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Influence of various concentrations of 24-epibrassinolide on the kinetic parameters during isothermal dehydration of two maize hybrids

H. Waisi [a,b,*], B. Janković [c], B. Nikolić [d], V. Dragičević [e], I. Panić [f], T. Tosti [g], J. Trifković [g]

[a] Faculty for Ecology and Environmental Protection, University Union-Nikola Tesla, Cara Dušana 62-64, 11000 Belgrade, Serbia
[b] Institute of Nuclear Sciences "Vinča", University of Belgrade, Department of Physical Chemistry, Mike Petrovica Alasa 12-14, 11351 Vinca, Belgrade, Serbia
[c] Faculty of Physical Chemistry, University of Belgrade, Department of General and Physical Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia
[d] Institute for Plant Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia
[e] Maize Research Institute "ZP", Slobodana Bajića 1, 11185 Zemun Polje, Serbia
[f] Directorate of Measures and Precious Metals, Mike Alasa 14, 11000 Belgrade, Serbia
[g] Faculty of Chemistry, University of Belgrade, Department of Analytical Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia

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ABSTRACT

24-epibrassinolide (24-EBL) is plant steroid hormone that can regulate several physiological effects in plants, including promotion of cell growth and induction of heat stress tolerance. The purpose of this work is to investigate the influence of various concentrations of 24-EBL on the dehydration mechanism of seedlings of two maize hybrids (ZP434 and ZP704). Control and treated samples were subjected to isothermal conditions for dehydration processes. The effects of carbohydrates and mineral contents on the possible changes in the dehydration mechanism (from kinetics standpoint) were investigated. Comparing control samples to samples treated with 24-EBL, it was found that different dehydration mechanisms exist. The differences arise from the influence of 24-EBL, that causes a significant increase of phosphorus values for ZP704, whilst the reverse was identified for ZP434. It was assumed that the plumule sensitivity to dehydration stress originates from the interaction of water with primary amino groups as cations in polyamines, for lower concentrations of 24-EBL. It was found that the temperature variation (105–130 °C) leads to situations where trehalose does not arrive fast enough to "replace the water", because its handicap to binds (by hydrogen bonds) to biomolecules instead of water. It was found that 24-EBL cause changes of carbohydrates properties, which are important for the defense mechanism from environmental stresses.

1. Introduction

Maize is recognized as one of the most important grains in the world, it has many products and it is used both for human consumption and livestock feeding. Drying is a complex thermophysical and to some extent biochemical process comprising simultaneous heat and mass transfer (Sharma and Pant, 1994; Hatamipour and Mowla, 2006; Asthir, 2015; Chen et al., 2016). So, the basic concept of drying is water removal. Removing water from maize grain samples is not easy, keeping in mind that there are a number of factors that could affect the "nature" of the physicochemical mechanism of water leaving, such as concentrations of various present chemical ingredients (proteins, dietary fibers (as cellulose, hemicellulose and lignin), non-fiber carbohydrates, oil, and in particular at content of trehalose), which can be very sensitive to thermochemical stress and have an impact on the behavior of water within maize grain (Lunn et al., 2014).

Brassinosteroids (BRs) involve a group of polyhydroxy steroidal phytohormones, and they can, beside other phytohormones (Agami, 2013; Bulak et al., 2014) influence biotransformation and reduce toxicity of potentially harmful elements (Khripach et al., 2000; Rao et al., 2002). These hormones are structurally equivalent to vertebrates steroidal hormones. The BR hormones assist in developing tolerance to elevated temperatures by maintaining protein synthesis machinery, and they play positive roles in resistance to both biotic and abiotic stresses such as extreme temperatures, drought and pathogen attacks throughout different mechanisms (Li and Van Staden, 1998; Waisi et al., 2015; Yusuf et al., 2017).

Given the scarcity of data related to maize plant dehydration mechanism, particularly in terms of kinetic modeling under thermal influence (Pérez-Maqueda et al., 2006), the maize seedlings were chosen as a model for this type of investigation. In scientific literature which is concerning plant dehydration mechanism (Buitink et al., 2002;

* Corresponding author.
E-mail address: hadiwaisi@yahoo.com (H. Waisi).

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Leprince and Golovina 2002; Pammenter et al., 2002; Sun 2002), no available data for dehydration mechanism of maize seedlings (as a representative model), analyzed through kinetics parameters, and particularly by under the influence of 24-epibrassinolide (24-EBL). This fact is particularly relevant to the examination of dehydration mechanism for maize seedlings treated with the different concentrations of 24-EBL.

The aim of this study is a detailed investigation of kinetics of dehydration under the isothermal thermal conditions in an air atmosphere, with appropriate reaction model considerations, for all maize seedling parts {plumule, radicule, and the rest of the seed (RoS)}. Kinetic studies in this paper are based on a phenomenological models approach, which is an alternative to the fundamental model, and this is formulated to the fit of experimental results in terms of one reactant.

2. Materials and methods

2.1. Plant material, treatments, and growth conditions

Different concentrations of 24-EBL {The product is active ingredient of the commercial preparation, "Epin-Extra", dissolved in ethanol (Steber and McCourt 2001) obtained from GALENIKA-FITOFARMACIJA a.d. Zemun Company} in concentrations of 5.2×10^{-9}–5.2×10^{-15} M were used in experiments. The mentioned concentrations were used due to their optimal role in different processes related to plant growth and improvement of abiotic stress defense mechanisms (Clouse and Sasse 1998; Kumar et al., 2010; Agami, 2013;Waisi et al., 2017).Two maize hybrids, ZP434 (drought tolerant) and ZP 704 (older generation hybrid, which is a standard hybrid, more susceptible to stressful conditions) were tested. The seeds were produced in the "Maize Research Institute ZEMUN POLJE", Republic of Serbia.

The 800 previously measured seeds were divided to 4 equal portions, a control group and groups intended to be treated with different targeted concentrations of 24-EBL (5.2×10^{-9}, 5.2×10^{-12} and 5.2×10^{-15} M). Seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite solution and washed thoroughly with several changes of sterile distilled water. Each group of 200 seeds was germinated in four replicates (50 seeds per one box) at 2 L plastic boxes, on filter paper sheets. Each replicate was topped at the beginning of the experiment with 60 mL of different concentrations of 24-EBL solution and the control was topped with distilled water (Vardhini and Rao, 2003), under the phytothrone (Loške tovarne hladilnikov Škofja Loka, d.d., Slovenia) conditions at 24 °C (over day) and 21 °C (over night), with 12 h of light (110–160 μmol photons m^{-2} s^{-1}) / 12 h of dark regime (ISTA, 2010; Waisi et al., 2017). After 7 days, 25 uniformly grown seedlings from the each box (totally 100 seedlings per treatment) were divided with a scalpel to plumule, radicle and RoS. Seedling parts chosen for the further experiments were measured using an analytical balance (Ohaus Pioneer, model PA413) and stored in deep frizz at -70 °C.

Isothermal dehydration kinetics from all seedling parts of both maize hybrids were investigated at three targeted concentrations of 24-EBL, such as 5.20×10^{-9}, 5.20×10^{-12} and 5.20×10^{-15} M, respectively. Likewise, kinetic analysis was conducted for control samples of both hybrids, in order to compare the results, and the impact assessment of 24-EBL on dehydrating mechanism under continuous influence of thermal stress (Jan et al., 2013).

2.2. Sugar content determination

2.2.1. Preparation of sample test solutions

The method used for extraction and isolation of sugar compounds was a modification of the method originally developed for peach kernels analysis (Stanojević et al., 2015). The fresh samples of seedling parts were placed into the previously heated oven (Carbolite Gero GmbH & Co. KG) at 130 °C in an air atmosphere. Samples were tested at 130 °C because that temperature does not have an effect on most of sugars. Approximately, 0.25 g of maize sample (radicle, plumule, and RoS) was suspended in 5 mL of ultrapure water (Millipore Simplicity 185 S.A., 67,120, Molsheim, France), then ultrasonicated for 30 min, and centrifuged at 4000 rpm for 10 min. The supernatant was collected and the solid residue was re-extracted. The supernatants were combined, filtered through a 0.45 μm poly-tetra-fluoro-ethylene membrane filter (Supelco, Bellefonte, PA, USA) and 1 mL of this solution was transferred into auto-sampler vial. Individual sugars were determined and quantified using high performance anion-exchange chromatography with a pulsed amperometric detector (HPAEC-PAD).

2.2.2. Preparation of standard solutions

The calibration was performed with standard solutions of sugars, sorbitol (Sor) (Sigma–Aldrich, Steinheim, Germany), trehalose (Tre), fructose (Fru), sucrose (Suc), glucose (Glu) (Tokyo Chemical Industry, TCI, Europe, Belgium). Each individual sugar standard was dissolved in ultrapure water, and the stock solutions with a concentration of about 1000 ng/mL were prepared. Dilution of the stock solutions with ultrapure water yielded the working solutions at the concentration ranges that corresponded to the content of each sugar in the maize samples. The quality control mixture used for monitoring of instrument performance was prepared by diluting standards to the concentrations in the range 0.9–100 ng/mL (depending on the concentration in the samples).

2.2.3. HPAEC-PAD measurements

The chromatographic separations were performed using an ICS 3000 DP liquid chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a quaternary gradient pump (Dionex). The carbohydrates were separated on a Carbo Pac®PA100 pellicular anion-exchange column (4 × 250 mm, particle size – 8.5 μm, pore size – microporous, <10 Å) (Dionex) at 30 °C. The mobile phase consisted of the following linear gradient (flow rate, 0.7 mL/min.): 0–5 min., 15% A, 85% C; 5.0–5.1 min., 15% A, 2% B, 83% C; 5.1–12.0 min., 15% A, 2% B, 83% C; 12.0–12.1 min., 15% A, 4% B, 81% C; 12.1–20.0 min. 15% A, 4% B, 81% C; 20.0–20.1 min. 20% A, 20% B 60% C; 20.1–30.0 min. 20% A, 20% B 60% C, where A – 600 mM sodium hydroxide, B – 500 mM sodium acetate and C was ultrapure water. Before analyses, the system was pre-conditioned with 15% A, 85% C, for 15 min. Each sample (25 μL) was injected with an ICS AS-DV 50 auto-sampler (Dionex). The electrochemical detector was consisted of gold as working and Ag/AgCl as the reference electrode (Stanojević et al., 2015).

2.3. Mineral content

2.3.1. Microwave digestion

A microwave-assisted acid digestion system (with pressure-resistant PFA-coated stainless steel housing, which provides the highest possible level of safety in all operating conditions) (Berghof, Speedwave®Four, Harretstraße 1, 72800 Eningen, Germany) was used to extract the elements from the samples (radicle, plumule and RoS). Approximately, 0.50 g of dry sample was digested. The digestion procedure was based upon recommendations by U.S. EPA guides for method 3051B (EPA, 2007) (with HNO_3, HCl, and H_2O_2).

2.3.2. ICP-OES determination

The resulting solutions were analyzed by a Spectro Genesis ICP-OES instrument with Smart Analyzer Vision software (SPECTRO Analytical Instruments GmbH, Boschstr. 10, 47533 Kleve, Germany). Curves were recorded on the basis of individual {Ultra Scientific, U.S.A. (concentrations of 1 g L^{-1})} and the multistandards (SPS-SW2, LGC, UK) for the target elements and molecular species, Fe, $PO_4^-$and K. The confirmation was carried out with matrix spike samples for a three concentration.