CHROMATOGRAPHIC AND PHYTOBIOLOGICAL ASSESSMENT OF SOME NATURAL BORON-CONTAINING COMPOUNDS

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Abstract

Boron (B) has many roles in the plant kingdom, being an essential element for fundamental metabolic pathways, with reference to carbohydrates, ribonucleic acid, phenolic compounds, indole acetic acid derivatives, respiration, as well as for the structure of cell wall and integrity of plasma membrane, cell division, differentiation, and elongation at the level of meristematic tissues, growth of the pollen tube, grain maturation. It has also been shown that the low amount of B can cause sterility during herbal reproductive stage.

For identification and quantitative determination of some natural B-containing compounds (BCCs), such as boric acid, fructoboric acid and its derivatives (Ca–fructose–B complex and Zn–fructose–B complex), sodium tetraborate, high-performance thin-layer chromatography (HPTLC)–ultraviolet (UV) densitometry method was used, based on the following experimental conditions: stationary phase HPTLC silica gel G 60 F_{254} precoated glass plates, mobile phase 2-propanol–water 8:2 (v/v), derivatization with chlorogenic acid.

The phytobiological assessment of natural BCCs was carried out using Triticum test. A slight stimulation of the mean radicular elongation of wheat-germinated caryopses, compared to the reference, was recorded for fructoboric acid and its derivatives at a concentration of 0.1% expressed in B.

Key words: *natural boron compounds, high-performance thin-layer chromatography, UV densitometry, phytobiological activity, Triticum assay*

INTRODUCTION

Boron (B) is normally found in the soil as boric acid (BA) or borate. It is an essential element for herbal growth and development. Numerous mono-, di-, and polyhydroxy compounds react with BA/borate to form many ester derivatives and complexes. Simple carbohydrates (glucose, fructose, galactose, ribose, apiose), polyols (mannitol, sorbitol, galactitol), polyphenols (phenolic acids, coumarins, flavonoids, tannins), amino acids, glycoproteins, glycolipids are

the main organic compounds that can bind with BA/borate. *Bis*-hydroxy acid–borate complexes have been identified in many herbal resources. Depending on plant species, vegetal organ and reproductive stage, the abundance of carbohydrates–B complexes is variable: *e.g.*, peach nectar contains significant amounts of fructose–B–fructose, fructose–B–sorbitol, sorbitol–B–sorbitol complexes (Hu et al. 1997, Matsunaga & Nagata 1995). Coming from various plant sources, natural B-containing compounds (BCCs) normally enter the human diet, sometimes being frequently consumed in relatively large amounts (Biţă et al. 2021).

In the plant kingdom, B represents an essential element for fundamental metabolic pathways (*e.g.*, carbohydrates, ribonucleic acid, phenolic compounds, indole acetic acid derivatives, respiration), as well as for the cell wall structure, integrity of plasma membrane, cell division, differentiation and elongation of meristematic tissues, growth of the pollen tube, grain maturation. It is well known that low amounts of B can cause sterility during herbal reproductive stage (Abdel-Motagally & El-Zohri 2018, Galindo et al. 2018, Kekec et al. 2010, Rehman et al. 2012, Rerkasem & Jamjod 2004, Schnurbusch et al. 2010).

The aim of the study was the identification and quantitative determination of some natural BCCs, such as BA, fructoboric acid (FBA) and its derivatives [Ca–fructose–B complex (CaFBC), Zn–fructose–B complex (ZnFBC)], sodium tetraborate decahydrate (STB), through high-performance thin-layer chromatography (HPTLC)–ultraviolet (UV) densitometry, as well as their phytobiological assessment using *Triticum* test.

MATERIALS AND METHODS

Chemicals and solvents

BA reference, BA and STB samples were bought from Merck Millipore (Darmstadt, Germany).

FBA, CaFBC and ZnFBC were obtained according to the general green chemistry method for the semisynthesis of various carbohydrates–borate complexes (Scorei 2011).

Chlorogenic acid and azomethine H were acquired from Sigma-Aldrich (Munich, Germany).

The chromatographic grade (LiChrosolv®) solvents (2-propanol, 99% ethanol, water) and HPTLC Si 60 F_{254} 20×10 cm precoated glass plates were purchased also from Merck Millipore.

HPTLC–UV densitometry analysis

For BA reference, as well as for the analyzed samples, 0.1% solutions were prepared in water. Two microliters of each sample solution and 1, 2, and 3 µL from BA reference solution were applied on the HPTLC plate as 8 mm bands. Application of reference and sample solutions was made through the semiautomatic TLC sample spray band applicator (CAMAG Linomat 5 – Muttenz, Switzerland). The identification and quantitative determination of BCCs was achieved using CAMAG TLC Scanner III connected with a computer system and visionCATS software (visionCATS ver. 2.5). For visualization, CAMAG UV cabinet was used. Nikon Coolpix S8000 digital camera of 14.2 million effective pixels' resolution and 10× optical zoom (Nikon Instruments Europe B.V.) was used for the image acquiring. For the sample preparation, Bandelin Sonorex Super ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Berlin, Germany) and Eppendorf 5804 centrifuge (Eppendorf, Hamburg, Germany) were also used. Mixture of 2-propanol–water (8:2, *v/v*, pH 7.53) was used as mobile phase, and the HPTLC plate was developed in a twin-trough glass chamber (20×10 cm). The developing distance was 42 mm from the application position. The CAMAG Chromatogram Immersion Device 3 was used for derivatization by automated dipping of the plate (the setting for speed was 1 and five seconds for immersion time) in 0.1% chlorogenic acid ethanolic solution. The HPTLC plate was dried with an air dryer for about one minute, and then visualized at 365 nm. Derivatization with azomethine H was applied only for quantitative analysis of CaFBC, and the UV densitometry method was performed at 420 nm, with deuterium and tungsten lamp as radiation source. All

experiments were done in triplicate (Biţă et al. 2017 & 2021).

Triticum assay

Five different concentrations of analyzed BCCs (0.01%, 0.05%, 0.1%, 0.5% and 1% – expressed in B) were used for the assessment of germination and mean radicular elongation of ∗ *Triticum aestivum* L. subsp. *aestivum* (*Poaceae*), Dropia variety caryopses. After filtration, 10 mL of each aqueous solution was added in Petri dishes over 10 previously germinated wheat caryopses, with 10 mm long main radicle. The analysis was performed using a reference sample obtained in the same conditions and treated with 10 mL of purified tap water per day. The linear measurements, performed in triplicate, were accomplished for five days, at 24-hour intervals (Rău et al. 2009 & 2016).

RESULTS AND DISCUSSIONS

HPTLC–UV densitometry analysis

The experimental results about the HPTLC analysis of natural BCCs were evidenced in Figures 1–5 and Table 1.

For quantitative analysis, the calibration curve for BA (Figure 5) was determined through linear regression mode, with 5% range deviation (RD), 2.03% coefficient of variation (CV), and with 0.9954 correlation coefficient (*R*), as follows: $y = 1.585\times10^{-9}x + 2.908\times10^{-2}$.

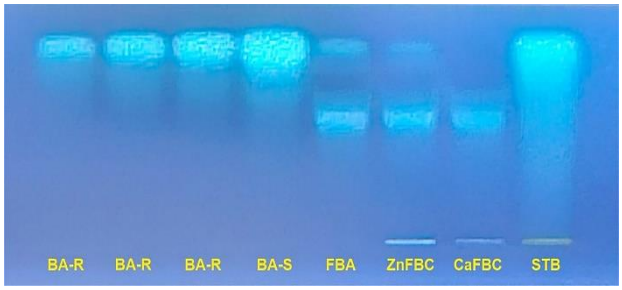

Figure 1. HPTLC chromatogram of natural boron-containing compounds, captured under UV light (365 nm), derivatization with chlorogenic acid.
BA-R: Boric acid reference; BA-S: Boric acid sample; CaFBC: Calcium–fructose–boron complex; FBA: Fructoboric acid; STB: Sodium tetraborate decahydrate; ZnFBC: Zinc–fructose–boron complex.

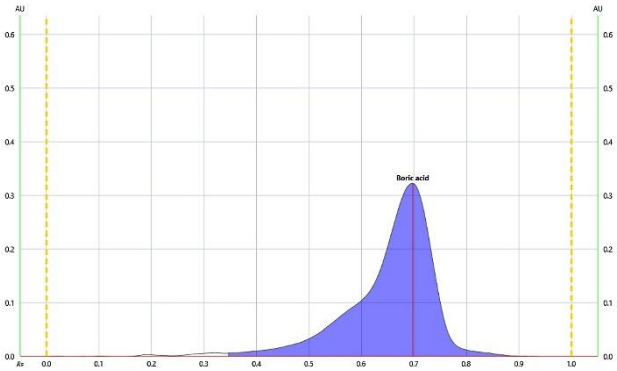

Figure 2. Densitogram of boric acid reference compound (R_f 0.70±0.01).

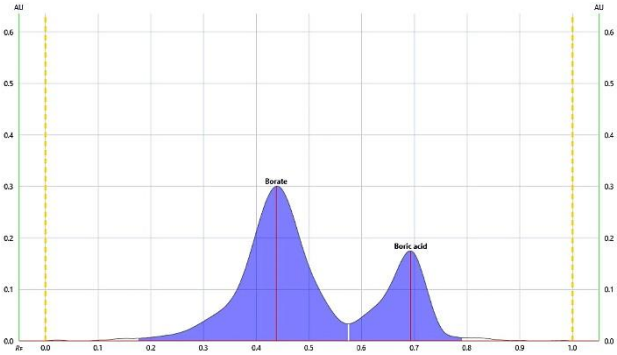

Figure 3. Densitogram of boric acid (R_f 0.70±0.01) and fructose–borate complex (R_f 0.43±0.01).

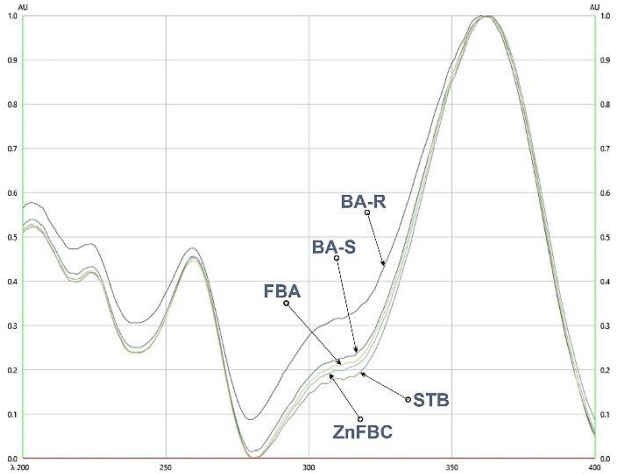

Figure 4. *In situ* UV spectra (365 nm) of the complex between boric acid and chlorogenic acid. BA-R: Boric acid reference; BA-S: Boric acid sample; FBA: Fructoboric acid; STB: Sodium tetraborate decahydrate; ZnFBC: Zinc–fructose–boron complex.

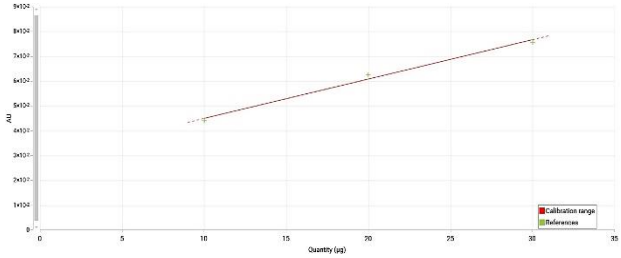

Figure 5. Boric acid reference calibration curve.

Table 1. Boron content of the analyzed samples

Sample	Analytical method	Boron content [mg/g]
BA-R	HPTLC–UV densitometry, derivatization with chlorogenic acid	177.46±3.55
BA-S		176.68±3.53
FBA		24.58±0.49
ZnFBC		25.39±0.51
STB		115.23±2.31
CaFBC	HPTLC–UV densitometry, derivatization with azomethine H	28.49±0.57

BA-R: Boric acid reference; BA-S: Boric acid sample; CaFBC: Calcium–fructose–boron complex; FBA: Fructoboric acid; HPTLC: High-performance thin-layer chromatography; STB: Sodium tetraborate decahydrate; UV: Ultraviolet; ZnFBC: Zinc–fructose–boron complex.

The results of HPTLC–UV densitometry analysis agree with the specialized research in the field of natural BCCs chemistry (Biţă et al. 2017 & 2021, Hu et al. 1997, Landi et al. 2019, Matsunaga & Nagata 1995, Scorei 2011, Shah et al. 2017).

Triticum assay

The experimental data of *Triticum* assay, compared with the reference, showed the inhibition of mean radicular elongation of wheat-germinated caryopses, determined by four concentrations of BCCs (0.01%, 0.05%, 0.5% and 1% – expressed in B). Only for 0.1% concentration (also expressed in B), a slight stimulation of the mean radicular elongation was observed in the case of FBA and its derivatives (Figure 6).

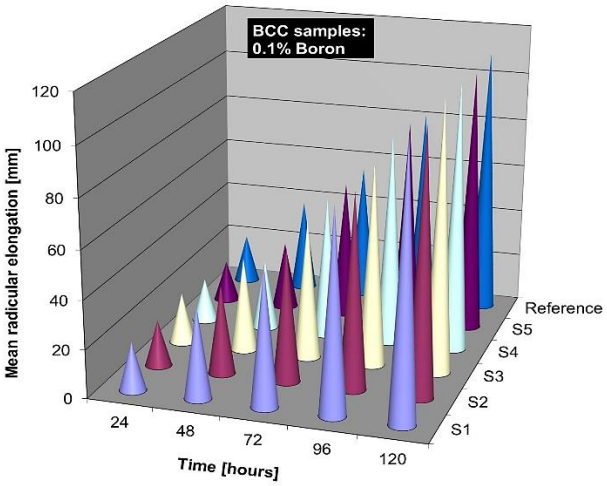

Figure 6. A slight stimulation of the mean radicular elongation, compared with the reference, was induced by 0.1% B concentration (*Triticum* assay). BCCs: Boron-containing compounds; S1: Boric acid; S2: Fructoboric acid; S3: Zinc–fructose–boron complex; S4: Calcium–fructose–boron complex; S5: Sodium tetraborate decahydrate.

Even if a specific biochemical function has not been identified in plants, B is essential for vegetal growth and development (Landi et al. 2019, Marschner 1995). B is usually applied as a foliar fertilizer to increase the nutritive value of cereals (Jin et al. 2008), as well as a component of hydroponic solutions for soilless vegetables growing (Johnson et al. 1957). Toxic symptoms, such as chlorosis/necrosis of the leaves and plant growth inhibition, can be caused by the high amounts of B in soil, growing substrates, and/or fertilizers (Biţă et al. 2021, Blevins & Lukaszewski 1998, Gupta & Solanki 2013, Shah et al. 2017).

B amounts in grains is improved through B fertilization, with the best results using top-dressing soil application of the bioactive nutrients (Galindo et al. 2018, Huang et al. 2000 & 2001, Wróbel 2009).

Wheat flowers fertility and grain yields can be improved by foliar spray applications of Ca–B composition, as follows: compared to the reference, the increasing of number of spikelets/spike (9%), number of grains/spike (24%), grain mass/spike (28%), spike mass (14%), number of spikes/m^2 (32%), grain yield (30%) (Camacho-Cristóbal et al. 2008, Zoz et al. 2016). Furthermore, in other field experiments, foliar application of Zn (as Zn

sulfate) and B (as BA) in wheat improved the main agronomic parameters: maximum plant height, grains/spike, thousand grains weight, grain yield, biological yield and the harvest index (Ali et al. 2009, Khan et al. 2019).

Considering its simplicity, reliability, and low cost, *Triticum* assay is useful mainly for the monitoring of environmental pollution, genotoxicity assessment, and phytotoxicity evaluation of different chemical compounds and nanomaterials (Jităreanu et al. 2019, Sahin et al. 2012).

CONCLUSIONS

Some natural BCCs, such as BA, FBA, CaFBC, ZnFBC, and STB, were identified and quantified by HPTLC–UV densitometry using 2-propanol–water 8:2 (*v/v*) as mobile phase and derivatization with chlorogenic acid. For the quantitative point of view, the calibration curve of BA was established by linear regression mode. *Triticum* test was applied for the phytobiological assessment of natural BCCs. At 0.1% concentration (expressed in B), FBA and its derivatives induced a slight stimulation of the mean radicular elongation of wheat-germinated caryopses, compared with the reference.

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