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Evaluation of GABA and GA Content in Cereals (Wheat, Spelt, Barley, Millet) Undergoing Germination

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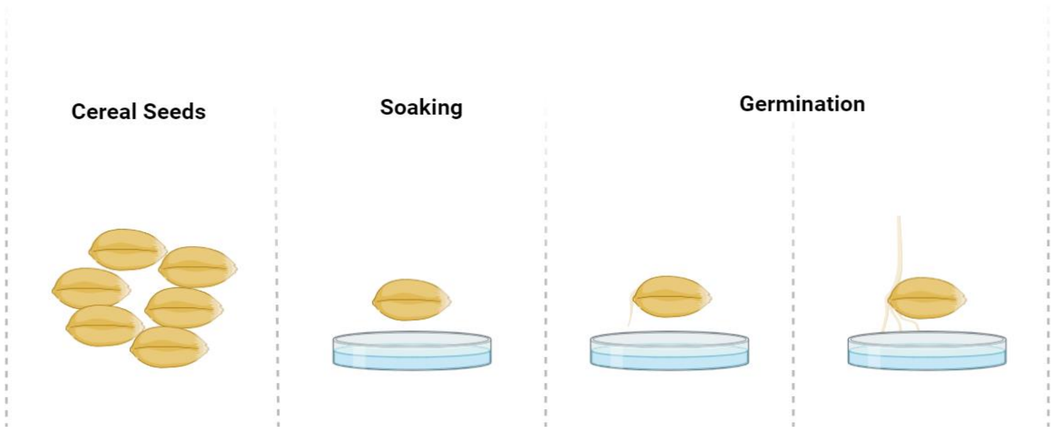
INTRODUCTION & AIM

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the mammalian central nervous system, regulating neuron excitability. GABA is synthesized from glutamic acid (GA) through decarboxylation, catalyzed by glutamic acid decarboxylase [1]. Given its numerous health benefits, increasing GABA levels in various food varieties, particularly in seeds, could lead to the development of functional foods or nutritional supplements [2].

Cereals, belonging to the *Poaceae* family, are essential plants that play a crucial role in providing sustenance for the global population, with around 65% of Bulgaria's total cultivated field area dedicated to cereal cultivation. Cereal crops are typically categorized into two main subgroups: temperate climate grains such as wheat, sorghum, rye, barley, triticale, and oats, and warm climate cereals including corn, sorghum, rice, and millet [3].

The aim of study was to investigate GABA and GA content in cereals (wheat, spelt, barley, millet) during soaking and germination, both with and without the application of cold stress.



METHOD

1.Plant Materials

Cereals (wheat, spelt, barley, millet) were acquired from local markets in Plovdiv, Bulgaria. The seeds of all germinated cultures underwent freezing and freeze-drying using the Alpha 1–4 LDplus laboratory freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Subsequently, the seeds were powdered before extraction, derivatization, and HPLC analysis.

2. Preparation of Extracts

The plant materials were ground into a fine powder just before the extraction and analysis. In the extraction phase, 1 g of powdered sample was combined with 15 mL of 75% ethanol and subjected to extraction on a magnetic stirrer for 1 h at room temperature. The resulting extracts were filtered, and the supernatants were collected for derivatization and subsequent HPLC analysis.

3. Derivatization of GABA and GA

The derivatization of GABA and GA followed a procedure outlined by Gong et al. [4], with slight adaptations. A derivatizing reagent for HPLC was prepared by blending 5 mg of dansyl chloride with 10 mL of acetone. Standard solutions of GABA and GA were prepared using ultra-pure water. During the derivatization step, 100 µL of the sample containing GABA and GA standard solutions or extracts were combined with 900 µL of a 0.1 M sodium hydrogencarbonate buffer (pH 8.7) and 1000 µL of the dansyl chloride solution to reach a final volume of 2 mL. The solution was homogenized by a vortex mixer and then heated for 1 h at 55°C. Subsequently, the solution was cooled to room temperature, filtered through a 0.45 µm RC syringe filter, and then used for HPLC analysis.

4. HPLC Analysis of GABA and GA Derivatives

The determination of GABA and GA derivatives was carried out using the UHPLC system Nexera-*i* LC2040C Plus from Shimadzu Corporation, Kyoto, Japan. The system was equipped with a UV-VIS detector and a binary pump. The column utilized was the Accucore (Thermo Fisher Scientific) C18 (2.1 mm × 150 mm, 2.6 µm), maintained at 30°C. Operational parameters included a flow rate of 0.2 mL/min and an injection volume of 5 µL. Derivative detection was performed at a wavelength (λ) of 254 nm. The mobile phase consisted of A: methanol and B: 900 mL of 0.05M sodium acetate in 100 mL of methanol (pH 8.0). The gradient elution profile commenced with 20% A from 0 to 10 minutes, increasing linearly to 60% A at 11 minutes and further to 100% A at 11 minutes. The system was run isocratically with 100% A from 12 to 17 minutes, followed by a linear decrease to 20% A from 18 to 25 minutes. The analytical method followed the protocol described by Pencheva et al. [5].

RESULTS & DISCUSSION

The experiments were conducted under two conditions: without the application of stress and with cold stress at -18°C for a period of 4 days. The HPLC analysis of the samples revealed a noticeable alteration in the levels of GABA and GA in the seeds following soaking and germination in both control groups (Figure 1 and Figure 2).

Wheat accumulated the highest content of GABA on the 3rd day of germination reaching 25.9 mg/100g DW, compared to 8.6 mg/100g DW in the non-germinated control. Germination increased GABA in all other cereals reaching 13.9 mg/100g DW in spelt, 8.8 mg/100g DW in barley, and 8.3 mg/100g DW in millet. Severe cold stress at -18°C was less conducive for GABA formation and did not result in further GABA increase. Interestingly, GA levels were notably higher than the GABA content, as wheat contained the highest amount of GA (130.3 mg/100g DW).

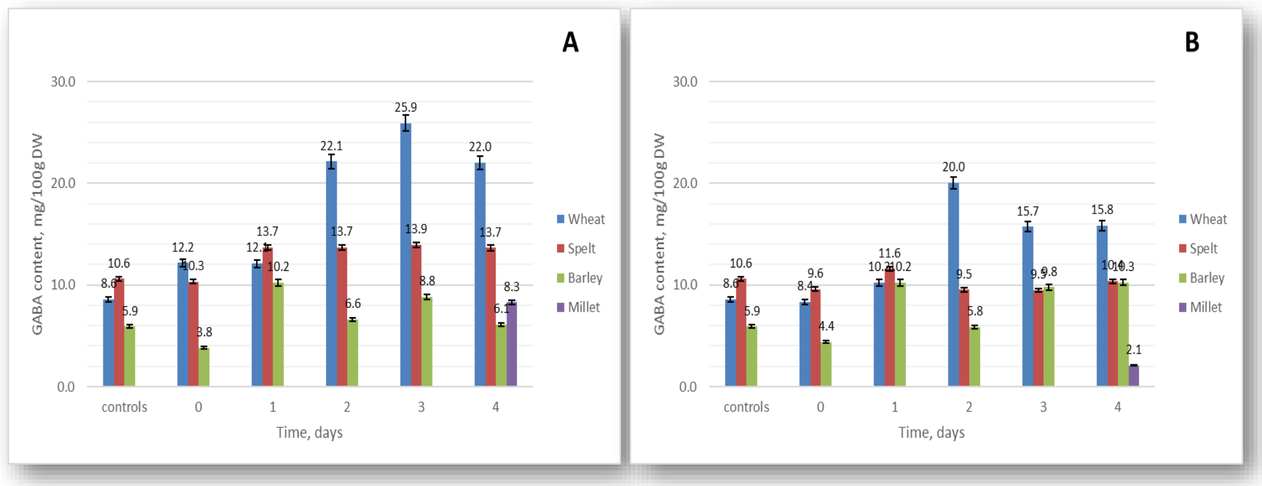


Figure 1. GABA content (mg/100g DW) in cereal seeds (Wheat, Spelt, Barley, Millet) during soaking and germination: A (no stress) and B (cold stress at -18 °C)

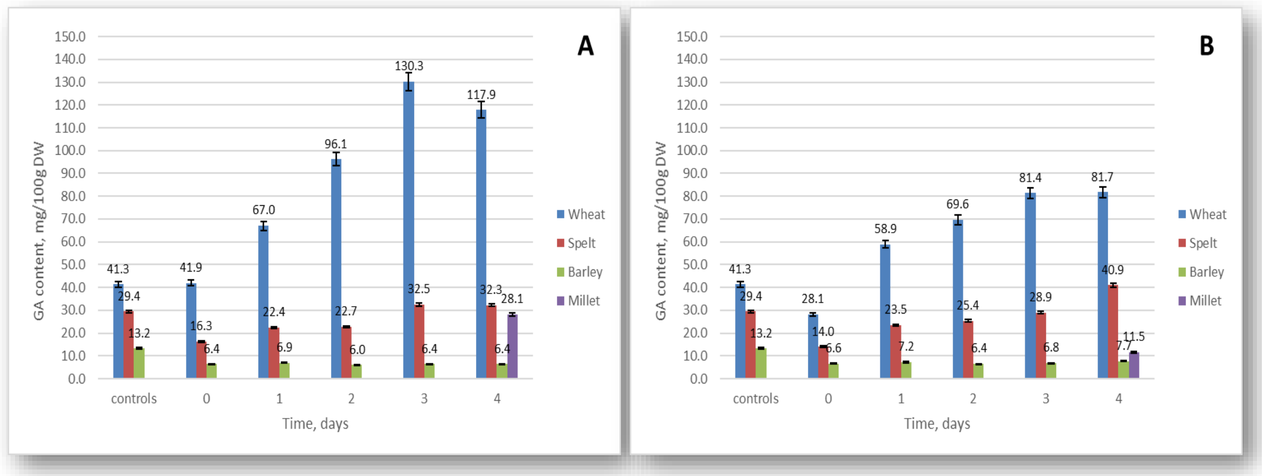


Figure 2. GA content (mg/100g DW) in cereal seeds (Wheat, Spelt, Barley, Millet) during soaking and germination: A (no stress) and B (cold stress at -18 °C)

CONCLUSION

Investigated cereals are capable of producing sufficient amounts of GABA after soaking and germination. The data indicates an insignificant difference between the non-stressed and stressed groups, suggesting that the application of cold stress in these cultures may be redundant. These optimistic results inspire further research to enhance GABA yields from natural food sources for nutritional supplements and functional foods.

FUTURE WORK / REFERENCES

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