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Original Scientific Paper

An insight into seasonal changes of carbohydrates and phenolic compounds within the moss *Polytrichum formosum* (Polytrichaceae)

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ABSTRACT:
The same population of the polytrichaceous moss *Polytrichum formosum* was studied over four different periods of the year, analysing its carbohydrate and polyphenolic content and dynamics related to environmental seasonal changes. A total of 18 different types of sugars (including mono-, di-, tri- and tetra-saccharides) and four sugar alcohols were determined. Chlorogenic acid was the most represented among the 10 detected phenolic compounds. As inferred by the sugar content, sucrose, fructose and glucose were the most dominant sugars, but it is worth mentioning the abundance of trehalose and turanose at least during one of the observed seasons. The presence of four trisaccharides and one tetrasaccharide within *P. formosum* should be highlighted, as well as the first reports of turanose, isomaltotriose, panose and rhamnose within this species. The quantitative changes over the year clearly demonstrate carbohydrate dynamics in relation to seasonal climatic variation. Sugars are shown to be significant constitutive molecules within *P. formosum,* but also physiologically active compounds, i.e. signalling and energy storage and supplier molecules. We assume that phenolics have moss-supportive effects during oxidative stress and biotic interaction.

Keywords:
bryophyte, chemical content, climate, sugars, phenolics

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INTRODUCTION

Bryophytes, a group of non-vascular plants among tracheophytes, with over 20,000 species worldwide, are known to harbour interesting chemical constituents. Despite being the second biggest group of terrestrial plants, their ecological and biological features continue to fascinate investigators.

Bryophytes are chemically very interesting due to their unique compound content of high biological and ecological relevance (Asakawa *et al.* 2013a). Additionally, the liverwort subgroup may chemically differ a great deal from the other two bryophyte subgroups (mosses and hornworts). They contain numerous oil bodies composed mainly of terpenoids and small aromatic compounds. Mosses, on the other hand, have more than twice the number of species compared to liverworts (ca. 14,000) and are chemically less investigated. Fewer chemical constituents were characterised in mosses than liverworts (Peters *et al.* 2018). In mosses, benzoic, cinnamic and phthalic acid derivatives, aromatic compounds containing nitrogen, coumarins and also terpenoids are found more frequently (Asakawa *et al.* 2013a).

A few hundred newly described compounds in recent years come from bryophytes (Peters *et al.* 2018). However, the ecological relevance of these remains unclear (Asakawa *et al.* 2013b), although there is evidence for some of them to be included in the defence against both biotic and abiotic stresses (Cornelissen *et al.* 2007). The chemical content of bryophytes is known to some extent, but remains underreported. Additionally, the chemical constituent records are rarely related to the plant environment and are thus insufficiently explained and remain obscure (Sabovljević *et al.* 2016).

Hence, despite considerable interest, the biochemical adjustments of bryophytes to environmental changes are poorly documented (Klavina 2015a). The link between the chemical constituents related to seasonal changes in bryophytes has been the subject of very few studies (e.g. Sun *et al.* 2013; Wagner *et al.* 2013; Mateo *et al.* 2016; Thakur & Kapila 2017; Klavina *et al.* 2018; Peters *et al.* 2018). Additionally, the sugar content and sugar dynamics within bryophyte species require further research (Klavina 2015a, b).

The aim of this study was to follow, document and discuss seasonal sugar profiles as well as phenolic dynamics during a one-year period within the same population of the hair-cap moss *Polytrichum formosum* Hedw., Polytrichaceae.

MATERIAL AND METHODS

Polytrichum formosum belongs to the tallest terrestrial mosses (Polytrichales) and can form tufts up to 5–10 cm high. It overgrows acidic to neutral soil and slopes within deciduous woodlands. It can be considered a mesophilic species in terms of humidity and light conditions.

The sampling was performed seasonally, starting in autumn 2017 and finishing in summer 2018 from the same patch (i.e. population) for further laboratory analyses. A patch moss (containing gametophores only, i.e. green parts in the haploid generation) from the same population was sampled at around noon, and kept in paper bags after the cleaning of mechanical impurities prior to further laboratory treatment under -70°C. Namely, the plants were collected in the Fruška Gora National Park, near the village of Rakovac (N 45.159094°, E 19.775948°; altitude 221m). The collections were made in mid-November 2017 (autumn season), mid-February 2018 (winter season), mid-May 2018 (spring season) and mid-July 2018 (summer season). Mid-season terms were chosen with the aim of avoiding seasonal climatic irregularity.

According to the data available from the Republic Hydro-Meteorological Institute of Serbia, the autumn and winter of 2017 were not significantly different from the long-term average in terms of both precipitation and temperature. The year 2018 was on average a slightly warmer year measured by the station nearest to the population studied (> 1°C). However, 2018 was very wet (starting already in January) and the summer period was also marked by a significantly higher amount of rain (ca. 50%) than normal (as regards the first half of the year).

Sample preparation. 100 mg moss (*P. formosum*) was extracted with 10 mL mixture of methanol/water (7/3 V/V) for 30 min in an ultra-sound bath (RK100, Bandelin, Berlin, Germany). Following extraction the suspensions were centrifuged at 12,000 rpm for 15 minutes. The supernatant was collected and the rest was re-extracted twice according to the procedure described below.

The solvents from the collected supernatants were lyophilyzer-evaporated (Freeze dryer GAMMA 1-16 LCS) and the solid residue was transferred to a 100 mL normal flask and diluted with ultrapure water to the mark. Before analysis the solutes were filtered through 0.22 μm syringe filters (Petrović *et al.* 2022).

Analysis of sugars. The standards of glucose, sucrose, fructose, maltose and trehalose were obtained from the Tokyo Chemical Industry, TCI, (Europe, Belgium); galactose, raffinose and maltotriose were purchased from the Tokyo Chemical Industry, TCI, (Tokyo, Japan); sorbitol, manose, galactitol, rhamnose and mannitol were purchased from Sigma-Aldrich (Steinheim, Germany); all the other reagents and solvents were of the highest available purity and were purchased from Merck (Darmstadt, Germany). All the aqueous solutions were prepared using ultrapure water (Thermofisher TKA MicroPure water purification system, 0.055 μS/cm).

The standard solutions of sucrose, glucose and fructose were prepared at a concentration of 1000 ng/mL, whereas the other standard compounds were prepared at a concentration of 100 ng/mL. The calibration standards were made by diluting these stock solutions with ultrapure water. The quality control mixture used for monitoring instrument performance was prepared by diluting the standards to concentrations in the range 0.9–100 ng/mL (depending on the concentration in the samples).

Chromatographic separations were performed using a DIONEX ICS 3000 DP liquid chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a quaternary gradient pump (Dionex, Sunnyvale, CA, USA). The carbohydrates were separated on a Carbo Pac®PA100 pellicular anion-exchange column (4 × 250 mm) (Dionex, Sunnyvale, CA, USA) at 30°C. The mobile phase consisted of the following linear gradients (flow rate, 0.7 mL/min): 0–5 min, 85% A, 15% B; 5.0–5.1 min, 83% A, 15% B, 2% C; 5.1–12.0 min, 83% A, 15% B, 2% C; 12.0–12.1 min, 81% A, 15% B, 4% C; 12.1–20.0 min 81% A, 15% B, 4% C; 20.0–20.1 min 60% A, 20% B 20% C; 20.1–30.0 min 60% A, 20% B 20% C; where A was ultrapure water, B – 300 mM sodium hydroxide and C was 300 mM sodium acetate. Prior to the analyses, the system was preconditioned with 85% A and 15% B for 30 minutes. Each sample (25 μl) was injected with an ICS AS-DV 50 au-

tosampler (Dionex, Sunnyvale, CA, USA). The electrochemical detector consisted of gold as the working and Ag/AgCl as the reference electrode.

The limits of detection (*LOD*) and limits of quantification (*LOQ*) were calculated from the regression line for points near the expected limit, using the following equations:

$$LOD = (3.3 \times SD) / a$$
$$LOQ = (10 \times SD) / a$$

where *SD* is the standard deviation of the response (the standard error value for the coefficient *a*) and *a* is the value of the slope obtained from the linear regression (Fortić Aškić *et al*. 2015).

Standard solutions of glucose, fructose, sucrose (25, 50 or 75 ng in 5 mL) and sorbitol, galactitol, mannitol, trehalose, rhamnose, manose, galactose, raffinose, maltose and maltotriose (2.5, 5.0 and 10 ng in 5 mL) were added to the nectar. The recoveries of the sugars and sugar alcohols are given by $F/(F_0 + A)\times100$ %, where F is the concentration of sugars or sugar alcohols in the spiked sample, F_0 is the concentration of sugars or sugar alcohols in the unspiked sample and A is the spiked concentration (Gorjanović *et al*. 2020).

The concentration of the carbohydrate obtained from the calibration curves was multiplied by the dilution and subtracted by the weight of the samples.

Analysis of phenolics. A Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Bremen, Germany) with a diode array detector (DAD) connected to a TSQ Quantum Access Maxtriple-quadrupole mass spectrometer (Thermo Fisher Scientific, Basel, Switzerland) was used to separate, identify, and quantify the methanol-soluble components of the *P. formosum* gametophores. Elution was performed at 30°C on a Hypersil gold C18 column (50 × 2.1 mm), with 1.9 µm particle size (Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of (A) 0.01% acetic acid and (B) acetonitrile (MS grade, Fisher Scientific, Loughborough, UK), which were applied in the following gradient elution: 5–20% B for the first 3.0 min, 20–40% B 3.0–5.0 min, 40–50% B 5.0–7.5 min, 50–60% B 7.5–8.5 min, 60-95% B 8.5–10.5 min, from 95% to 5% B until 12th min and 5% B until 15th min. The flow rate was set at 0.4 mL min^{-1} and the detection wavelengths at 260 and 320 nm. The injection volume was 15 µL. The analyses were performed in triplicate. For more details, please refer to Mišić *et al*. (2015).

The phenolic acids and flavonoids were identified by direct comparison with commercial standards. The total amount of each targeted compound in the gametophyte material was calculated based on calibration curves of pure compounds (calibration levels in the range from 1 ng mL^{-1} to 50 µg mL^{-1}) and expressed as ng per 100 mg fresh weight.

RESULTS AND DISCUSSION

During the seasonal analyses of the *P. formosum* chemical constituents, a total of 18 different types of sugars (including mono-, di-, tri and tetra-saccharides) and 4 sugar alcohols were determined. The sugars for the seasonal analyses were chosen based on previous literature data and the standards available. Although they were present within the target population throughout all the seasons, the quantities were changeable due to seasonal conditions.

The total amount of sugar molecules varied with the plants accumulating the highest value of the total sugars in the autumn period when preparing the whole organisms for the period of low assimilation or even complete inactivity i.e. overwintering. During the winter period the total sugar content decreased indicating usage in minimally physiological active processes (such as respiration). In the spring time the decline of total sugars discontinued, and with the environmental changes, started to increase slightly with the recovery of full physiological activity. The peak of the total sugar molecule accumulation was achieved by autumn when the plants were preparing for winter inaction. Frost tolerance is also related to total sugar content in some bryophytes (e.g. Rütten & Santarius 1992). However, we analysed individual sugars in all the seasons and it was found that the autumn was the season to accumulate more sugars than winter. The difference may be a result of the methodology applied and the calculation per dry weight. Nevertheless, the sugars in both cases are assumed to be related to the overwintering of *P. formosum*.

The results obtained clearly showed that the main sugars, i.e. glucose, fructose and sucrose vary among the seasons as a consequence of changing environmental conditions (Fig. 1). Glucose and fructose demonstrate similar changing patterns, in contrast to sucrose. Sucrose was highly presented within the plants of *P. formosum* during summertime, but decreased manyfold in the winter period. Within the transitional seasons of spring and autumn the amount of sucrose was almost equal. Interestingly, the concentration of fructose and glucose were the highest within the plants of *P. formosum* during winter time.

These results are expected to some extent due to the fact that sucrose is accumulated during summer time and transformed into its monomers (i.e. fructose and glucose) in the winter period. This winter increase of monosaccharide (namely fructose and glucose) in *P. formosum* is probably related to adaptation to the cool period. Higher levels of osmoprotectants within the moss cells prevent water loss, the cytoplasm gains in viscosity and the phenomena of plasmolysis and cell damage are rather rare (e.g. Ljubešić *et al*. 2005).

The interchangeability of the two monomer sugar units during the transitional seasons (spring and au-

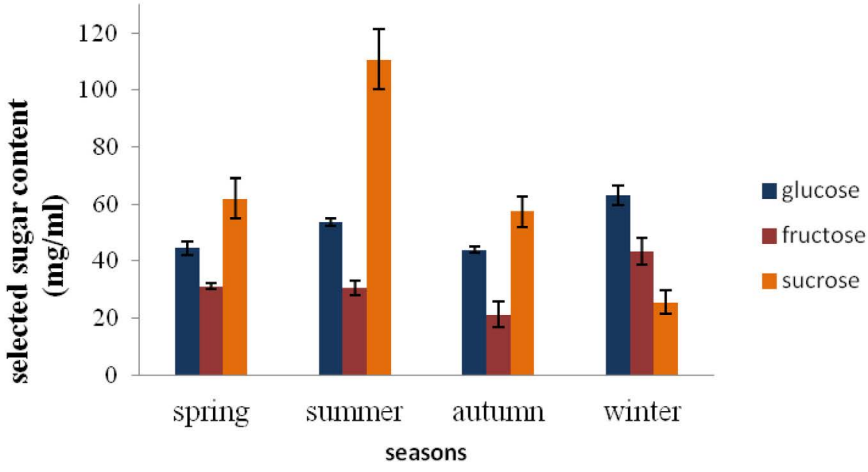

Fig. 1. The comparison of sugar contents (sucrose, fructose and glucose) in four seasons within the same population of *Polytrichum formosum*. Each data point represents the mean of three independent assays.

	Detected sugars	Spring	Summer	Autumn	Winter
Monosaccharides	Arabinose	8,534	6,998	4,449	2,129
	Glucose	44,558	53,733	43,994	62,967
	Fructose	31,137	30,397	21,119	43,340
	Ribose	1,411	2,804	3,705	1,166
	Galactose	14,545	11,927	1,516	3,628
	Rhamnose	3,710	3,059	2,575	2,415
	Xylose	2,806	2,298	8,057	1,827
	Sucrose	61,910	110,831	57,332	25,501
Disaccharides	Trehalose	1,894	5,346	83,751	2,567
	Melibiose	2,365	3,038	1,119	1,633
	Turanose	29,840	10,023	31,649	30,797
	Maltose	6,887	0,270	0,649	0,341
	Isomaltose	0,140	2,344	0,193	1,597
Trisaccharides	Raffinose	11,815	1,256	1,566	1,127
	Isomaltotriose	0,438	0,580	0,580	0,414
	Maltotriose	1,015	0,966	0,965	0,939
	Panose	2,837	3,533	8,229	3,276
Tetrasaccharides	Stachyose	1,125	1,056	1,140	1,563
Sugar alcohols	Sorbitol	8,519	12,167	10,303	12,966
	Galactitol	0,897	1,310	1,019	0,456
	Glycerol	9,840	8,100	11,488	3,882
	Mannitol	4,454	4,055	2,360	4,937

min max

Fig. 2. The seasonal changes of sugars (mg/mL) per fresh weight of *Polytrichum formosum* gametophores represented by the heat map graph.

tumn) is assumed, bearing in mind that there are no significant variations in the total concentrations of glucose plus fructose in comparison to sucrose. However, it should be taken into account that some of those compounds also act as signalling molecules and/or participate in the overall stress response of many plants (e.g. Rolland *et al.* 2002).

Fig. 2 shows the content and dynamics of various sugars during the four-season cycle. Within the heat map (Fig. 2) the presence of trehalose in autumn is in-

dicative. This is a dimer sugar containing two glucoses. It is known to be overrepresented in plants exposed to cold, but also to those under salt stress (Lunn *et al.* 2014). Further, its function in plants is to signal attacks by pathogens, insects and other herbivores. It is also involved in the interaction of plants with beneficial microbes, and other symbionts (e.g. Rodriguez-Salazar *et al.* 2009). Although trehalose was found in cryptogams (algae, bryophytes and ferns) (Figueroa & Lunn 2016), with the exception of desiccation-tolerant resurrection plants, there were few reports of trehalose being present in angiosperms (Iturriaga *et al.* 2000). However, Phan *et al.* (2020) stated that not much is known even in model moss *Physcomitrium patens* (Hedw.) Mitt. The same authors stated that highly accumulated trehalose in resurrection plants, to which numerous mosses belong, participates in the survival of extreme heat and oxidative stress as well as dehydration. The presence of enzymes essential for sensing and signalling sugars and growth regulators are documented in *P. patens*, and are thus assumed to play a role in the developmental balance between caulonema and chloronema (Phan *et al.* 2020). Previously, trehalose was identified in some moss species. Smirnoff (1992) studied sugars in *P. formosum,* but trehalose was not detected. The same author reported trehalose to be known only from sporophyte tissue from related *Polytrichum juniperinum* Hedw. However, the presence of trehalose was documented in *P. formosum* by Renault *et al.* (1992), both in gametophytes and sporophytes, and we confirm its presence in gametophore tissue.

Turanose is a reducing disaccharide, present within the tested population throughout the year, but significantly less in the summertime. It is an analogue of sucrose and as a non-metabolisable sugar is shown to affect growth patterns in tracheophytes (e.g. Gonzali *et al.* 2005). Loreti *et al.* (2001) even speculated that distinct sensors sense trehalose and sucrose analogues. We were not able to find any documentation of this sugar reported in bryophytes, and thus not in *P. formosum*. Its function in mosses, i.e. seasonal changes, is yet to be elucidated.

Sorbitol is present in higher concentrations in winter and summer periods and lowest in spring time. Its role in osmotic adjustment, the prevention of osmotic stress and accumulation in the vacuoles are documented for vascular plants. Similarly, mannitol is an isomer of sorbitol and is evenly distributed over the seasons, with slightly lower amounts detected in the autumn period. This suggests the interchangeable physiological function of the two isomers. Additionally, two sugar alcohols, namely glycerol and galactitol, are detected with their peak seen in the autumn. Sorbitol is rather widely documented in liverworts (e.g. Marschall *et al.* 1998) but also in mosses (e.g. Pejin *et al.* 2012). We assume these to be related to the rapid change of the hydration/dehydration state, due to the absence of protective cuticles.

Galactose is a monosaccharide, combined with glucose and fructose in trisaccharide raffinose. It was detected in significant amounts during the spring and summer seasons, interfering with raffinose, which is probably accumulated in spring when the available water, light and temperature conditions are close to optimal, before the period of harsh conditions (other seasons - the time of physical and physiological drought), when it is probably used to increase the osmolarity of the moss cell plasma by hydrolysis. A similar pattern was detected for another trisaccharide, maltotriose, but to a lesser extent.

Additionally, a further two trisaccharides, isomaltotriose and panose, were also identified in the moss *P. formosum*, evenly distributed over the seasons. These were not previously reported in this moss species.

Isomaltose is a structural isomer of sucrose, highly accumulated in the summer period in the moss *P. formosum*, while maltose is the intermediate product of starch degradation by hydrolysis, highly present in the spring when growth is vigorous and an energy source required.

Xylose and panose, in similar amounts, are predominant in the autumn season, but present to a lesser extent throughout the year. This is rather expected since xylose is a structural carbohydrate which is included in the stability of the moss stem and to some extent in the formation of conductive elements.

The ribose peak, screened in autumn within the plants of *P. formosum,* is probably related to the formation of gametangia and the increased reproductive efforts, i.e. cell division, vigorous and energy efficient growth, since ribose forms part of the ATP energy molecule as well as nucleic acids.

In plants, arabinose is a key component of cell wall polymers and glycoproteins, as well as flavonoids, and signalling peptides. It was evenly present through the seasons in the studied population.

Rhamnose, a monosaccharide, forming part of the polymers in the cell walls of vascular plants, is also known as part of the decoration of various specialised metabolites. We are not aware of previous reports in *P. formosum*. In the studied population, it was highly present in the spring when the extension and growth of the stem occurs.

Raffinose and stachyose are known to be energy acting molecules in seed plants. Galactinol together with raffinose produce stachyose and *myo*-inositol. Galactosyl oligosaccharides, such as raffinose and stachyose are known to have many important biological functions, such as promoting the growth of beneficial bacteria (Wu *et al.* 2021). Stachyose was slightly more present in the analysed moss plants during the summer time.

Slightly higher concentrations of melibiose were detected in the summer. This compound is known to play a role in the plant-fungus interface in tracheophytes (e.g. Lingner *et al.* 2011). Indeed, fungal hyphae were observed within the basal part of the material sampled.

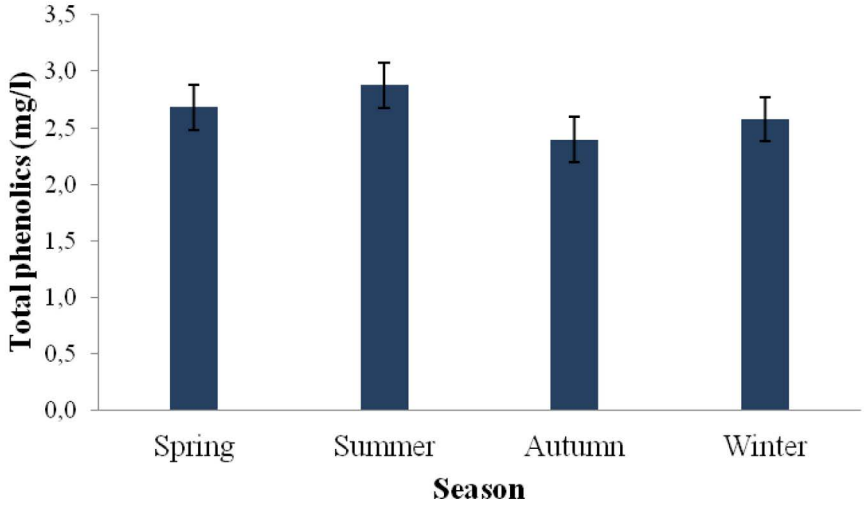

Fig. 3. The comparison of the total phenolic contents in four seasons within the same population of *Polytrichum formosum*. Each data point represents the mean of three independent assays.

	Spring	Summer	Autumn	Winter
Gallic acid	0,057	0,037	ND	0,016
Protocatechuic acid	0,254	0,319	0,233	0,251
Chlorogenic acid	0,980	1,258	0,991	1,193
p-Hydroxybenzoic acid	0,409	0,429	0,362	0,317
Caffeic acid	0,487	0,473	0,517	0,505
Vanillic acid	0,199	0,198	0,184	0,163
p-Coumaric acid	0,197	0,077	0,073	0,074
Quercetin 3-O-glucoside	0,024	0,014	ND	0,011
Ferulic acid	0,038	0,035	0,025	0,027
Quercetin 3-O-rhamnoside	0,036	0,035	0,010	0,018

min ND max

Fig. 4. The seasonal changes of phenolics (mg/L) per fresh weight of *Polytrichum formosum* gametophores represented by the heat map graph.

A rather small number of moss species are characterised by the presence of carbohydrates, even fewer by the carbohydrate content and dynamics related to environmental factors.

As poikilohydric organisms many species of mosses can rapidly adjust cellular water content in relation to air and environmental humidity (Proctor *et al.* 2007). The moss *P. formosum* belongs to this group of bryophytes, and this study confirms sugars to be included in the desiccation tolerance and freezing and drought survival of this species.

Phenolics are non-enzymatic protective antioxidant compounds in many plant systems (including mosses). It is evident that only a few of the detected phenolics display differential season-dependent qualitative content, while the majority are distributed evenly throughout the tested seasons (Fig. 3).

Chlorogenic acid was the most abundant phenolic compound in the gametophytes of *P. formosum* regardless of the sampling season (Fig. 4). The amount of chlorogenic acid was season-dependent, and it reached its peak in the samples harvested during the summer. Slightly lower amounts were recorded in the samples collected in the spring and autumn, suggesting summer and winter functional significance. It has been reported that the soft drying of tissues is crucial for the accumulation of chlorogenic acid in vascular plants (Moreira *et al.* 2018), and since multiple dehydration/hydration cycles occurred during the tested period, this can, at least partially, explain the variations observed between the seasons. It can be presumed that the dehydration/rehydration process occurs more often in the studied *P. formosum* population during the summer.

Additionally, p-hydroxybenzoic acid and caffeic acid were rather more represented in *P. formosum* compared

to the other identified compounds (Fig. 4). However, there was a slight decrease in p-hydroxybenzoic concentrations in the autumn and winter seasons, while caffeic acid increased. Caffeic acid may be obtained by the hydrolysis of chlorogenic acid, and these interchanges are also noticed in Fig. 4. In tracheophytes, it is related to lignification, as an intermediate to lignin, but in mosses its role remains obscure. P-hydroxybenzoic acid is known to possess biological activities such as antimicrobial and antifungal effects (Chong *et al.* 2009), thus higher amounts in warmer and wet periods of the year are not expected, suggesting its role in moss-microbe/fungal interactions.

Interestingly, quercetin-3-*O*-glucoside and gallic acid were undetectable in the samples collected during the autumn season. No variations in the content of these two compounds were recorded between the samples collected during the spring, summer, and winter. In the moss *Hypnum cupressiforme* Hedw., for example, they are shown to vary seasonally (Lunić *et al.* 2022)

The other detected phenolic compounds were evenly distributed over the seasons. Moreover, they also expressed a similar range in the amounts present in this moss population in terms of both different periods of the year and in comparison with each other (Fig. 4).

Both primary and secondary metabolism depends on climatic, i.e. environmental conditions, in some tested boreal species. However, Klavina *et al.* (2018) found that the seasonal changes exhibited by 57 secondary metabolites studied in four moss species reflected species-specific features. This is rather expected having in mind that optimal, suboptimal and high stress conditions are also related to the ecological niche of each species.

The fluctuation in carbohydrate content, both qualitatively and quantitatively, is evidently related to the environment. Indeed, Klavina *et al.* (2018) showed that carbohydrates increased during the rainy season. The same authors stated that in a less variable climate fewer changes were observed in the carbohydrate content. They inferred that both primary and secondary metabolism are climate- dependent, based on the strong correlation between the climatic parameters (season, precipitation, and temperature) and chemical constituents of mosses. The results obtained in our study also support the carbohydrate content and dynamics to be seasonal-dependent in the moss *P. formosum*.

As regarded phenolics, no significant deviation was documented and chlorogenic acid was predominant among the phenolic compounds identified. Since this phenolic acid plays anti-pathogen roles in plants, and has supportive effects during oxidative stress (e.g. Ćosić *et al.* 2023), it is not surprising that it was detected in all the samples from the tested population which, at least during the spring and summer seasons (wet/hot), was also under the threat of strong cohabitant fungal development.

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REZIME

Botanica SERBICA

Uvid u sezonske promene ugljenih hidrata i fenola kod mahovine *Polytrichum formosum* (Polytrichaceae)

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Sezonska dinamika ugljenih hidrata i fenola kod mahovine iz porodice vlasaka *Polytrichum formosum* praćena je tokom jedne godine unutar iste populacije. Kod ove vrste je zabeleženo 18 različitih ugljenih hidrata (mono-, di-, tri- i tetraharidi), te četri šećerna alkohola. Među 10 identifikovanih fenolnih jedinjenja, najzastupljenija je bila hlorogena kiselina. Među šećerima najzastupljeniji su bili saharoza, fruktoza i glukoza, ali i trehaloza i turanoza. Treba istaći da su kod *P. formosum* zabeleženi jedan tetra- i čak četiri trisaharida, a po prvi put su kod ove vrste konstatovane turanoza, izomaltoza, panoza i ramnoza. Sezonske promene ugljenih hidrata direktno su u relaciji sa sredinskim faktorima, a šećeri osim konstitutivne uloge verovatno imaju i signalnu funkciju odnosno ulogu energetskih molekula. Fenoli sa druge strane verovatno imaju potpornu ulogu tokom oksidativnog stresa, ali i u biotičkim interakcijama ove vrste.

Ključne reči: briofite, hemijski konstituenti, klima, šećeri, fenoli