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Changes in the content of D-*chiro*-inositol and its α-D-galactosyl derivatives during vegetation and desiccation of common buckwheat (*Fagopyrum esculentum* Moench)

Zmiany zawartości D-*chiro*-inozytolu i jego α-D-galaktozydowych pochodnych
podczas wegetacji i desykacji gryki zwyczajnej
(*Fagopyrum esculentum* Moench)

Summary. Common buckwheat (*Fagopyrum esculentum* Moench) is the only crop that contains D-*chiro*-inositol (DCI) in significant contents in vegetative tissues and its α-D-galactosyl derivatives in seeds. Besides DCI, buckwheat tissues contain small contents of D-pinitol (PIN) and *myo*-inositol (MIN) and their α-D-galactosyl derivatives. D-*chiro*-inositol is a health-promoting cyclitol of increasing importance in the treatment of some human diseases. However, changes in DCI content in stems, leaves and maturing buckwheat seeds during plant vegetation and under desiccation were not known. The present study analyzed the concentration of cyclitols and their galactosides in the stems, leaves and seeds of plants harvested on 79th, 94th and 123th days after sowing (DAS) and after desiccation at ambient temperature (23° ±2°C). D-*chiro*-inositol content in stems and leaves increased with vegetation, while the opposite trend was found in developing and maturing seeds. In the seeds, the accumulation of mono-galactosyl DCI derivatives increased, but at the same time, the content of mono-galactosyl PIN and MIN derivatives decreased.

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The desiccation process drastically increased the content of di-galactosyl derivatives of DCI and MIN in the seeds. The obtained results suggest a protective role of DCI and MIN di-galactosides against desiccation stress in buckwheat tissues.

Key words: common buckwheat, cyclitol galactosides, D-*chiro*-inositol, desiccation, *myo*-inositol, vegetation

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) is a dicotyledonous plant that is classified as a pseudo-cereal species due to its seed chemical composition similar to cereals, i.e. high content of starch and low proteins, and very low lipids [Podolska et al. 2021]. Buckwheat is a species that has retained many of the features of a wild plant, such as continuous growth and a long growing season [Kreft et al. 2020]. Buckwheat is used for food purposes as seeds or flour and its benefits for human health have been highlighted in several reviews [Giménez-Bastida and Zieliński 2015, Pirzadah et al. 2019, Huda et al. 2020].

Buckwheat tissues contain various flavonoids including flavonols (rutin and quercetin) and flavones (vitexin, *iso*-vitexin, orientin and *iso*-orientin) [Wiczkowski et al. 2014, Horbowicz et al. 2015, Dziadek et al. 2016]. Other components of buckwheat have received less research attention. Among these, for both scientific and health-promoting reasons, are important carbohydrates, especially α-D-galactosyl derivatives of D-*chiro*-inositol (DCI), called fagopyritols [Obendorf et al. 2012b, Brenac et al. 2013]. Seeds of common buckwheat accumulate a unique set of six fagopyritols, which are mono galactosides of the DCI (fagopyritol A1, fagopyritol B1), di-galactosides (fagopyritol A2, fagopyritol B2), as well as tri-galactosides (fagopyritol A3 and fagopyritol B3) [Horbowicz et al. 1998, Szczeciński et al. 1998, Steadman et al. 2001, Obendorf et al. 2000, Horbowicz and Obendorf 2005, Ma et al. 2005, Gui et al. 2013]. Fagopyritols belong to two distinct series, A and B, which differ from each other in the bonding position of galactose to DCI [Obendorf et al. 2012a, 2012b].

Fagopyritols are accumulated in buckwheat embryos during seed development and maturation on the basis of DCI produced in leaves and transported to seeds [Horbowicz et al. 1998]. However, supplying *myo*-inositol (MIN) to buckwheat stem explants markedly increases the concentration of DCI in leaf tissues indicating that this cyclitol is formed from MI [Ma et al. 2005]. On the other hand, delivery of DCI to plant explants elevated fagopyritol concentrations in soybean, pea and buckwheat seeds [Obendorf et al. 2004, Gomes et al. 2005, Lahuta et al. 2010]. Cereal plants also can translocate cyclitols to the synthesis of their galactosides in seeds [Lahuta and Goszczyńska 2010]. However, supplying buckwheat explants with D-pinitol (PIN) caused its accumulation in buckwheat seeds, but not PIN galactosides [Ma et al. 2005]. This may indicate that in addition to sucrose, sorbitol, raffinose and stachyose transport of both DCI and PIN is possible [Noiraud et al. 2001, Ma et al. 2005, Obendorf et al. 2004, Lahuta et al. 2010, Thomas et al. 2016]. The relatively low temperature during seed maturation resulted in an increase in fagopyritol A1 and fagopyritol B1 content in the buckwheat seeds, compared to the higher temperatures [Horbowicz and Obendorf 2005]. Accumulation of fagopyritol B1 was also associated with the acquisition of desiccation tolerance during seed development and maturation in planta [Horbowicz et al. 1998]. This wide range of biological properties of inositols has led to extensive research resulting in the publication of many reports and comprehensive reviews on these compounds and their derivatives [Antonowski et al. 2019, Wiśniewski et al. 2020, Siracusa et al. 2022].

Osmolytes such as amino compounds (proline) and saccharides (trehalose, fructans and polyols) play a major role in protecting the cytoplasm during the dehydration stress of plants [Yang et al. 2021]. Of these, the raffinose family oligosaccharides (RFO) as well as the galactosides of cyclitols are of particular interest [Sengupta et al. 2015]. These carbohydrates provide osmotic regulation and protect macromolecules and membranes. Moreover, they can be a carbon source for energy metabolism when photosynthesis is limited and play an important role in regulating biosynthesis and activating plant hormones [Eveland and Jackson 2012]. The protective effect of polyols is more effective than mono- and disaccharides due to the ease of forming hydrogen bonds between them and macromolecules, which protects the three-dimensional structures of macromolecules [Shen et al. 1997, Caramelo and Iusem 2009]. For instance, D-pinitol concentrations were higher in all organs of soybean [Dumschott et al. 2019] and transgenic *Arabidopsis thaliana* [Ahn et al. 2018] grown under water deficit.

On the other hand, cyclitols indicate a broad range of health-promoting properties, due to their blood glucose-lowering activity [He et al. 2021], anti-oxidative, anti-atherogenic, anti-inflammatory, and anti-cancer properties [Owczarczyk-Saczonek et al. 2018]. They are considered to be a dietary supplement for the treatment/prevention of gestational diabetes mellitus and type-2 diabetes, polycystic ovary syndrome [Antonowski et al. 2019, Gambioli et al. 2021]. Thus, the natural sources rich in some cyclitols, like DCI in common buckwheat, seems to be important for human nutrition [Giménez-Bastida and Zieliński 2015] and health [Gambioli et al. 2021] and a rational goal for breeding programs [Suzuki et al. 2020].

The aim of the research undertaken was to evaluate how aging of plant tissues and the process of their desiccation affect the content of saccharides, especially the content of D-*chiro*-inositol and its α-D-galactosyl derivatives in various organs of the buckwheat plant. The results obtained may be relevant during the potential use of buckwheat tissues as a source for the production of pharmacological preparations rich in DCI and its α-D-galactosyl derivatives.

MATERIALS AND METHODS

Plant cultivation and desiccation

A field experiment was carried in 2016 near Olsztyn (53°38′22″N, 20°23′12″E) using seeds of common buckwheat (*Fagopyrum esculentum* Moench, cv. Kora) purchased from on-line garden store Sadowniczy.pl. The seeds were sown on pseudopodsolic soil, which is characteristic for the region of Warmia and Mazury, at a density of about 250 seeds m^{-2}. The area of the harvested plot was 10 m^2. The buckwheat seeds were sown on 16 May 2016, and no mineral fertilizers or pesticides were applied during the cultivation. Plant samples were taken to the analysis and to desiccation experiment on 79th, 94th and 123th days after sowing (DAS). The total number of plants taken at each time period for analysis and desiccation was between 150 and 200. Intact plants were subjected to desiccation process (4 weeks, temperature 23 ± 2°C; RH = 40–50%; laboratory room) to an air-dry state and then seeds, leaves and stems were analysed separately.

Analyses of cyclitols and their galactosides

Samples of freshly harvested seeds, leaves and stems were frozen at –76°C, and then freeze-dried. Dried or freeze-dried samples of plant tissues were pulverized in a mixer mill (MM200, Retsch, Verder Group, Netherlands). Soluble carbohydrates were extracted from 40 to 45 mg of flour with 800 μl of ethanol: water (1:1, v/v, at 90°C for 30 min with continuous shaking at 300 rpm), containing 100 μg of xylitol (internal standard). Homogenates were centrifuged (20,000 × *g* at 4°C for 20 min) and aliquots (400 μl) of clear supernatants were deionized (with a 300 μl of a mixture of Dowex ion-exchanger resins, Sigma-Aldrich, St. Louis, USA) and brought to dryness in a speed vacuum rotary evaporator. The dry residues were derivatized with a 200 μl of mixture of TMSI/pyridine (1:1, v/v, Sigma-Aldrich, St. Louis, USA) at 80°C for 45 min. The TMS derivatives of carbohydrates were analyzed with the high-resolution gas chromatography method on a capillary column (Rtx-1, 15 m length, 0.25 mm diameter, 0.1 μm thickness of 100% dimethyl polysiloxane layer, Restek, Anchem Plus, Warsaw, Poland) in a gas chromatograph (GC2010, Shimadzu, Kyoto, Japan), under conditions described previously [Lahuta and Górecki 2010].

The results were calculated using the internal standard method. Standards of *myo*-inositol, D-pinitol, D-*chiro*-inositol were obtained from Fine Chemicals (Auckland, New Zealand), and galactinol was supplied by Wako Pure Chemicals Industries Ltd. (Osaka, Japan). Galactosides of D-pinitol (galactosyl pinitol A and B), were extracted from seeds of winter vetch *(Vicia villosa* Roth.) and purified as described previously [Szczeciński et al. 2000]. Fagopyritols A1, B1, A2 and B2 were isolated and purified from seeds of common buckwheat [Horbowicz et al. 1998, Szczeciński et al. 1998, Obendorf et al. 2000, Steadman et al. 2001, Gui et al. 2013].

Analyses of the buckwheat tissues were performed in three replicates. Analysis of variance (one way ANOVA) and Tukey's post-hoc test were used to check the significance of the differences. These calculations were performed using Statistica 12PL software (StatSoft, Tulsa, USA).

RESULTS

The high-resolution gas chromatography method used in this study demonstrated the presence of common carbohydrates (fructose, glucose, galactose, sucrose, maltose, cellobiose, raffinose, stachyose, sorbitol), *myo*-inositol (MIN), D-pinitol (PIN), D-*chiro*-inositol (DCI) and α-D-galactosyl derivatives of MIN, PIN and DCI in buckwheat seeds, leaves and stems. The obtained results of analyses were shown in Fig. 1–3 and Tables 2 and 3. Weather conditions occurring during the vegetation of buckwheat plants were shown in Table 1.

Table 1. The weather conditions in the buckwheat growing area (May – August 2016)

Month	Air temperature (°C)			Soil temperature (°C)		Number of rainy days	Exposure to sunlight, hours
	mean	maximal	minimal	mean	minimal		
May	14.7	27.5	-0.5	17.7	4.5	12	290
June	17.7	33.7	-0.2	21.9	6.8	11	366
July	18.3	31.0	8.5	21.5	11.4	19	167
August	17.3	29.5	5.0	19.6	9.5	19	172

Myo-inositol content was maintained during buckwheat vegetation in leaves and stems at a similar level, whereas during desiccation of plants collected on 79th and 94th day after sowing (DAS) a decrease in its content occurred (Fig. 1, Tab. 2 and 3). On the other hand, the desiccation of plants collected on 123th DAS did not cause a decrease in MIN. *Myo*-inositol content in immature (green) buckwheat seeds (79th DAS) was several times higher than in more mature seeds collected on 94th and 123th DAS (Fig. 1). Seeds desiccation, regardless of the degree of their maturity, caused MIN losses. Also DCI was present in immature buckwheat seeds (79th DAS) in the highest content, but the desiccation process led to a large decrease in its level. However, desiccation of more mature seeds (94th and 123th DAS) did not cause DCI losses (Fig. 1).

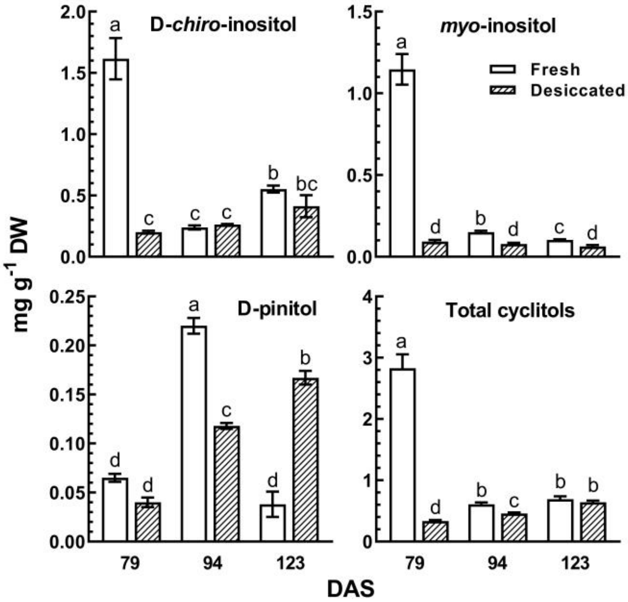

Fig. 1. Content of cyclitols in buckwheat seeds harvested on 79th, 94th and 123th day after sowing (DAS) and after desiccation by drying of whole plants at temperature 23 ±2°C. Mean results ± SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test

D-pinitol contents in buckwheat seeds were lower than DCI or MIN and fluctuated to some extent during seed maturation and desiccation (Fig. 1). The PIN content in stems was higher than that of the leaves and seeds of buckwheat, but its decreased after desiccation. It is interesting to note that clearly lower contents of DCI and PIN were shown in buckwheat seeds than in stems on 94th and 123th DAS. These results indicate a different physiological role of both cyclitols in the analyzed organs.

The cyclitols in buckwheat seeds are delivered from leaves. However, cyclitol monogalactosides may also be transported from leaves to seeds, as indicated by their presence in the stem. The contents of mono-galactosides of MIN and PIN were highest in young buckwheat seeds (79th DAS), and decreased sharply in more mature ones (93th and 123th DAS) (Fig. 2). The desiccation of young seeds (79th and 94th DAS) resulted in a marked decrease of their content, while an increase was observed in the mature seeds (123th DAS). An increase in PIN mono-galactosides content also occurred in the leaves and stems desiccated at late vegetative stage (123th DAS) (Tab. 2 and 3).

Table 2. Content (mg g^{-1} DW) of cyclitols and their α-D-galactosides in buckwheat leaves harvested on 79th, 94th and 123th day after sowing (DAS) and after desiccation at temperature 23 ±2°C. Mean results in rows ± SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test

Carbohydrate	79th DAS		94th DAS		123th DAS	
	fresh	desiccated	fresh	desiccated	fresh	desiccated
DCI	1.11 ±0.03^{d}	0.64 ±0.02^{e}	1.50 ±0.03^{c}	1.13 ±0.02^{d}	2.86 ±0.04^{b}	3.76 ±0.02^{a}
Fag A1	0.17 ±0.02^{b}	0.19 ±0.03^{b}	0.05 ±0.01^{c}	0.09 ±0.02^{bc}	0.36 ±0.01^{a}	0.04 ±0.01^{c}
Fag B1	0.21 ±0.03^{a}	0.13 ±0.04^{a}	0.07 ±0.02^{a}	0.13 ±0.01^{a}	0.10 ±0.03^{a}	0.18 ±0.04^{a}
Fag A2	0.12 ±0.03^{a}	0.09 ±0.01^{a}	0.08 ±0.01^{a}	0.09 ±0.02^{a}	0.10 ±0.02^{a}	nd
Fag B2	0.10 ±0.02^{a}	0.09 ±0.01^{a}	0.05 ±0.01^{a}	0.05 ±0.01^{a}	0.04 ±0.01^{a}	nd
myo-inositol	1.48 ±0.05^{b}	0.68 ±0.01^{e}	1.79 ±0.03^{a}	1.00 ±0.01^{d}	1.21 ±0.02^{c}	1.20 ±0.01^{c}
Galactinol	0.26 ±0.05^{a}	0.07 ±0.02^{b}	0.09 ±0.01^{b}	0.07 ±0.01^{b}	0.08 ±0.01^{b}	0.30 ±0.03^{a}
DGMI	nd	nd	nd	nd	nd	nd
Pinitol	0.10 ±0.01^{bc}	0.19 ±0.02 ^{b}	0.06 ±0.01^{c}	0.14 ±0.02^{b}	0.41 ±0.02^{a}	0.40 ±0.04^{a}
GPA	0.36 ±0.07^{bc}	0.38 ±0.07^{bc}	0.20 ±0.03^{c}	0.37 ±0.03^{b}	0.47 ±0.06^{b}	0.78 ±0.03^{a}
GPB	0.03 ±0.01^{c}	0.39 ±0.03^{a}	0.01 ±0.01^{c}	0.31 ±0.07^{a}	0.05 ±0.02^{bc}	0.10 ±0.01^{b}
Total cyclitols	2.69 ±0.09^{d}	1.51 ±0.07^{e}	3.35 ±0.07^{c}	1.78 ±0.05^{e}	4.48 ±0.08^{b}	5.36 ±0.07^{a}
Total galactosyl-cyclitols	1.15 ±0.21^{a}	1.34 ±0.19^{a}	0.55 ±0.07^{b}	1.11 ±0.11^{a}	1.20 ±0.12^{a}	1.40 ±0.11^{a}

Abbreviations: DCI – D-*chiro*-inositol; Fag A1 – fagopyritol A1; Fag B1 – fagopyritol B1; Fag A2 – fagopyritol A2; Fag B2 – fagopyritol B2; Galactinol – galacto-*myo*-inositol; DGMI – di-galacto-*myo*-inositol; GPA – galacto-pinitol A; GPB – galacto-pinitol B; nd – not detected

Table 3. Content (mg g^{-1} DW) of cyclitols and their α-D-galactosides in buckwheat stems harvested on 79th, 94th and 123th day after sowing (DAS) and after desiccation at temperature 23 ±2°C. Mean results in rows ± SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test

Carbohydrate	79th DAS		94th DAS		123th DAS	
	fresh	desiccated	fresh	desiccated	fresh	desiccated
DCI	1.31 ±0.07^{d}	1.02 ±0.02^{e}	1.32 ±0.05^{d}	2.28 ±0.02^{c}	2.57 ±0.04^{b}	3.02 ±0.02^{a}
Fag A1	0.21 ±0.02^{a}	0.05 ±0.01^{b}	0.02 ±0.01^{b}	0.19 ±0.02^{a}	0.09 ±0.02^{b}	0.27 ±0.03^{a}
Fag B1	0.17 ±0.01^{a}	0.06 ±0.01^{b}	0.03 ±0.01^{b}	0.08 ±0.01^{b}	0.03 ±0.01^{b}	0.08 ±0.01^{b}
Fag A2	nd	nd	nd	nd	nd	nd
Fag B2	nd	nd	nd	nd	nd	nd
myo-inositol	0.31 ±0.03^{a}	0.17 ±0.01^{b}	0.35 ±0.02^{a}	0.25 ±0.02^{a}	0.33 ±0.01^{b}	0.32 ±0.01^{b}
Galactinol	0.16 ±0.01^{a}	0.01 ±0.01^{b}	0.09 ±0.02^{b}	0.01 ±0.01^{b}	0.02 ±0.01^{b}	0.02 ±0.01^{b}
DGMI	nd	nd	nd	nd	nd	nd
Pinitol	1.53 ±0.31^{a}	0.05 ±0.01^{d}	0.62 ±0.11^{b}	0.19 ±0.03^{c}	2.03 ±0.34^{a}	0.40 ±0.14^{bc}
GPA	0.31 ±0.04^{a}	0.04 ±0.01^{b}	0.16 ±0.03^{a}	0.13 ±0.03^{a}	0.16 ±0.02^{a}	0.32 ±0.07^{a}
GPB	0.05 ±0.01^{b}	0.01 ±0.01^{b}	0.01 ±0.01^{b}	0.05 ±0.01^{b}	0.01 ±0.01^{b}	0.11 ±0.01^{a}
Total cyclitols	3.15 ±0.29^{ab}	1.24 ±0.04^{c}	2.29 ±0.17^{b}	2.72 ±0.06^{b}	4.93 ±0.48^{a}	3.74 ±0.15^{a}
Total galactosyl--cyclitols	0.90 ±0.09^{a}	0.17 ±0.04^{c}	0.31 ±0.06^{bc}	0.46 ±0.07^{b}	0.31 ±0.06^{bc}	0.90 ±0.11^{a}

Abbreviations: DCI – D-*chiro*-inositol; Fag A1 – fagopyritol A1; Fag B1 – fagopyritol B1; Fag A2 – fagopyritol A2; Fag B2 – fagopyritol B2; Galactinol – galacto-*myo*-inositol; DGMI – di-galacto-*myo*-inositol; GPA – galacto-pinitol A; GPB – galacto-pinitol B; nd – not detected

The presence of mono-galactosides of DCI (fagopyritols A1 and B1) was confirmed in the present study in maturing seeds and during vegetation of buckwheat leaves and stems (Fig. 2, Tab. 2 and 3). The content of Fag A1 (Gal-*chiro*-inositol A1) and Fag B1 (Gal-*chiro*-inositol B1) in buckwheat leaves and stems was low (0.03–0.36 mg g^{-1} dry weight (DW) and fluctuated slightly during vegetation and desiccation. Considerably higher contents of Fag A1 (0.7–1.4 mg g^{-1} DW), and especially Fag B1 (2.0–8.5 mg g^{-1} DW) were found in buckwheat seeds (Fig. 2). In immature seeds (79th DAS), desiccation caused an increase in Fag A1 and Fag B1 contents, while in more mature seeds (94th and 123th DAS) their decrease. The content of Fag B1 gradually increased during seed maturation from ca. 2.0 mg g^{-1} DW to ca. 8.5 mg g^{-1} DW, but desiccation of young seeds (79th DAS) increased its content, while this process in more mature seeds (94th and 123th DAS) caused a decline to 5 mg g^{-1} DW.

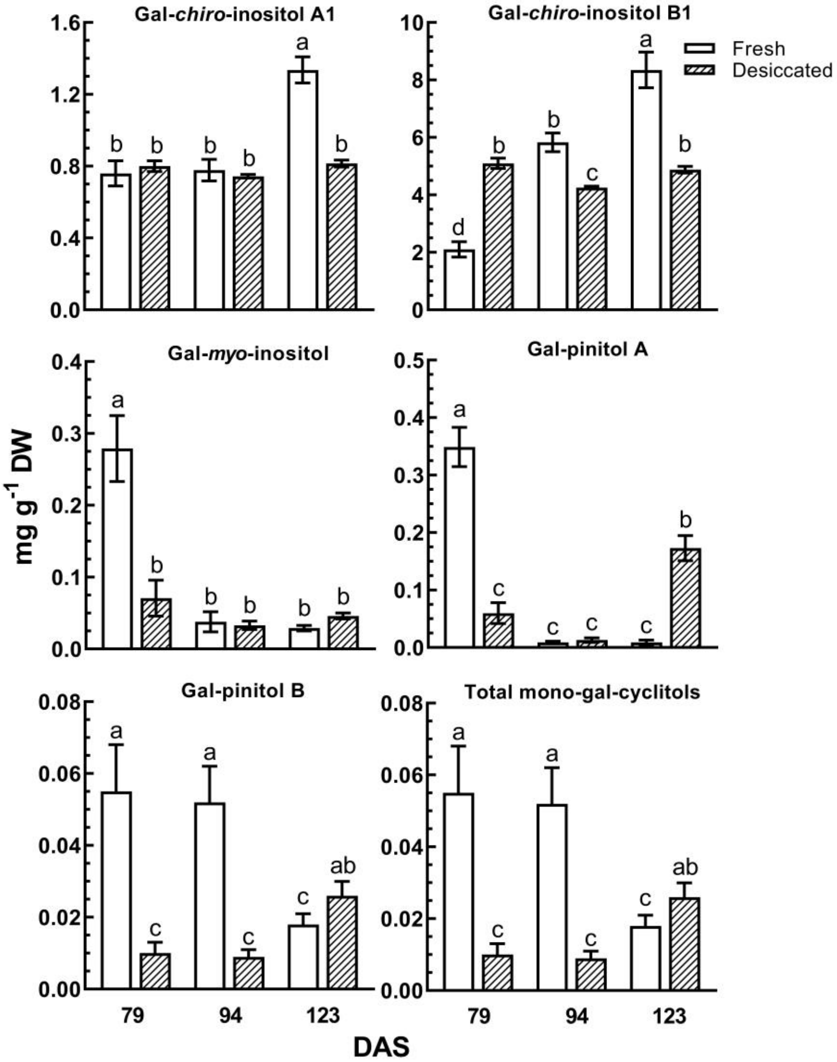

Fig. 2. Content of mono-galactosyl cyclitols in buckwheat seeds harvested on 79th, 94th and 123th day after sowing (DAS) and after desiccation by drying of whole plant at temperature 23 ±2°C. Mean results ± SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test

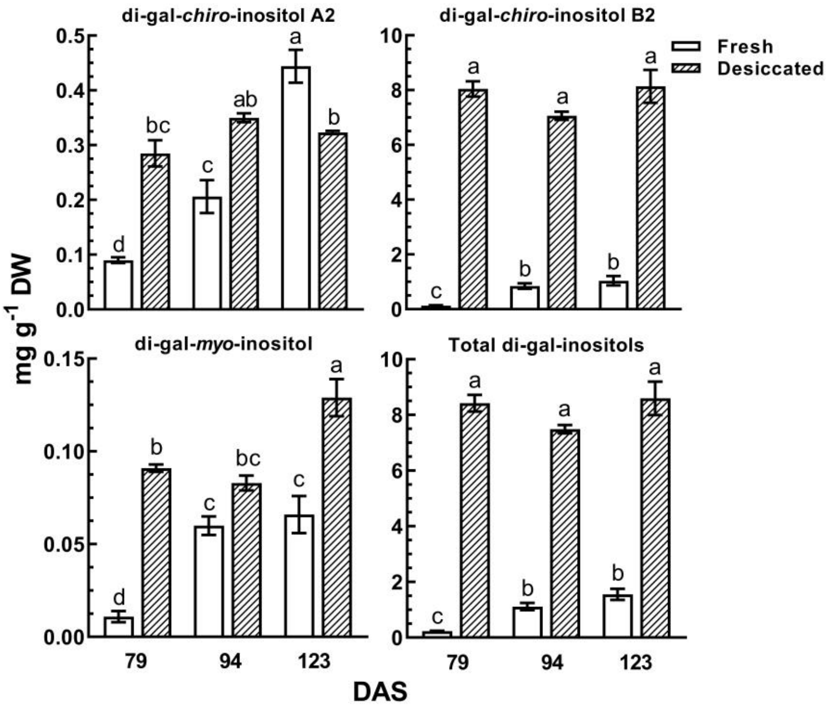

Fig. 3. Content of di-galactosyl cyclitols in buckwheat seeds harvested on 79th, 94th and 123th day after sowing (DAS) and after desiccation by drying of whole plant a temperature 23 ±2°C. Mean results ± SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test

In present study the presence of di-galactosides of MIN and DCI was demonstrated in the maturing buckwheat seeds (Fig. 3), as well as in leaves, but not in stems (Tab. 2 and 3). The contents of MIN and DCI galactosides gradually increased during maturation of seeds, and the desiccation process generally increased their levels as well. This process resulted in a particularly large increase in the content of Fag B2 (di-gal-*chiro*-inositol B2) (Fig. 3). Digalactosides of MIN and DCI are present in small contents in buckwheat leaves and are absent in stems.

DISCUSSION

Myo-inositol (MIN), D-*chiro*-inositol (DCI) and D-pinitol (PIN) are natural compounds involved in many biological pathways [Thomas et al. 2016]. The MIN and PIN are commonly found in plant tissues while DCI is found more rarely. Recently, using modern methods, the presence of measurable amounts of DCI in many plant species has been confirmed [Ratiu et al. 2019]. The well-known plant source of DCI is the genus *Polygonaceae*, especially seeds of *Fagopyrum* species [Obendorf et al. 2012a, 2012b].

The content of cyclitols in other organs of this species has not been analyzed so far [Ma et al. 2005]. During the completion of this study all buckwheat tissues examined contained measurable contents of the MIN, DCI and PIN (Fig. 1, Tab. 2 and 3). The presence of PIN as well as α-D-galactosyl derivatives of DCI in buckwheat leaves, stem and inflorescences was shown for the first time.

During desiccation of plants tissue, polyols and other sugars can partially replace lost of water to protect important macromolecules and biological membranes from destruction [Caramelo and Iusem 2009]. However, in the case of young buckwheat organs/tissues the stress due to desiccation is distinct from that of mature ones. Particularly large decreases in DCI and MIN occurred after desiccation of young seeds and leaves, which may indicate that these tissues are particularly susceptible to such stress due to the lack of defense systems or their incomplete activity in combating it. This means that metabolic processes also take longer and then cyclitols can be further metabolized.

Among osmolytes accumulated during the stress caused by drought stress and cold stress is D-pinitol (PIN) [Bertrand et al. 2007, Hornyák et al. 2022]. The decrease in PIN content after desiccation of young leaves, stems and seeds of common buckwheat indicates that PIN there does not play a protective role or, that such young tissues do not have fully developed defense mechanisms against this stress. The presence of PIN in buckwheat tissues may be due to the putative involvement of this cyclitol as an intermediate in the biosynthesis of DCI from MIN [Horbowicz et al. 1998, Horbowicz and Obendorf 2005]. DCI in leaf tissues is formed from MIN, as demonstrated by supplying MIN to buckwheat explants, which resulted in a marked increase in DCI content [Ma et al. 2005]. DCI is transported from leaves to seeds, as increasing the supply of DCI to buckwheat explants increases the accumulation of fagopyritols in seeds [Ma et al. 2005]. Supplying the DCI to soybean, peas and cereal explants also elevated fagopyritols concentration in seeds of these species [Gomes et al. 2005, Lahuta and Goszczyńska 2010, Lahuta et al. 2010].

The presence of PIN has not yet been found in seeds and other organs of common buckwheat [Ma et al. 2005, Brenac et al. 2013]. These mentioned studies were conducted under controlled laboratory conditions and on another cultivar of buckwheat than the present one in which plants from a field cultivation were used. Field conditions with wide temperature fluctuations (Tab. 1) could have caused a response that resulted in a relatively high PIN content (Tab. 3). The presence of MIN, PIN and DCI in buckwheat stem indicates probabaly that these cyclitols are transported to seeds where they can be used for the synthesis of their α-D-galactosides. This confirms previous data showing that multi-day supplementation of buckwheat explants with PIN and DCI resulted in a significant increase in their content in leaves and massive accumulation in maturing seeds [Ma et al. 2005].

The most important in buckwhat, in terms of their content, are the α-D-galactosides of DCI (fagopyritols) [Obendorf et al. 2012a, 2012b]. Among broadly cultivated plants, only seeds of common buckwheat contain a unique set of mono-, di-, and tri-α-D-galactosides of the DCI [Horbowicz et al. 1998, Obendorf et al. 2012b] instead of galactosides of sucrose – raffinose family oligosaccharides, common for legume and cereal seeds [Obendorf et al. 2012a]. Fagopyritols were found as a major soluble carbohydrates in buckwheat seed embryos and their accumulation is associated with the acquisition of desiccation tolerance during seed development and maturation

[Horbowicz and Obendorf 2005, Obendorf et al. 2012b]. The current study demonstrates that fagopyritols are present in both seeds and other organs of buckwheat such as leaves, stem and inflorescences, which are new data.

A large increase in the content of fagopyritol B2 may indicate its important protective role in seeds when the whole plant being exposed to dessication. This process probably results in a gradual inhibition of DCI transport to the seeds and other physiological disorders including changes in enzymatic activity. This may cause enzymes associated with galactosylation to target the attachment of galactosyl residues to mono-galactosides of MIN and DCI (galactinol and fagopyritol B1, respecively) resulting in the production of their di-galactosides. This resulted in an increase in MIN and DCI di-α-D-galactosides, while mono-galactosides levels decreased (Fig. 2 and 3). However, this hypothesis requires further detailed studies.

The phenomenon of reduction of DCI mono-galactosides content in seeds during whole plant dessication is different from that observed during physiological maturation and desiccation of seeds on the intact living buckwheat plant [Horbowicz et al. 1998, Ma et al. 2005]. In these previous experiments, a marked increase of Fag. B1 content in embryos was demonstrated. It has also been suspected that this may be related to the acquisition of desiccation tolerance by the seeds of this species. The results obtained in the current study show that the physiological desiccation involving a growing plant is different from that occurring in a plant desiccated as a whole.

The increase mono-galactosides of PIN after desiccation of fully mature seeds, leaves, and stem of buckwheat may support suggestions that the older tissues have developed defense mechanisms against such type of stress. It was previously reported that buckwheat tissues do not contain PIN or its galactosides [Horbowicz et al. 1998, Ma et al. 2005]. However, in those experiments buckwheat plants were grown in a greenhouse or in temperature-controlled growth chambers. In the present study, buckwheat was grown in an open field and the air temperature varied from 0°C to 25°C (Tab. 1). This may be the reason for the differences that occurred, as well as genetic diversity between cultivars. It is known that cold stress during vegetation may be responsible for the enhanced accumulation of PIN and DCI in plants [Bertrand et al. 2007, Zuluaga et al. 2020].

Comparing the results of the saccharide profile of buckwheat seeds obtained in this study, we can see their similarity with those obtained previously [Horbowicz et al. 1998, Horbowicz and Obendorf 2005, Ma et al. 2005]. However, obtained results are not consistent with those recently published by Nešović et al. [2021]. The reasons for these discrepancies are unknown, but probably an important factor is the analytical methods used as well as the ability to use pure standards. They used to analyses a HPLC method equipped with classical 250 mm x 4 mm column and pulsed amperometric detector. Using this method, they found in buckwheat grain the presence of almost 265 g kg^{-1} DW and 394 g kg^{-1} DW of fructose and glucose, respectively, 48.1 g kg^{-1} DW of sucrose, and the absence of DCI, MIN and PIN, as well as their galactosides. The occurrence of such large amounts of monosaccharides is unprecedented in mature seeds. Previously, the presence galactosides of DCI, PIN and MIN was confirmed by their isolation, purification, and structural analyses by ^{13}C nuclear magnetic resonance (NMR) [Szczeciński et al. 1998, Obendorf et al. 2000, Steadman et al. 2001, Gui et al. 2013].

SUMMARY

All plant organs of buckwheat plant had the same saccharide profile, with the exception of the leaves, which had no measurable levels of di-α-D-galactoside *myo*-inositol (DGMI), and the stems, which had no DGMI, as well as fagopyritols A2 and B2. The contents of D-*chiro*-inositol (DCI) in young seeds were markedly higher than in more mature ones. In contrast, in buckwheat stem and leaves, DCI contents increased with vegetation, and the desiccation process further increased its levels, confirming the protective role of this cyclitol in response to such stress.

Desiccated stems and leaves of buckwheat from the final phase of their vegetation can be a source for obtaining preparations containing DCI and other cyclitols. The presence of free cyclitols in buckwheat seeds indicates that these cyclitols are translocated from the leaves and undergo galactosylation there, confirming previously published data. The desiccation process at ambient temperature leads to a slow loss of water in the leaves and stem and this causes the metabolic processes to be continued for some time, as a result of which the saccharide content can change markedly.

The desiccation process of the whole buckwheat plant very clearly increased the di-α-D-galactosides content of both DCI and MIN in seeds, indicating their protective role against such stress.

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