid by the glutamic acid decarboxylase (GAD) enzyme using vitamin B6 as a co-enzyme [15]. Vitamin B6 is found in six different forms: pyridoxine, pyridoxamine, pyridoxal, pyridoxine-5-phosphate, pyridoxamine-5-phosphate, and pyridoxal-5-phosphate [16]. Pyridoxal phosphate (PLP) is employed as a cofactor in the synthesis and degradation of GABA, as well as in the modulation of its action [17]. To increase GABA accumulation in

grains, activation of the rate-limiting enzymes, glutamate decarboxylase (GAD) and the GABA shunt, is essential. These enzymes convert endogenous and/or exogenous glutamic acid (Glu) to GABA [18].

Mung beans (*Vigna radiata* L.) are one of the healthiest foods due to their high protein and fiber contents. They are rich in functional components, with moderate starch and fat contents [19]. Moreover, mung bean sprouts are popular as an ingredient in many kinds of foods. They are a potential source of GABA at high levels, which is primarily produced during seed germination [20–22]. Among various seeds, the GABA content in germinated mung beans is higher than those found from germinated soybeans, red beans, and sesame [22,23]. Even though GABA is naturally synthesized by enzymatic action during germination, vitamin B6 may promote effective GABA production. Therefore, the effect of vitamin B6 in the form of pyridoxine hydrochloride (PN) on growth and GABA accumulation in mung bean sprouts was investigated for developing GABA-enriched products.

2. Materials and methods

2.1 Pre-germination (soaking)

PN solutions were prepared and added at various concentrations (0, 1, 10, and 50 mg/L) during mung bean soaking. Washed beans were soaked in PN solutions at room temperature for 12 h at a water:bean ratio of 1:5 (w/v). GABA was extracted from soaked beans with 4% (v/v) acetic acid [24]. The collected samples were then frozen for further analysis.

2.2 Germination (sprouting)

The germination procedure was slightly modified from that of Vann et al. [23]. Briefly, pre-germinated beans were allowed to sprout in the dark (enclosed in a paper container) for 5 days. Various concentrations (0, 1, 10, and 50 mg/L) of pyridoxine (in tablet form) were added to the soaking solutions. Moist beans were placed in a plastic receptacle with gauze on the bottom and tissue sheets on top to aid in germination. Every 12 h, the germinated seedlings in each group were moistened with 50 mL of a PN solution (0, 1, 10, or 50 mg/L). Data were collected every 24 h during germination. The lengths of 30 sprouts were measured daily. Two grams of bean sprouts were used for extraction of GABA with 8 mL of 4% acetic acid. The extracted samples were stored in a freezer prior to GABA analysis.

2.3 Determination of GABA contents

2.3.1 GABA extraction

The GABA extraction method was slightly adapted from Bai et al. [24] and Wang et al. [25]. In this procedure, 2 g of samples (raw, soaking and germinated beans) were suspended in 8 mL of 4% (v/v) acetic acid at room temperature with continuous vortex agitation. After an hour of extraction, materials were centrifuged at 10,000g for 10 minutes at room temperature. Finally, the supernatants were collected and kept at -20 °C in a freezer for further analysis.

2.3.2 GABA determination by high-performance liquid chromatography (HPLC)

GABA levels were determined using an HPLC (Waters Corporation, USA) equipped with an Inertsil ODS-3 (5 m, 4.6 x 250 mm, Japan) column, as described by Vann et al. [23]. The extracted samples were filtered (0.45 um) and placed in a vial for automatic sample insertion into the HPLC. Sodium dihydrogen phosphate hydrate and Na-P-toluenesulfonate were completely dissolved in deionized water and the solution pH adjusted to 3.5, with stabilization using ethanol. This served as a phosphate buffer. Then, a reagent buffer, fluoraldehyde o-phthaldialdehyde (OPA) was dissolved in ethanol and added into another reagent buffer that consisted of boric acid, sodium hydroxide, N-acetyl-L-cystein. The analytical condition was isocratic elution with a flow rate of 0.5 mL/min for pumps A and B and 0.2 mL/min for the solution pump. The column temperature was adjusted to 40°C. A multi- and fluorescence detector was used. GABA (5, 10, 20, 40, 60, and 80 mg/L) and glutamate (10, 20, 40, 80, 100, and 160 mg/L) reference curves were produced.

2.4 Statistical analysis

Three replicates were used to express all study data as mean ± standard deviation (SD) values. Analysis of Variance (ANOVA) employed IBM SPSS Version 28.0.1.0 software to analyze the significance levels between the means. Duncan's analysis with a 95% confidence level was then performed.

3. Results and discussion

3.1 Effect of pyridoxine hydrochloride (PN) on GABA content in soaked mung beans

GABA is essential for plant growth and development, regulating pH, nutritional balance (nitrogen supply), and stress responses [26]. Soaking creates a stressful environment due to a lack of oxygen, which affects metabolic activity and GABA accumulation in beans [27]. The concentration of GABA significantly increased after soaking compared to raw beans (Figure 1).

PN, a type of vitamin B6, was added to mung beans in concentrations of 1, 10, and 50 mg/L and soaked for 12 h. The results showed that after soaking, the highest GABA content, 80.18 ± 4.62 mg/100 g (dry basis), was found in a control group (with no PN addition), whereas the GABA content in raw beans was only 0.75 ± 0.20 mg/100 g. An increased PN concentration resulted in a negative effect on GABA accumulation in soaked mung beans. The GABA contents decreased to 70.41 ± 2.95, 63.70 ± 3.04, and 40.03 ± 2.04 mg/100 g as the PN concentration was increased from 1 to 10 and 50 mg/L during soaking, respectively.

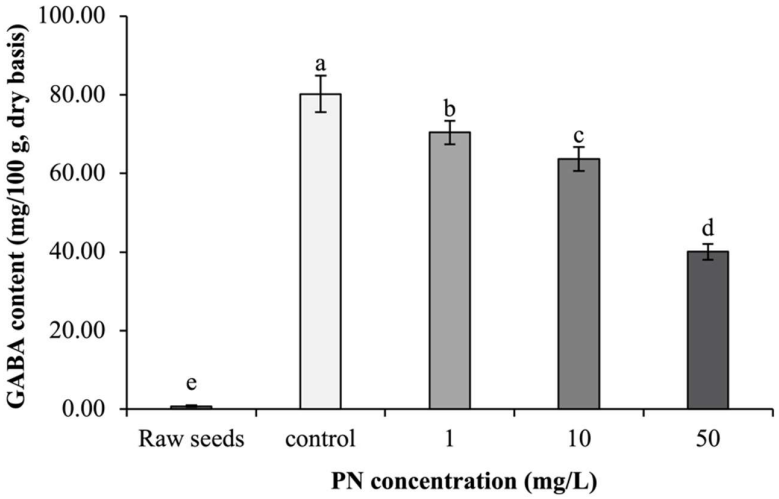

Figure 1 The effect of pyridoxine hydrochloride (PN) concentrations on GABA content in soaked mung beans. Data from triplicate experiments are expressed as mean ± SD (error bars). Values not sharing the same letter are significantly different at $p < 0.05$.

Soaking is the first step in germination. At this stage, the beans have an abiotic stress from a lack of oxygen. When water was absorbed into the seeds, metabolic activity from many enzymes started in response to stress, including GAD that promotes GABA production. However, our finding was that supplementation of PN provided a negative effect on GABA production in mung beans. In human metabolism, PN is taken by patients for stress release and brain improvement purposes. PN is absorbed in the human intestine and the liver turns a large portion of PN into pyridoxal phosphate (PLP), another form of vitamin B6 [28, 29]. As a result, PN becomes an active form (PLP) as a coenzyme for GAD. A plant cannot convert PN into the PLP form when its seeds are soaked. A high concentration of PN could contaminate the cell and block the active form of pyridoxal phosphate that already exists [30]. Consequently, raising the concentration of PN reduces the GABA content in soaked beans.

Normally, when plants are exposed to stressful conditions, GABA levels are increased. However, Chen and Xiong [31] postulated the necessity of PN for osmotic and oxidative stress tolerance. Therefore, it might be possible that when PN was added to the seed-soaking solution, it could reduce stress and stimulate plant growth, resulting in lower GABA accumulation.

3.2 Effect of pyridoxine hydrochloride (PN) on mung bean germination

Growth of mung bean sprouts was evaluated by measuring the bean sprout lengths (stems) every 24 h for five days. As shown in Figure 2, there is a slow extension in the length of bean sprouts on day 1 until day 3 of germination. The bean sprouts started growing leaves and roots on day 3 and expanded stem length very rapidly on days 4-5.

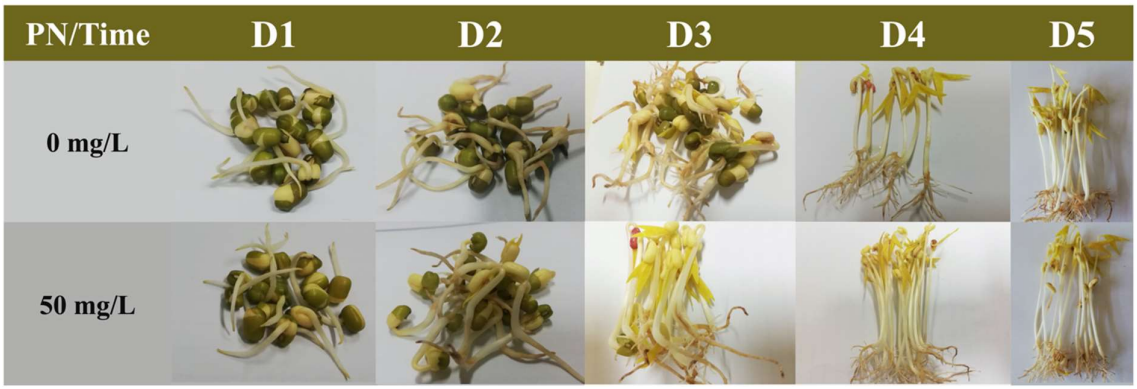

Figure 2 Mung beans stems undergoing germination for 5 days.

To determine the effects of PN addition on the growth of germinated mung beans, different concentrations of PN (0, 1, 10, or 50 mg/L) were applied every 12 h for 5 days. As indicated in Figure 3, the lengths of the mung bean sprouts were significantly extended with increasing PN concentrations. The group with a 50 mg/L PN treatment showed the greatest length, followed by the 10 mg/L PN treatment. The shortest stem length was the control, which was not different from those of the 1 mg/L PN treatment. Our findings clearly demonstrate the effect of PN on promoting the growth of mung bean sprouts.

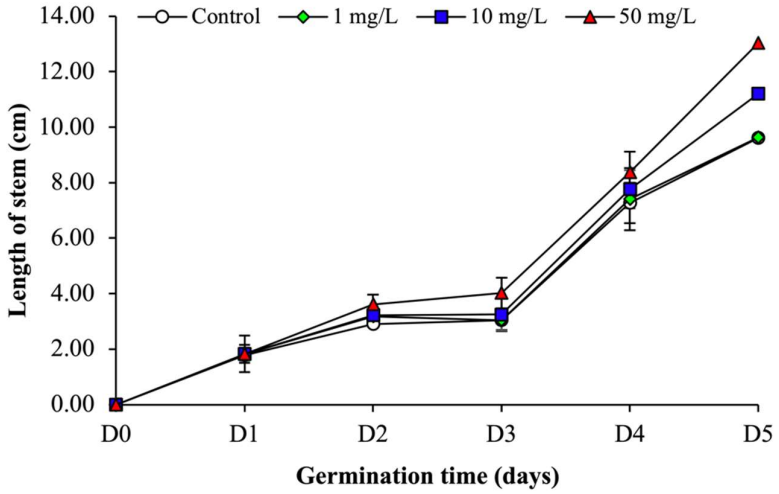

Figure 3 The effect of pyridoxine (PN) on stem length of germinated mung beans after 5 days in a dark place at room temperature. Germinated beans were sprayed every 12 h with various concentrations of PN (0, 1, 10 or 50 mg/L). Thirty bean sprouts were sampled and data expressed as mean ± SD (error bars).

Further investigation of the effect of PN focused on GABA accumulation in mung beans during germination. As shown in Figure 4, the GABA content profiles upon exposure to various PN concentrations (0, 1, 10, and 50 mg/L) show the same pattern. Soaked mung beans (D0) produced GABA only in the early stage, at day 1. From days 2 to 4, the GABA level decreased rapidly. On day 4, the GABA content was approximately 35% that of day 1. Moreover, the GABA content in germinated mung beans decreased with an increased PN concentration (Figure 4). Bean sprouts in the control group showed the highest GABA content, followed by those supplemented with 1, 10, and 50 mg/L of PN, in descending order. This indicates that PN supplementation has a negative effect on GABA accumulation in germinating mung beans.

When PN, with a critical role in plant development and stress tolerance [31], was added to germinating mung beans, it directly stimulated plant growth. Our findings indicate that at 50 mg/L, PN induced a 35.6% elongation of stem length over that of a control, while accumulation of GABA as a reserve protein was decreased with greater PN concentrations (Figure 4) due to GABA utilization for growth in germinating mung beans.

Similarly, increased GABA levels in the early germination stage were also observed in rice and maize seeds [20, 21]. GABA is synthesized from L-glutamic acid and stored as a reserve protein for plant growth. After 24 h, sprouted beans showed increased root extension, nutrients from the endosperm (which are sometimes found in

the cotyledons) were metabolized. While L-glutamic acid was limited, GABA was consumed in the Kreb's cycle, resulting in lower GABA levels [32].

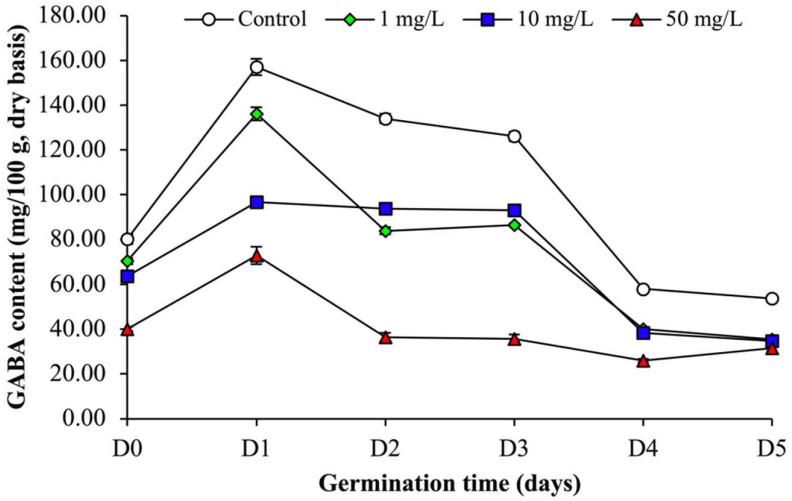

Figure 4 Effect of pyridoxine (PN) at different concentrations (1, 10, and 50 mg/L) on GABA production in germinating mung beans. The data are expressed as the mean ± SD of triplicate measurements.

According to our findings, PN supplementation during soaking and germination had no favorable effect on GABA enhancement in mung bean sprouts. However, this is the first report demonstrating PN growth promotion of these sprouts. Our findings will encourage future studies about the effect of PN in other kinds of sprouts.

4. Conclusions

Various PN concentrations were supplemented during mung bean soaking and germination to increase GABA accumulation. PN had a negative effect on GABA production during soaking and germination. GABA levels were dramatically decreased at greater PN concentrations. In contrast, PN showed an unexpected effect promoting the growth of mung bean sprouts. The findings of this study confirm that PN plays an important role in stress tolerance and is an effective growth stimulating substance during mung bean germination.

5. Acknowledgements

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