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THE DYNAMICS OF SOLUBLE CARBOHYDRATES IN UNDERGROUND ORGANS OF SISYRINCHIUM VAGINATUM (IRIDACEAE) VARIES ACCORDING TO PHENOLOGY

A DINÂMICA DOS CARBOIDRATOS SOLÚVEIS NOS ÓRGÃOS SUBTERRÂNEOS DE SISYRINCHIUM VAGINATUM (IRIDACEAE) VARIA DE ACORDO COM A FENOLOGIA

LA DINÁMICA DE LOS CARBOHIDRATOS SOLUBLES EN ÓRGANOS SUBTERRÁNEOS DE SISYRINCHIUM VAGINATUM (IRIDACEAE) VARÍA SEGÚN LA FENOLOGÍA

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RESUMO:

Os órgãos subterrâneos são importantes para a persistência das plantas em ambientes sazonais, pois armazenam substâncias e podem conter gemas. Os órgãos subterrâneos de *Sisyrinchium vaginatum* armazenam carboidratos solúveis, mas não se sabe como a fenologia afeta sua dinâmica. O objetivo deste trabalho foi analisar o conteúdo e a composição de carboidratos solúveis em órgãos subterrâneos de *S. vaginatum* em diferentes fases fenológicas. Os teores e composição de carboidratos solúveis foram analisados durante a brotação, crescimento vegetativo I e II, dispersão de sementes e senescência. O armazenamento de carboidratos foi maior quando o crescimento vegetativo estabilizou, com os níveis mais baixos nas fases com maior demanda energética. Foram identificados *mio*-inositol, glicose, frutose, sacarose e um açúcar tipo-rafinose, sendo os dois últimos predominantes. A proporção da maioria desses açúcares diferiu entre as fases fenológicas. As variações dos carboidratos solúveis foram associadas às mudanças fenológicas e podem contribuir para a persistência de *S. vaginatum* em ambientes sazonais.

PALAVRAS-CHAVE: HPAEC-PAD, carboidrato não estrutural, variação sazonal, armazenamento, sacarose

ABSTRACT:

Underground organs are important for the persistence of plants in seasonal environments, as they store substances and may contain buds. The underground organs of Sisyrinchium vaginatum store soluble carbohydrates, but how phenology affects their dynamics is unknown. This study aimed to analyze the contents and composition of soluble carbohydrates in underground organs of S. vaginatum at different phenological phases. The contents and composition of soluble carbohydrates were analyzed during sprouting, vegetative growth I and II, seed dispersal and senescence. Carbohydrate storage was greater when vegetative growth stabilized, with the lowest levels occurring during more energy-demanding phases. myo-Inositol, glucose, fructose, sucrose, and raffinose-like sugar were identified, with the last two being predominant. The proportion of majority of these sugars differed among phenological phases. The variations in soluble carbohydrates were associated with phenological shifts and may contribute to the persistence of S. vaginatum in seasonal environments.

KEYWORDS: *HPAEC-PAD, non-structural carbohydrate, seasonal variation, storage, sucrose*

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RESUMEN

Los órganos subterráneos son importantes para la persistencia de las plantas en ambientes estacionales, ya que almacenan sustancias y pueden contener yemas. Los órganos subterráneos de Sisyrinchium vaginatum almacenan carbohidratos solubles, pero se desconoce cómo afecta la fenología a su dinámica. El objetivo de este estudio fue analizar el contenido y composición de carbohidratos solubles en órganos subterráneos de S. vaginatum en diferentes fases fenológicas. Se analizó el contenido y composición de carbohidratos solubles durante la brotación, crecimiento vegetativo I y II, dispersión de semillas y senescencia. El almacenamiento de carbohidratos fue mayor cuando el crecimiento vegetativo se estabilizó, y los niveles más bajos se produjeron durante las fases de mayor demanda de energía. Se identificaron mioinositol, glucosa, fructosa, sacarosa y azúcar tipo rafinosa, siendo los dos últimos los predominantes. La proporción de la mayoría de estos azúcares difirió entre las fases fenológicas. Las variaciones en los carbohidratos solubles se asociaron con cambios fenológicos y pueden contribuir a la persistencia de S. vaginatum en ambientes estacionales.

Palabras clave: *HPAEC-PAD, carbohidratos no estructurales, variación estacional, almacenamiento, sacarosa*

INTRODUCTION

In plants, carbon occurs predominantly in the form of carbohydrates. Carbohydrates that constitute the cell wall are the major constituents of phytomass and are termed structural carbohydrates (Schädel *et al.*, 2010). Structural carbohydrates are stable, insoluble, and resistant to degradation (Taylor, 2008). In contrast, non-structural carbohydrates are dynamic, transient, and constantly metabolized in synthesis and hydrolysis reactions. They include soluble carbohydrates such as soluble mono-, di-, oligo-, and polysaccharides, including starch, a water-insoluble polysaccharide (Schädel *et al.*, 2010).

As they are dynamic, non-structural carbohydrates are fundamental to plant metabolism. They perform numerous functions, such as providing substrates for metabolism and development, transporting carbon between sources and sinks, regulating osmotic potential, signaling intracellular reactions, and protecting against stresses (Halford *et al*., 2011; Quentin *et al*., 2015). The main function of non-structural carbohydrates is to store energy (Chapin *et al.*, 1990), especially when they occur in specialized organs such as bulbs, rhizomes and corms or in other parenchyma-rich organs (Morris *et al.*, 2016; Plavcova *et al.*, 2016). These structures provide space for long-term storage until reserves are recruited to meet some demands of the plant (Chapin *et al.*, 1990).

Underground reserve organs store organic compounds, water, and nutrients and possess buds, so they occupy an important position in ecosystem processes (Pausas *et al*., 2018; Silva and Rossatto, 2019). As they have structures and energy for regrowth, underground organs contribute to the perennialization of herbs, especially in environments with seasonal climates and/or that are subject to frequent disturbances (Klimesova *et al.*, 2018; Lubbe *et al.*, 2021), such as the Cerrado.

The dry season limits the water supply for Cerrado plants, especially for the herbaceous (Rossato *et al.*, 2013). Climatic seasonality also influences phenology (Mantovani and Martins, 1988; Munhoz and Felfili, 2005). At the onset of drought, many herbs and subshrub species lose their aerial organs and remain with only their dormant underground systems. When the rainy season starts, carbohydrates are mobilized and provide energy and carbon blocks for the development of new aerial organs. Once these organs are fully developed and with sufficient photosynthesis, they produce and allocate sugars to underground organs (Silva *et al.*, 2013, Rigui *et al.*, 2015; Almeida *et al.*, 2017). Thus, the storage of non-structural carbohydrates is a key trait to enable plant growth and survival facing disturbances (Furze *et al.*, 2018).

Different types of carbohydrates can be stored in underground organs of plants, but the presence of a given type is a feature restricted by phylogeny and common to closely related taxa (Campos *et al.*, 2021; Verluys *et al.*, 2018; Lubbe *et al.*, 2021). Underground storage organs are common in Iridaceae (Rudall 1995), accumulating fructans, starch and glucomannans individually or combined (Ranwala and Muller, 2008). Starch, glucose, fructose and sucrose are the main carbohydrates found in species that belong to *Trimezia* Salisb. ex Herb., *Cipura*

Aubl. and *Sisyrinchium* L. (Almeida *et al.,* 2015); genera whose species are representative of Iridaceae in the Cerrado (Dantas-Queiroz *et al.,* 2016). These studies show the great diversity of Iridaceae since this family typically accumulates several types of non-structural carbohydrates.

Among the species of Iridaceae from the Cerrado, *Sisyrinchium vaginatum* Spreng. has soluble carbohydrates in its underground organs (Almeida *et al.,* 2015). In that study, however, the species was analyzed during only one phenological phase, whereas soluble carbohydrate contents can be influenced by both phenology and environmental conditions (Ruiters and McKenzie, 1994; Silva *et al.,* 2013; Almeida *et al.,* 2017; Sharma *et al.,* 2019). Therefore, it is necessary to expand the study of carbohydrates stored in underground organs in different phenological stages to assess their dynamics.

Since the largest fraction of carbon stored in *S. vaginatum* comprises soluble carbohydrates (Almeida *et al.,* 2015), this study hypothesized that soluble carbohydrates will be stored in the underground organs of *S. vaginatum* during the vegetative growth phases and used in phases with greater energy demand. Therefore, the objective was to analyze the contents and composition of soluble carbohydrates in underground organs of *S. vaginatum* at different phenological stages.

MATERIALS AND METHODS

Plants of *Sisyrinchium vaginatum* Spreng. were collected at Reserva Biológica Prof. José Angelo Rizzo of the Universidade Federal de Goiás (16° 06’ 07’’ S, 50° 12’ 36’’W), located in the municipality of Mossâmedes, Goiás (GO), Brazil. Altitudes in the reserve range 700 – 1080 m and there are different Cerrado physiognomies, including open savannah, where the plants of *S. vaginatum* were found (Almeida *et al.,* 2015).

Field trips were carried out in May, July, November, and December of 2015 and in February and July of 2016, with authorization for collection and transport (Sisbio 51327-1) and registration for access to genetic heritage (SISGEN AD2294F). On the last field trip, during the dry season, the aerial organs of the species were not found, which made it impossible to collect the underground organs. Climatic data for the collection period were obtained from the nearest meteorological station (A014, Goiás, GO) at the Instituto Nacional de Meteorologia (Table 1) (www.inmet.gov.br).

During each field trip, five different specimens, spaced at least 2 m apart, representative of the phenological phase in progress were collected. The sampling occurred at sprouting, vegetative growth I (beginning of vegetative development), vegetative growth II (end of vegetative development), seed dispersal and senescence (Table 1). The flowering period for this species lasts from 1 to 4 days (Freitas and Sazima, 2003). Due to the scheduled fieldwork dates not aligning with the species' rapid flowering, collecting flowering plants

was impossible. The collection carried out at the time of seed dispersal was the only sampling during the sexual reproduction period.

The soil was carefully excavated to remove the underground system, consisting of little evident corms and predominantly adventitious roots with a fibrous consistency (Menezes *et al.*, 2012).

In the laboratory, separating corms and roots proved to be difficult, so samples consisted of a combination of both organs. Approximately 10 grams of fresh matter was washed in water to remove soil particles, and excess water was removed with a paper towel. The samples were cut into small pieces and divided into two portions. The first one was oven-dried (50 °C) until constant mass, to determine water content, calculated as the percentage of fresh mass. The second portion was used for the extraction of soluble carbohydrates.

Table 1 – Monthly means of climatic parameters during the period of collection of underground organs of *Sisyrinchium vaginatum* Spreng. in different phenological phases.

Phenological phase	**Date**	**Rainfall (mm)**	**Relative air humidity (%)**	**Monthly mean temperatures (°C)**		
				minimum	**mean**	**maximum**
Sprouting	Nov/2015	81.8	66.8	22.8	26.9	34.7
Vegetative growth I	Dec/2015	148.5	71.6	21.8	26.6	33.8
Vegetative growth II	Feb/2016	126.3	76.1	22.0	26.1	33.3
Seed dispersal	May/2015	70.7	68.9	19.9	24.5	32.1
Senescence	July/2015	2.0	53.9	18.2	24.5	33.5

Source: A014 meteorological station, Goiás, GO, Brazil

Soluble carbohydrates were extracted according to the methods described by Almeida *et al.* (2015), with some modifications. Samples of 1 g of fresh mass, cut into small pieces, were placed in centrifuge tubes with 10 ml of ethanol (80%), which were then placed in a water bath at 80 °C for 15 min for enzyme inactivation. The material was then filtered through nylon, and the liquid phase, consisting of ethanol and soluble compounds, was saved. Ethanol (80%) was

again added to the plant material for processing in a tissue homogenizer and then placed in a water bath at 80°C for 15 min. After filtering through nylon fabric, the liquid phase was saved. The extraction with 80% ethanol was done once more. To ensure exhaustive extraction, 10 mL of distilled water was added and mixed with the plant material, which was then placed in a water bath at 60°C for 30 min, in two successive steps. All liquid phases obtained in each sample's ethanolic and aqueous extraction steps were combined and constituted the crude extracts, which were concentrated under vacuum in a rotary evaporator at 39°C. The volume of concentrated extracts was adjusted to 5 mL. Total soluble carbohydrates were quantified in crude extracts with phenol reagent (5%) and sulfuric acid (98%, P.A.), using a standard curve established with predetermined glucose concentrations (Dubois *et al.*, 1956). The absorbances were determined in triplicate using a spectrophotometer at 490 nm.

Aliquots of 1 mL of the crude extracts were applied to ion exchange resins (Amberlite IRA 120, cationic + Amberlite IRA 410, anionic). Each extract was then eluted with 10 mL of ultrapure water (18.2 MΩ) and the pH of the deionized extracts was neutralized with ammonium hydroxide. The deionized extracts were vacuum dried (39°C), solubilized in 1.5 mL of ultrapure water, filtered through cellulose ester membranes (0.45 µm) and stored at -20 °C for the following analysis.

The samples were analyzed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a CarboPac PA1 column (4 x 250 mm) with its guard column, connected to a Dionex ICS 5000 chromatograph. Elution was done with sodium hydroxide (100 mM), isocratically at 1 $mL.min^{-1}$ for 20 min. Potential PADs were applied according to the manufacturer's recommendation. Sugars were identified by comparison with the retention time of the following authentic standards: *myo*-Inositol, glucose, fructose, sucrose, raffinose and stachyose, all from Sigma®. When necessary, deionized extracts were diluted to obtain peaks with heights in the appropriate range for quantification or were co-chromatographed with authentic standards to validate sugar identification. The individual quantification of the sugars that constitute the samples was done with calibration curves obtained with predetermined concentrations of the standards, generated by Chromeleon software (v 6.8). One of the most relevant peaks in the samples was not identified (raffinose-like) and thus made it impossible to obtain a specific standard curve. Thus, estimates of quantities were obtained from the standard raffinose curve and expressed in raffinose equivalents. Data were evaluated based on the proportion of each sugar in relation to the total amount of sugars identified in the sample, to normalize possible variations in mass and volume.

Data were evaluated for normality of residuals by the Shapiro-Wilk test and homoscedasticity of variances by Levene's test. As they did not meet the assumptions for parametric analysis, differences between medians were evaluated by the Kruskal-Wallis test, followed by Dunn's post hoc test ($p < 0.05$), for the variables: water content, total soluble sugars, proportions of *myo*-inositol, glucose, fructose and raffinose-like sugar. These analyses were performed in

Microsoft Excel 365®, using Real Statistics Resource Pack software (Release 7.9.1) (Zaiontz, 2020).

A principal component analysis was performed to assess the effects of the phenological phase on the evaluated variables. This analysis aims to gather the set of explanatory variables and reduce the number of orthogonal axes that express part of the variability contained in the original variables (Legendre and Legendre, 1998). Principal component analysis was performed with a correlation matrix in PAST software version 4.0 (Hammer *et al.*, 2001).

RESULTS

The water content of the underground organs differed among phenological phases ($H_{(4)}$ = 15.48; p = 0.0038). Water content was lower in senescence than in the phases of vegetative growth I (p = 0.0003) and vegetative growth II (p = 0.0077). Water content in the seed dispersal phase was lower than that in vegetative growth I (p = 0.0173) (Figure 1).

Total soluble carbohydrates in the underground organs of *S. vaginatum* differed among phenological phases ($H_{(4)}$ = 11.73; p = 0.0194). Total soluble carbohydrates in senescence phase were lower than that in seed dispersal (p = 0.0127) and vegetative growth II (p = 0.0091). Total soluble carbohydrates in sprouting were lower than that in vegetative growth II (p = 0.0294) and seed dispersal (p = 0.0385) (Figure 1).

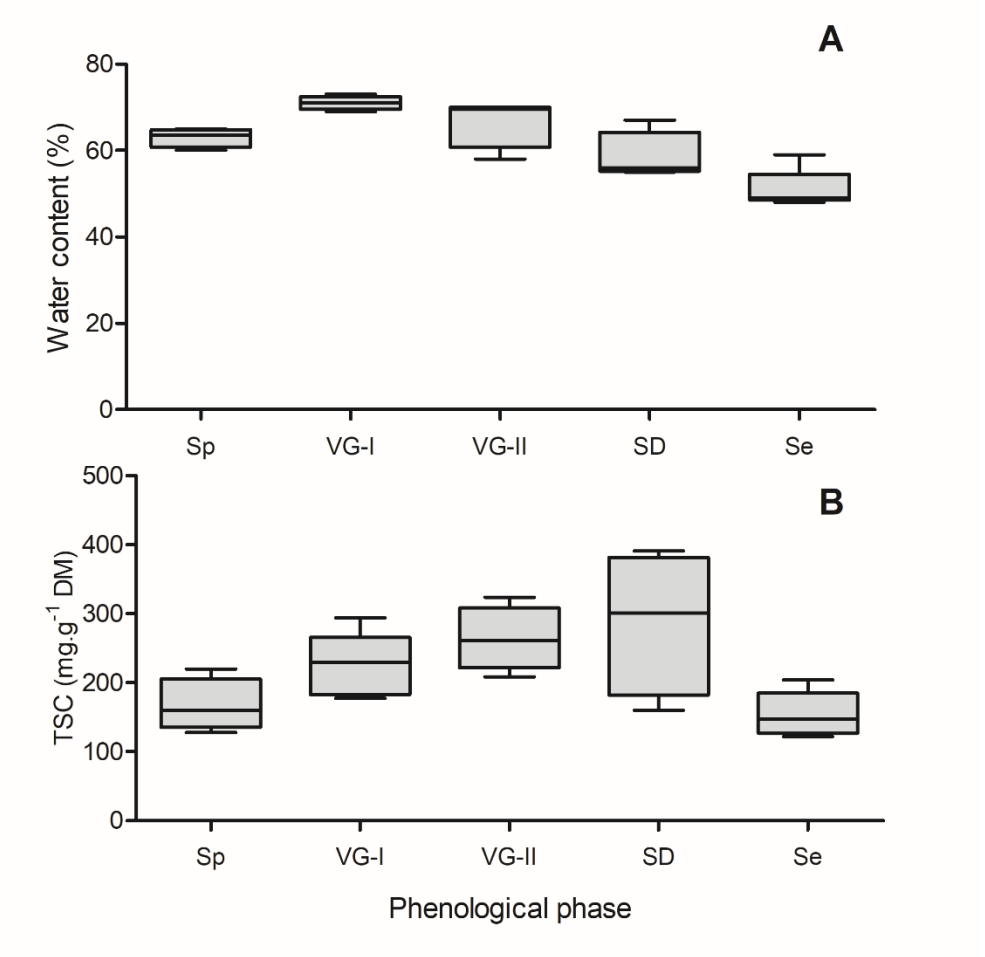
myo-Inositol, glucose, fructose and sucrose were identified in the extracts of soluble carbohydrates obtained from the underground organs of *S. vaginatum* (Figure 2). Small peaks close to *myo*-inositol were observed but did not correspond to the standards used. In addition, a sugar eluted in the raffinose region was verified in all samples. This sugar had a different retention time compared to raffinose and stachyose standards in the co-chromatography (Figure 3), thereby indicating it as distinct from the raffinose series oligosaccharides. From this point forward in this study, this unidentified sugar will be termed “raffinose-like”. Other soluble oligosaccharides and polysaccharides were not observed in the extracts of the phenological phases.

Individual quantification of the sugars revealed sucrose and raffinose-like to be predominant in the underground organs in all phenological phases. Among the monosaccharides, fructose occurred in a greater proportion than glucose. *myo*-Inositol was the minor component of the set of identified sugars (Figure 4).

Although in smaller amounts than the other constituents of the extracts, the proportion of *myo*-inositol differed among phenological phases ($H_{(4)}$ = 14.3; p = 0.0063); it was greater in seed dispersion was greater than in sprouting (p = 0.0197), vegetative growth I (p = 0.0231) and vegetative growth II (p = 0.0034). The proportion of *myo*-inositol in senescence was also greater than that in

sprouting (p = 0.0364), vegetative growth I (p = 0.0421) and vegetative growth II (p = 0.0071).

Figura 1 – Box-plot with **A**. water content and **B**. total soluble carbohydrates (TSC) content in underground organs of *Sisyrinchium vaginatum* Spreng. collected at different phenological stages in an area of cerrado: sprouting (Sp), vegetative growth I (VG-I), vegetative growth II (VG-II), seed dispersal (SD), and senescence (Se). Differences among medians (n = 5) were detected by Dunn's test ($p < 0.05$).



Source: The authors

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Figura 2 – HPAEC-PAD profiles of carbohydrates in the underground organs of *Sinsyrichyum vaginatum* Spreng. collected in different phenological phases from an area of cerrado: *myo*-inositol (I), glucose (G), fructose (F), sucrose (S), raffinose (R), stachyose (St), and raffinose-like (R-L). Asterisks indicate non-identified peaks.

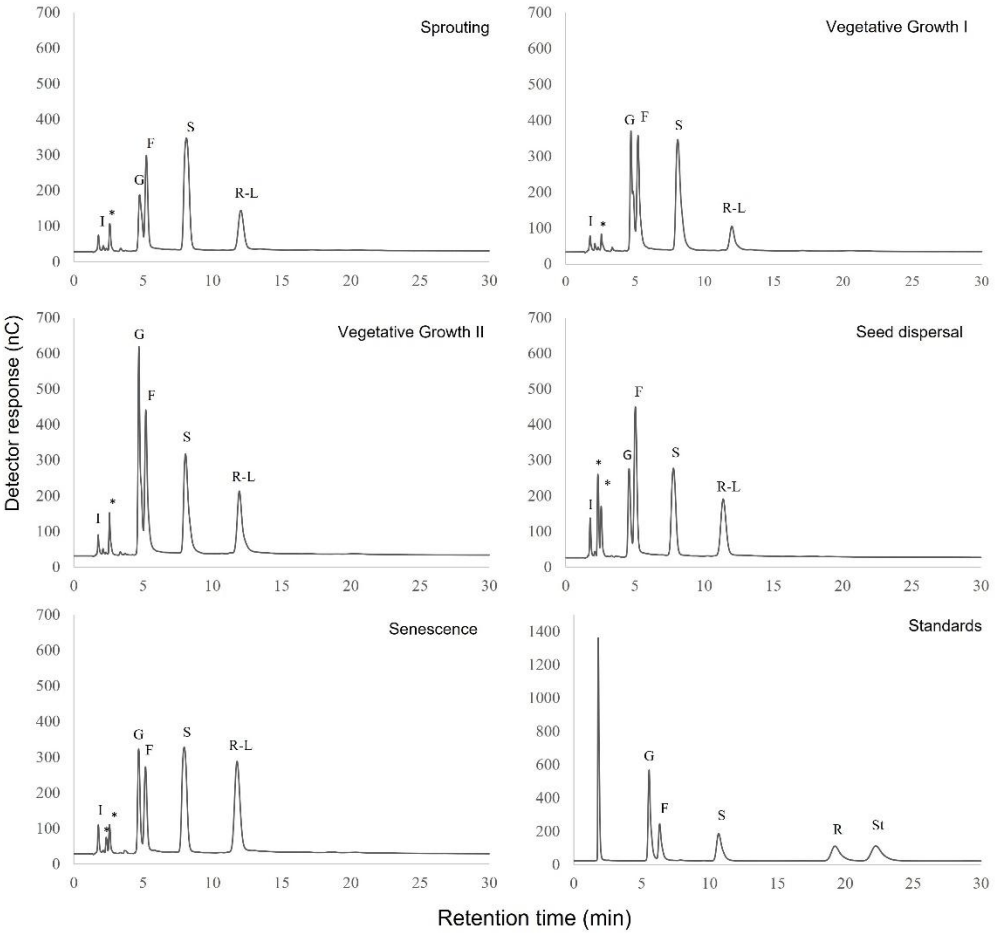

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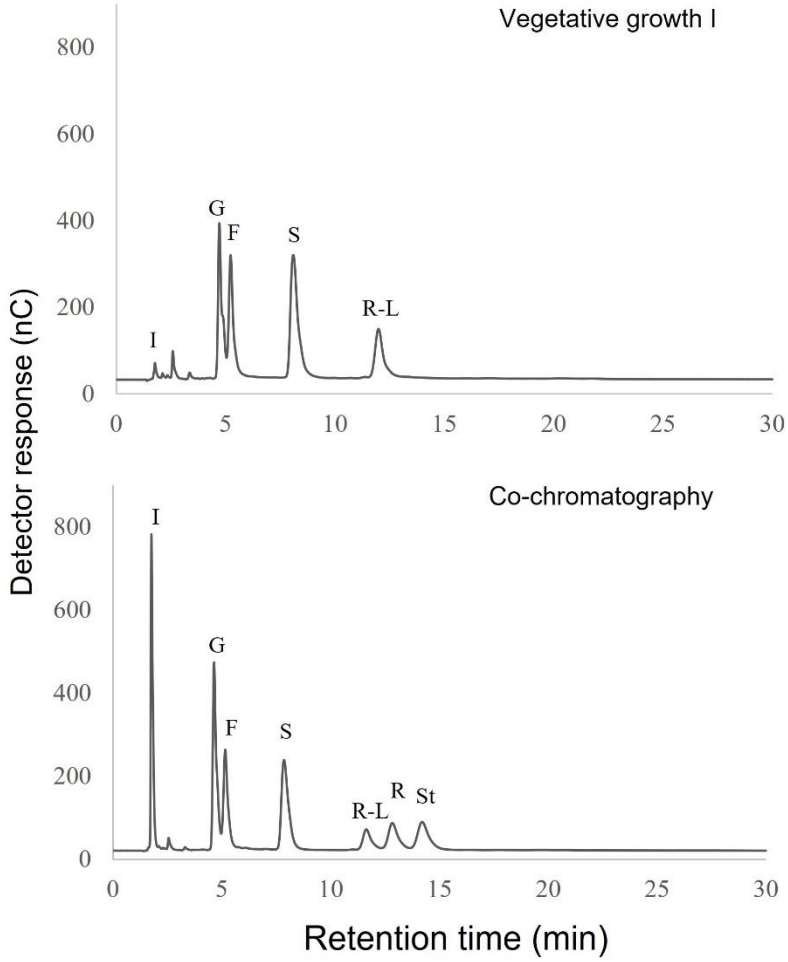
Figura 3 – Co-chromatography of carbohydrates from the underground organs of *Sinsyrichyum vaginatum* Spreng. collected in vegetative growth I phase and authentic standards, obtained by HPAEC-PAD Brazil: *myo*-inositol (I), glucose (G), fructose (F), sucrose (S), raffinose (R), stachyose (St), and raffinose-like (R-L).

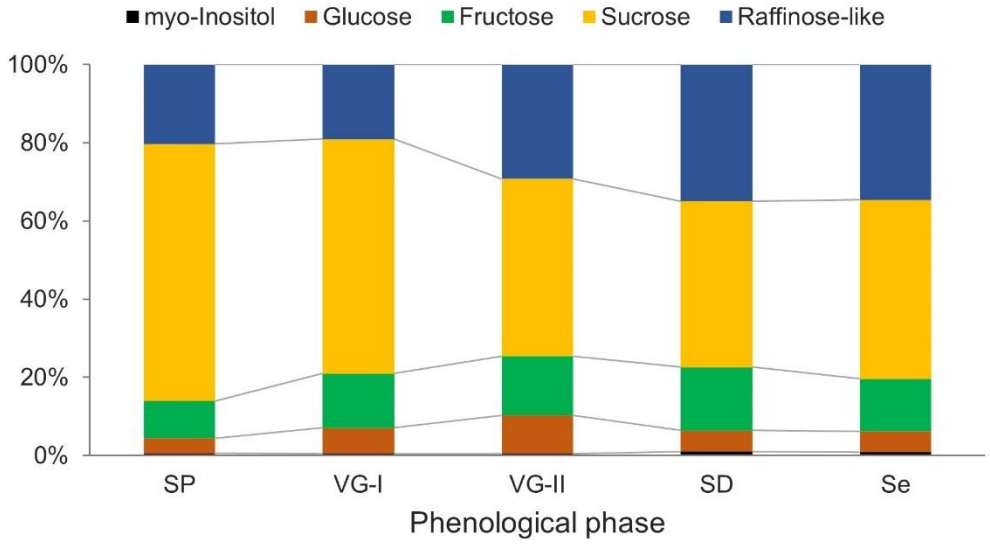


Source: The authors

The proportion of glucose differed among phenological phases ($H_{(4)}$ = 13.74; p = 0.0081), with the proportion being greater in vegetative growth II than in sprouting (p = 0.000211); however, the proportion of glucose did not differ significantly among the other phenological phases. The proportion of fructose did not differ significantly among phenological phases, according to the Kruskal-Wallis test ($H_{(4)}$ = 7.68; p = 0.1037).

The proportion of sucrose in the underground organs differed among phenological phases ($H_{(4)}$ = 14.142; p = 0.0068), with it being greater in sprouting than in vegetative growth II (p = 0.0101), seeds dispersal (p = 0.0023) and senescence (p = 0.027). The proportion of sucrose in vegetative growth phase I was greater than that in vegetative growth II (p = 0.0421) and seed dispersal (p = 0.0121).

Figure 4 – Proportions of sugars in the set of soluble carbohydrates detected in extracts from underground organs of *Sisyrinchium vaginatum* Spreng. collected at different phenological stages in an area of cerrado: sprouting (Sp), vegetative growth I (VG-I), vegetative growth II (VG-II), seed dispersal (SD), and senescence (Se). Differences among medians (n = 5) were detected by Dunn's test ($p < 0.05$).

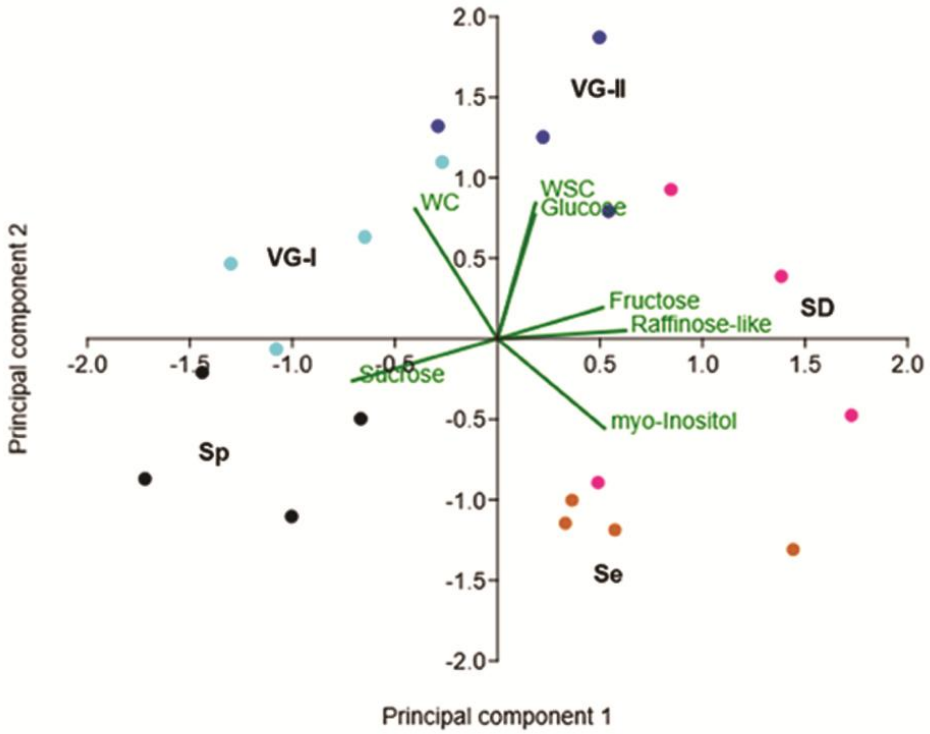


Source: The authors

As occurred with the sucrose proportion, the proportion of raffinose-like in the underground organs differed among phenological phases ($H_{(4)}$ = 14.6; p = 0.0056), being greater in seed dispersion than in sprouting (p = 0.0041) and vegetative growth I (p = 0.0028). Likewise, raffinose-like was greater in senescence than in sprouting (p = 0.0364) and vegetative growth I (p = 0.027). Raffinose-like was greater in vegetative growth II than in vegetative growth I (p = 0.0314) and sprouting (p = 0.0421).

Principal component analysis revealed two distinct clusters, which are distinguishable by phenological phase (Figure 5). The first two components (PCA1 and PCA2) explain 70.2% of the ordination. For the first axis (40.98%), the proportion of raffinose-like (0.485) was the variable that contributed the most positively, while the proportion of sucrose contributed the most negatively (-0.543). For the second axis (29.22%), the variables that contributed positively were the soluble carbohydrate content (0.555), glucose proportion (0.543), and water content (0.454). Analysis of the most influential variables enabled discrimination between the phenological phases of seed dispersal and senescence on the right side of the graph and the sprouting and vegetative growth phases I on the left, while vegetative growth II was intermediate between the two groups.

Figure 5 – Principal component analysis performed with biochemical variables obtained from underground organs of *Sisyrinchium vaginatum* Spreng.: water content (WC), total soluble carbohydrates contents (TSC), proportions of *myo*-inositol, glucose, fructose, sucrose and raffinose-like in different phenological phases: sprouting (Sp), vegetative growth I (VG-I), vegetative growth II (VG-II), seed dispersal (SD) and senescence (Se).



Source: The authors

DISCUSSION

Climatic seasonality in the Cerrado is marked by the rainy season, which occurs from October to April, and by the dry season, from May to September (Silva *et al.*, 2008). The phenological pattern of *Sisyrinchium vaginatum* is influenced by seasonal changes. Sprouting and vegetative growth occur during the rainy season, while seed dispersal occurs in the transition between the rainy and dry seasons. Then, at the beginning of the dry season, the aerial organs become senescent, and the underground organs become dormant. This pattern also occurs in other herbaceous species from the Cerrado (Mantovani and Martins 1988, Munhoz and Felfili, 2005). Throughout the entire fieldwork period, the species was not found with flowers; collections only began during the seed dispersal phase. Therefore, flowering likely occurred prior to the start of collections.

Almeida *et al.* (2015) also did not find *S. vaginatum* flowering during different field expeditions.

The water content of the underground organs of S. *vaginatum* varies along phenological phases. The lowest water content occurs in senescence, during the dry season when there is low rainfall and low relative air humidity (Table 1). These results corroborate the observations made by Almeida *et al.*, (2015), who reported similar water content in underground organs of *S. vaginatum* in the dry season. Variation in the water content of underground organs occurs in other species, such as the Asteraceae *Ichthyothere terminalis* (Spreng.) S.F.Blake (Almeida *et al.*, 2017), possibly in response to variation in soil water content (Dimitrakopoulos and Bemmerzouk, 2003).

In addition to storing water, the underground organs of *S. vaginatum* serve as storage sites for soluble carbohydrates, which constitute more than 15% of the dry mass of these structures. These contents vary along phenological phases, increasing with vegetative growth until seed dispersal. Species with determined growth, such as *S. vaginatum*, have a sigmoid growth curve with acceleration, linear, and saturation phases, the last of which is reached at maximum vegetative growth of the aerial organs (Yin *et al.*, 2003). When growth slows, the allocation of photoassimilates for this purpose is reduced. If photosynthesis continues to operate and exceeds the demand for maintenance and growth, surplus sugars are stored (Ludewig and Flüge, 2013). When present, underground reserve organs, such as those of *S. vaginatum*, constitute important sinks for photoassimilates. Therefore, the highest levels of soluble carbohydrates in underground organs are found during the saturation periods of vegetative growth, represented in this study by vegetative growth II.

Otherwise, during senescence, the degradation of leaf tissues, cells and chloroplasts leads to a gradual reduction in photosynthesis until cell death (Wojciechowska *et al.*, 2018). The lowest levels of soluble carbohydrates in the underground organs of S. *vaginatum* occur during senescence. This suggests the use of stored reserves due to the maintenance of metabolic activity in underground organs, combined with the lower contribution of photoassimilates through the degradation of photosynthetic tissues. This profile is different from that observed in other herbaceous species from the Cerrado, which show an increase in soluble carbohydrates in underground organs at the beginning of dormancy, resulting from the increased allocation of photoassimilates to these storage structures (Silva *et al.*, 2013; Rigui *et al.*, 2015; Almeida *et al.*, 2017).

In adverse conditions, such as water deficit, the movement of carbohydrates from sources to sinks is essential to adjust plant growth (Chang and Zhu 2017). Carbohydrate storage in underground organs is an important feature of plants with the ability to regrow (Clarke *et al.*, 2013); reserve compounds are used to support the development of buds and new aerial organs in the favorable growing

season. Therefore, the storage of carbohydrates in organs that have buds contributes to the permanence of these species in savanna environments, which are often subject to disturbances (Klimesova *et al.*, 2018).

In addition to providing energy to support development, soluble carbohydrates can have other roles in plants (Keunem *et al.*, 2013). Therefore, it is necessary to know the distribution and dynamics of these sugars.

The present study confirmed that sucrose is the main soluble carbohydrate stored in the underground organs of *S. vaginatum*, as previously reported by Almeida *et al.*, (2015). Sucrose is an important reserve carbohydrate in the underground organs of other species, such as *Beta vulgaris* L., the sugar beet (Getz, 2000) and *Amaranthus viridis* L. (Campos *et al.*, 2021). Sucrose is the main product of photosynthesis and performs numerous functions in plants: carbon transport by phloem, storage, and signaling for development and stress responses (Salerno and Curatti, 2003; Ruan *et al.*, 2014). Sucrose is an osmotically active sugar, which reduces osmotic potential and, consequently, water potential, which in turn mitigates the gradient of water potential between cells and the environment (Keunem *et al.*, 2013), thus preventing plant tissues from losing water during water restriction. Therefore, the presence of sucrose stored in underground organs of *S. vaginatum* suggests it may have a role in the protection of these structures during the dry season.

The hydrolysis of sucrose produces glucose and fructose, so these monosaccharides are expected to be present in equivalent proportions in sucrose-containing organs (Koch, 2004). Glucose and fructose were identified in the underground organs of *S. vaginatum*, but the proportion of fructose was higher than that of glucose, indicating that fructose is related to other metabolic pathways.

Almeida *et al.* (2015), who used the CarboPac PA-100 column and a gradient of sodium acetate in sodium hydroxide as eluent in the HPAEC-PAD, observed a sugar with a retention time close to the trisaccharides, which was identified as raffinose by co-chromatography. The authors reported that this sugar was stained by the specific reagent for fructose in thin-layer chromatography, which reinforced its probable identification as raffinose. In the present study, a different eluent (sodium hydroxide) and a different column (CarboPac PA1) were used to separate the sugars of the raffinose series to confirm their presence. Co-chromatography showed the raffinose-like peak and the sugars of the raffinose series eluted at different retention times, which confirms that they are distinct. However, it is likely that the structure of raffinose-like may be close to raffinose, as the higher proportion of fructose may be related to raffinose-like metabolism.

The presence of raffinose-like in *S. vaginatum*, as far as our concern, a sugar not reported in other Iridaceae, highlights the biochemical diversity of this

family. Studies report that starch, fructans, and glucomannans are the main reserve carbohydrates found in underground organs of species of the Iridaceae (Ruiters and McKenzie 1994; Ranwala and Miller 2008; Almeida *et al.*, 2015). However, isolation and additional analyses are needed for the correct identification of raffinose-like.

The *myo*-inositol found in the underground organs of *S. vaginatum* is a cyclitol that has several functions in plants: cell signaling, intermediation in biosynthetic pathways, removal of free radicals and protection against stresses (Valluru and Van den Ende 2011). *myo*-Inositol is used in the synthesis of galactinol, a galactosyl residue donor substrate for the synthesis of galactooligosaccharides (Gangola *et al.*, 2014; Van den Ende 2013). Its presence in the underground organs of *S. vaginatum* indicates the possibility that it acts as an intermediary for raffinose-like biosynthesis. Additionally, since the highest proportion of *myo*-inositol was observed at the beginning of the dry season, it is possible that it exerts some cellular protection function.

Phenological phases do indeed influence the dynamics of soluble carbohydrates and the water content of the underground organs of *S. vaginatum*. This was evidenced by the formation of distinct groups in the principal component analysis. On axis 1, the proportions of sucrose have opposite tendencies to those of raffinose-like and fructose, which is supported by the analysis of the dynamics of these sugars proportions and suggests that raffinose-like is synthesized from sucrose and releases fructose.

The relationship between the composition and dynamics of soluble carbohydrates in the underground organs of *S. vaginatum*, its development, and climatic seasonality may play a role in the species' ability to persist in seasonal environments.

CONCLUSIONS

The soluble carbohydrates in *S. vaginatum* underground organs were influenced by the phenology. Storage increased along vegetative growth, consistent with the initial hypothesis. The extracts contained significant amounts of sucrose, raffinose-like sugar, glucose, and fructose in all the growth stages. Higher levels of sucrose were associated with sprouting, while raffinose-like content was linked to seed dispersal. The changes in soluble carbohydrates in the underground organs of *S. vaginatum* may contribute to the species' seasonal growth, allowing it to thrive in water-limited environments.

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