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DATA TO THE NON-STRUCTURAL CARBOHYDRATES OF THE FRUCTAN ACCUMULATING SPHAGNUM SPECIES FROM THE TROPICAL RÉUNION ISLAND IN RELATION TO THEIR ALTITUDE ZONE AND DISTRIBUTION

Marianna Marschall* & Andrea Sass-Gyarmati

Eszterházy Károly Catholic University, Institute of Biology, Department of Botany and Plant Physiology, 3300 Eger, Leányka u. 6, Hungary;
**E-mail: marschall.marianna@uni-eszterhazy.hu*

Abstract: The prevalence of fructan-producing plants is primarily restricted to the temperate climate, while they are virtually absent from the tropical regions. Although the *Sphagnum* genus has a worldwide distribution and considerable ecological importance, it is relatively rare for the whole of tropical Africa. The island of Réunion is located in the tropical climate and has a rich *Sphagnum* flora containing 14 species. *Sphagnum* species – as an exception amongst mosses – synthesise fructan and have a considerable amount of sucrose as well. No data on fructan content of *Sphagnum* occuring in tropical habitats have been reported so far. This short paper provided data first, based on experimental results, on the non-structural carbohydrate pool, especially fructans in *Sphagnum* species from a tropical region, from Réunion Island. Fructans accounted for 2–4% of the total soluble carbohydrates in the examined *Sphagnum* species. We compared the fructan content of eight *Sphagnum* species collected from Réunion in relation to their altitude zone and their distribution. The species generally increased their fructan accumulation as the altitude increased. Our preliminary experimental results show that fructans as an alternative storage carbohydrate to starch may play a role in the ecological niche preference of the *Sphagnum* species according to altitudinal zones.

Keywords: *Sphagnum* species, non-structural carbohydrates, fructan, tropical climate, altitude, Réunion

INTRODUCTION

Fructan, a third type of carbohydrate and energy storage component, is involved in metabolism, produced in a small fraction (15%) of the Angiosperms (van der Meer *et al.* 1994; van den Ende *et al*. 2011). The non-vascular bryophytes also synthesise fructans (Maass and Craigie 1964; Suleiman *et al*. 1979; Marschall 1998;

Marschall 2010). Fructans as non-structural carbohydrates are the primary products of photosynthesis, key regulators of adaptation to environmental stress, provide substrates for growth and metabolism (Liu *et al.* 2018), act as antioxidants, scavenging reactive oxygen species and preventing cell damage under abiotic stress conditions (Peshev *et al*. 2013). The role of fructans as storage carbohydrates, their function in desiccation tolerance and low temperature stress is well emphasized in higher plants (Marschall 2010), but there is only few number of articles focusing on the physiology of fructan producing bryophytes (Marschall *et al.* 1998; Marschall 2010). Fructan-accumulating species contain only traces (~1%) of starch, which means that fructan is a real alternative to starch. In the plant cells, fructans accumulate in the vacuole (Wagner and Wiemken 1986), where they play an important role in turgor regulation (Pontis 1989). Fructans were later discovered in the apoplast also (Livingston and Hanson 1998; van den Ende *et al.* 2005). More molecules mean that these cells are more resistant to osmotic pressure or even cold, so these plants are better adapted to sudden climate changes. The size of fructan polymers can be altered quickly; this could be an explanation for their role in osmotic adjustment. Polimerization or breakdown of fructan will alter vacuolar osmotic potential, and hence may alter turgor pressure (Marschall 2010). The DP (degree of polymerization) appears to closely track changes in the external environment.

It is likely that fructans protect plants from various environmental stresses such as frost and drought (Valluru and van den Ende 2008) by stabilizing membranes. It was found that fructans inserted between the headgroups of different kinds of phospholipids with some preference for phosphatidylethanolamine (Vereyken *et al.* 2001; Hincha *et al*. 2002, 2003). Pollock's (1986) study shows that starch synthesis drops dramatically when the temperature decreases below 10°C, but photosynthetic processes and fructan production are much less sensitive to low temperatures, suggesting that fructan production benefits those plants, which actively photosynthesize during the winter and early spring. The protection of the photosynthetic apparatus as temperature rises, mobilization of carbohydrates stored in fructans for rapid growth are strong influencing factors in the evolution of fructan production (Vijn and Smeekens 1999).

As well as being the main storage carbohydrate, vacuolar fructans with their synthesis can regulate the concentration of sucrose in plant cells, thereby preventing the inhibition of photosynthetic sugar-induced feedback (Pollock 1986). Global distribution shows that the temperate climate is particularly rich in fructan-producing plants, whereas in the tropical regions they are virtually absent (Hendry and Wallace 1993). Drought, high irradiance or/and low temperature favours fructan accumulation in Angiosperms, so their relevance is linked to desiccation and freezing resistance or emphasized in response to cold and dry seasons (Marschall 2010).

Sphagnum is a large genus of about 380 accepted species worldwide. *Sphagnum* mosses occur mainly in the Northern Hemisphere in peat bogs, conifer forests, and moist tundra areas. Their northernmost populations lie in the archipelago of Svalbard, Arctic Norway. In the Southern Hemisphere, the largest peat areas are in southern Chile and Argentina, part of the vast Magellanic moorland (Arroyo *et al.* 2005). Peat areas are also found in New Zealand and Tasmania. In the Southern Hemisphere, however, peat landscapes may contain many moss species other than *Sphagnum*. *Sphagnum* species are also reported from "dripping rocks" in mountainous, subtropical Brazil. *Sphagnum* species are common in climates with more precipitation and higher relative humidity, mainly in the boreal region and high mountains. Although the *Sphagnum* genus has a worldwide distribution and considerable ecological importance, it is relatively rare in tropical Africa. Plants, including *Sphagnum* species, produce a wide range of metabolites (Sytiuk *et al.* 2023) in response to environmental changes and impacts. The prevalence of fructan-producing plants is primarily restricted to the temperate climate, while they are virtually absent from the tropical regions (Hendry and Wallace 1993). The island of Réunion is located in the tropical climate and has a rich *Sphagnum* flora. *Sphagnum* species – as an exception amongst mosses – synthesise fructan and have a considerable amount of sucrose as well. No data on fructan content of *Sphagnum* occuring in tropical habitats have been reported so far. This prompted us to investigate the fructan content of *Sphagnum* species from Réunion. Reunion *Sphagnum* species included in the study, with the exception of one species endemic to Réunion (*Figure 1*), also occur on the island of Madagascar. In Mauritius, only three of our study species are found (*Table 1*). Their distribution is also variable. They include species with an Afro-alpine, East African, South African, mountain, pantropical or island endemic distribution (*Table 1*).

Table 1. *Sphagnum* species included in the study with their habitat altitude zone, their island occurrence and their type of distribution.

Species included in the study with their habitat altitude zone	Occurrence of species on these islands			Distribution
	Réunion	Madagascar	Mauritius	
*Sphagnum davidii***	+	+	–	Afro-alpine
*Sphagnum ceylonicum***	+	+	–	East Afr.
*Sphagnum violascens***	+	+	+	South, East Afr. mountain
*Sphagnum capense***	+	+	–	South Afr.
*Sphagnum pappeanum***	+	+	–	pantropical
*Sphagnum rutenbergii**	+	+	+	East Afr. island endemism
Sphagnum tumidulum var. *tumidulum**	+	+	+	East Afr. island endemism
Sphagnum tumidulum var. *confusum**	+	–	–	Réunion

Notes: **2000–2500 m, *900–1600 m.

According to the studies so far (Marschall 2010), *Sphagnum*' fructan belongs to the inulin type. Inulin-type chains are generally between DP 2–60 (Sissons and Fellows 2014), while levan types are slightly longer (DP < 200) (Avigad and Dey 1997). These values vary with the current weather conditions and the physiological requirements of the plant. The accumulation and reduction of fructans in plants is mainly dependent on the temperature, i.e. the season and the amount of precipitation. Cold-adapted species synthesized fructan permanently, whereas species adapted to warmer climates accumulated it only under cold stress.

The aim of this paper is *1*) to obtain data about the non-structural carbohydrate pool, especially fructans in *Sphagnum* species from a tropical region, from Réunion Island; *2*) to compare the fructan content of the *Sphagnum* species collected from Réunion in relation to their altitude zone and distribution; *3*) Greeting Tamás Pócs on his 90th birthday, bringing back fond memories of a previous expedition to Reunion.

MATERIALS AND METHODS
Plant material

Sphagnum species were collected from the following different locations of Réunion Island in 1994. *Sphagnum davidii* Warnst., *Sphagnum ceylonicum* Mitten ex Warnst., *Sphagnum violascens* C. Müll., *Sphagnum capense* Hornsch, *Sphagnum pappeanum* (C. Müll.) Eddy (NE ridge of the PITON DES NEIGES summit. Subalpine ericaceous bush with Philippia and Stoebe, at 1800–2500 m altitude, between Caverne DUFOUR and Crete Riv. des MARSOUINS. 21°5–6' S, 55°30–31' E 01.09.1994.). *Sphagnum rutenbergii* C. Müll. (PLAINE DES PALMISTES. Lava flow at the E edge of village called „PREMIER VILLAGE LES BAS" at 900 m altitude. Heathlike *Philippia*, *Pandanus* vegetation with peaty ground. 21°6'33" S, 55°39' E, 30.08.1994.). *Sphagnum tumidulum* var. *tumidulum* Bescher, *Sphagnum tumidulum* var. *confusum* A. Eddy (Forêt de BÉBOUR, in the V. of Rivière MARSOUINS. Mossy montane rainforest with *Dombeya* ssp. and treeferns, with annual rainfall above 8000 mm at 1300-1550 m altitude. 21°5–6' S, 55°33–34' E, 31.08.1994.) (*Figure 1*). Nomenclature and distribution of the collected *Sphagnum* species was checked according to Ah-Peng and Bardat (2005) and Michaelis (2011).

Samples for carbohydrate analysis were taken in the middle of the photoperiod. All the collected samples represented the full turgor status of the plants in their original habitats. Laboratory measurements were carried out on air-dried samples, containing capitula and stem parts.

Preparation of plant extracts

Capitula and stem parts of *Sphagnum* species were extracted with 80% ethanol followed by hot water (Marschall *et al.* 1998). Supernatant and sediment extracts were also used for assays. 0.0363 g of air-dried plant tissues (capitula and stem parts) were homogenized with 2 ml of 80% ethanol, then were centrifugated for 10 minutes (10.000G). To the sediment remaining in the tube 1 ml of hot distilled water was added for further extraction and after shaking it was incubated at 70°C for 2 hours. During incubation time, the sample was shaken every 30 minutes. Then the tube was centrifuged for 10 minutes at 10.000G and the supernatant

(=sediment extract) was kept for assays. Supernatants and sediment extracts were used for fructan and total soluble carbohydrate assay.

Determination of total soluble sugar and fructan content

Total soluble sugars were detected in plant extracts by the Dubois method (1956). Fructans were quantified using a ketose-specific method with resorcinol (Farrar 1993; Marschall *et al*. 1998).

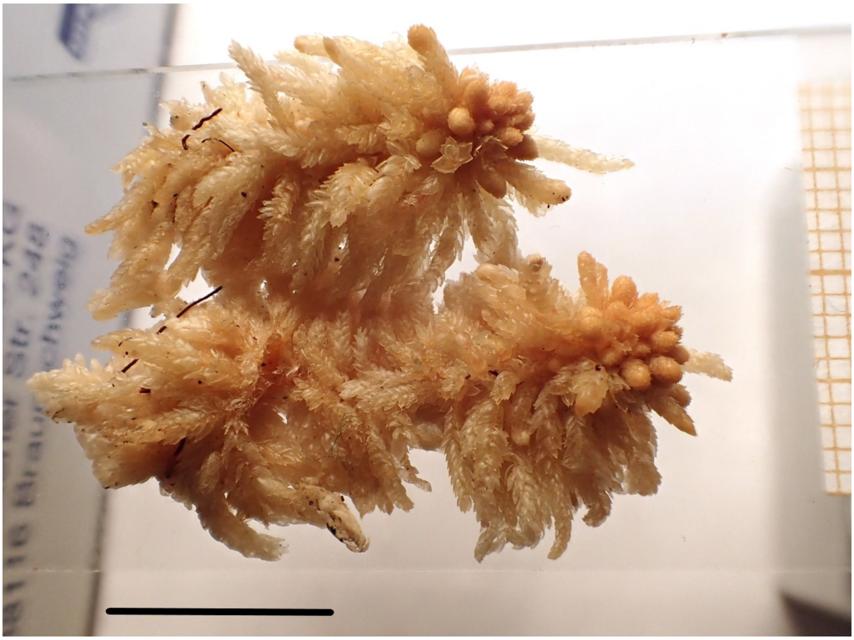

Figure 1. Habitus image of *Sphagnum tumidulum var. confusum* A. Eddy (9481/EH, EGR) endemic to Réunion on an EGR herbarium specimen. Scale bar=10 mm (Photo: C. Reeb).

RESULTS

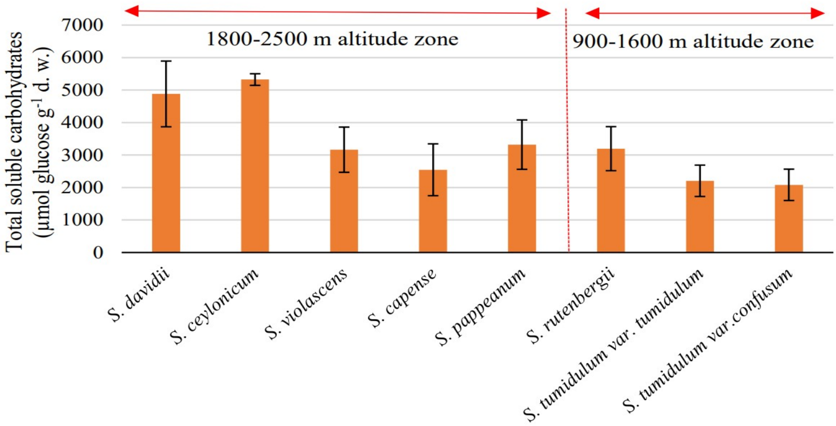

Figure 2. Total soluble carbohydrate content (µmol glucose g^{-1} d.w.) of *Sphagnum davidii, S. ceylonicum, S. violascens, S. capense, S. pappeanum, S rutenbergii, S. tumidulum* var. *tumidulum, S. tumidulum* var. *confusum* relation to their habitat altitudinal zone. Error bars are STDs, where n=3.

The total fructan content at altitudes above 2000 m was at least 1.5 times, and for some species more than 2 times, higher than that of *Sphagnum* species in the 900 and 1600 m altitudinal zone (*Figure 3*). In the two species (*S. davidii, S. ceylonicum*) occurring in the highest regions, fructan constitutes 2–3.5% of the total dry biomass content, in four species it is around 1.5%, while in the species occurring in the lowest altitudinal zone it is only below 1% (*Figure 4*). The fructan content as a % of total dry biomass is of the same order of magnitude as the fructan content of peat mosses from the Northern Hemisphere published by Hendry (1996).

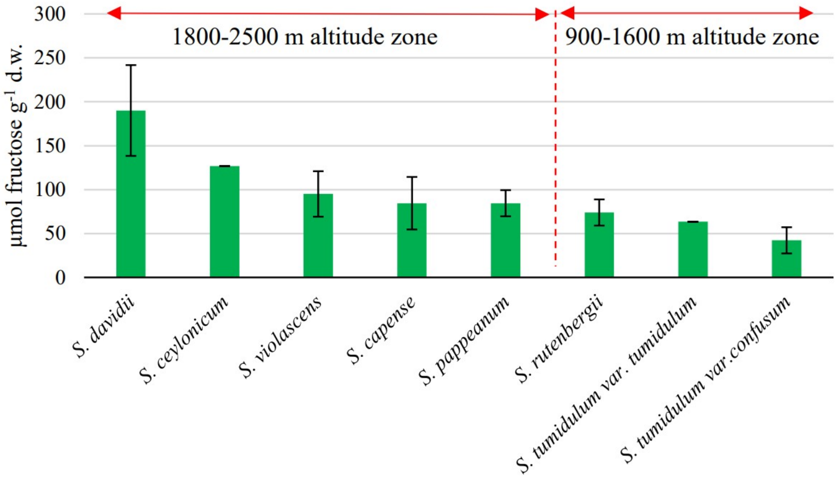

Figure 3. Total fructan content (µmol fructose g^{-1} d.w.) of *Sphagnum davidii, S. ceylonicum, S. violascens, S. capense, S. pappeanum, S rutenbergii, S. tumidulum var. tumidulum, S. tumidulum var. confusum* relation to their habitat altitudinal zone. Error bars are STDs, where n=3.

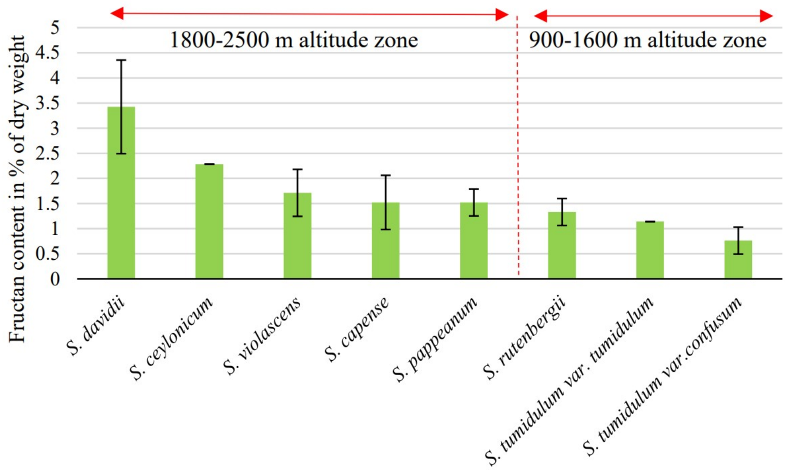

Figure 4. Total fructan content in % of dry weight of *Sphagnum davidii, S. ceylonicum, S. violascens, S. capense, S. pappeanum, S rutenbergii, S. tumidulum var. tumidulum, S. tumidulum var. confusum* relation to their habitat altitudinal zone. Error bars are STDs, where n=3.

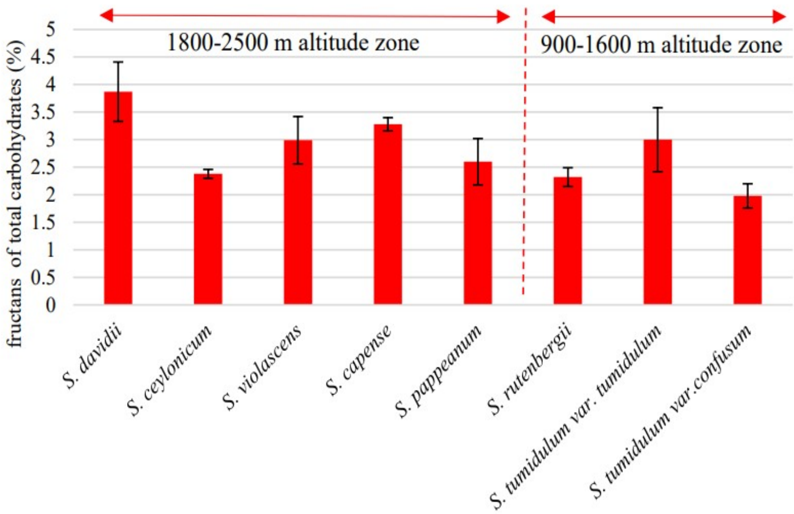

Figure 5. Fructan content in % of total carbohydrates of *Sphagnum davidii, S. ceylonicum, S. violascens, S. capense, S. pappeanum, S rutenbergii, S. tumidulum var. tumidulum, S. tumidulum var. confusum* relation to their habitat altitudinal zone. Error bars are STDs, where n=3.

If we consider the fructan content as a % of the total soluble carbohydrate content, the fructan content of *S. davidii* is close to 4, while for the other seven species it ranges between 2 and 3.2% (*Figure 5*). Compared to vascular plants, the % fructan content relative to the total soluble carbohydrate content is comparable to the fructan content of *Dactylis glomerata* leaves in spring, although cock's-foot accumulates non-inulin-type fructans. Fructans accounted for less than 3% of the total soluble carbohydrates in the spring leaf of *D. glomerata* (Marschall *et al.* 2019).

The island of Réunion has a tropical climate, but in the mountains of the island, the temperature can be 10–15°C cooler. As altitude increases, temperature decreases by 0.6–1 degree Celsius per 100 metres, depending on the relative humidity of the air. Although no microclimate measurements were associated with the *Sphagnum* plant sampling, and the number of samples was not sufficiently large for statistical purposes, a positive correlation between higher fructan content and decreasing temperature with increasing altitude is clearly visible.

CONCLUSION

This short paper provided data, based on experimental results, on the non-structural carbohydrate pool, especially fructans in *Sphagnum* species from a tropical region, from Réunion Island. We compared the fructan content of the Réunion *Sphagnum* species in relation to their altitude zone and their distribution. The species generally increased the synthesis of fructans as the altitude increased. It would be interesting to check how fructan content do change with altitude in the same *Sphagnum* species at the same time. In terms of fructan accumulation, the different *Sphagnum* species are located in their own ecological niches in terms of altitudinal zones. The fructan production of the plants investigated is mainly influenced by the temperature factors, daily irradiation and also the amount of precipitation, as these vary with altitude in their microhabitats.

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