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Short communication

Diversity of non-structural carbohydrates in the underground organs of five Iridaceae species from the Cerrado (Brazil)

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ABSTRACT

South America has a great diversity in some tribes of the Iridaceae family. Most of the Iridaceae are geophytes, with underground organs bearing buds and reserve compounds, which favor their occurrence in seasonal environments, such as the Cerrado. Non-structural carbohydrates (NSC) are the main reserves in geophytes, essential to support phenological events, and protect plants against abiotic stresses. NSC may also reflect taxonomic relationships among plant groups. The objective of this study was to determine the contents and composition of NSC in underground organs of five Iridaceae species from the Cerrado (*Cipura paludosa*, *Cipura xanthomelas*, *Trimezia cathartica*, *Trimezia juncifolia* and *Sisyrinchium vaginatum*), representing the tribes Tigridieae, Trimezieae and Sisyrinchieae. Soluble carbohydrates and total fructose in oligo and polysaccharide fractions, and the starch contents were determined, and sugar composition was analyzed by HPAEC-PAD. The species from the tribes Trimezieae and Tigridieae showed similar NSC profiles, with considerable accumulation of starch and glucose, fructose, sucrose and maltose. Additionally, *T. juncifolia* also presented raffinose and 1-kestose. In contrast, *S. vaginatum* of the Sisyrinchieae has very low starch content and a distinct carbohydrate profile, predominating soluble carbohydrates, possibly of the raffinose family.

1. Introduction

The Cerrado covers approximately 20% of Brazil surface area, comprising several physiognomies, from open grasslands to woodlands in a mosaic determined by edaphic factors and fire (Coutinho, 2002). Plants growing in the Cerrado are subjected to seasonal rainfall, fire events and nutrient-poor soils, leading to different adaptations, like the presence of underground organs storing carbohydrates. In Cerrado, the rainy season lasts from October to March, and the dry season from April to September (Silva et al., 2008). Among the storage carbohydrates, fructans are typically found in Asteraceae, one of the most abundant families (Carvalho et al., 2007; Rossatto et al., 2013; Silva et al., 2008). In another abundant family in the Cerrado, Poaceae, with many fructan accumulators, these sugars have not been found in any of the 24 accessions in a restrict area of Cerrado (Moraes et al., 2013).

Iridaceae is a species-rich herbaceous and petaloid monocot family well represented in the Cerrado and with distinct morphology (Goldblatt, 1990; Eggers et al., 2010). Southern Africa is the major radiation site for this family. However, the family is also highly diverse in South America (Goldblatt et al., 2008). In the Neotropics, only the subfamily Iridoideae occurs and includes the tribes Tigrideae and Trimezieae, which are restricted to the Neotropics, and the New World and Australasian Sisyrinchieae (Goldblatt et al., 2008; Lovo et al., 2012). In Brazil, there are 18 genera and 160 species of Iridaceae (Eggers et al., 2010).

One noteworthy feature of many of the Iridaceae species is the geophytic life form with the presence of underground organs with perennating buds and reserve compounds. This provides conditions that allow their occurrence in environments with climatic seasonality regarding temperature and/or water restriction (Kamenetsky et al., 2005). During the unfavorable periods, geophytes lose their aerial parts and become dormant (Dafni et al., 1981; Raunkiaer, 1934). As soon as the appropriate environmental conditions for growth are reestablished, geophytes re-grow and complete their life cycle within this period, which may be considered short for some species. For this reason, the main focus of a geophyte should be keeping the underground organs alive (Dafni et al., 1981; Rossa and von Willert, 1999). From a physiological perspective, geophytes go through several cycles of allocation and mobilization of reserves and nutrients to and from the storage organ (Rossa and von Willert, 1999; Ruiters, 1995). For this reason, the metabolism of the storage compounds is essential to ensure their success in a seasonally impacted environment, such as the Cerrado.

The main reserves in geophytes are the non-structural carbohydrates (NSC), such as starch, sugars and fructans; however, glucomannans may also be found (Kamenetsky et al., 2005; Miller, 1992; Ranwala and Miller, 2008). Such reserves support sprouting

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during the favorable season and growth when overall photoassimilate production is low; therefore, NSC are essential to maintain geophytes phenological events (Dafni et al., 1981). Changes in the pool of NSC triggered by phenology may lead to increases in low molecular weight sugars to attend developmental demands (Kamenetsky et al., 2003). Moreover, soluble carbohydrates may provide protection against harsh conditions due to their involvement in the protection against abiotic stresses (Patrick et al., 2013; Valluru and Van den Ende, 2008).

In addition to the physiological roles, NSC composition may be influenced by genetic traits, the geographical origin of the species and their taxonomic relationships (Hendry, 1993; Moraes et al., 2013). Among the Poaceae, fructans have been found typically in the Pooideae subfamily, predominant in temperate regions, while in the tropical subfamilies, these carbohydrates are absent (Chatterton et al., 1989; Hendry, 1993; Moraes et al., 2013). Although the storage of carbohydrates in underground organs is a typical characteristic of Iridaceae, not all species of this family accumulate fructans (Hendry, 1993), indicating evolutionary traits and metabolic peculiarities of such plants to overcome the wide range of environmental conditions in habitats where they occur.

In the present study, we determined the contents and composition of NSC in underground organs of Iridaceae species occurring in the Cerrado, aiming to increase the knowledge on the distribution, characterization and function of these compounds in wild species, still scarcely studied in this monocot family. In addition, plants from two populations were collected in distinct locations, one of them slightly higher in altitude but with similar floristic composition, aiming to associate putative differences with the predominant environmental forces.

2. Materials and methods

2.1. Study sites

This study was conducted in two Cerrado areas of Goiás State, Brazil, one located at the Reserva Biológica Prof. José Angelo Rizzo (16°22′–15°48′S and 50°44′–49°55′W), in Serra Dourada (SD) (Mossâmedes, GO, Brazil), with altitudes between 700 m and 1080 m. The other site was Serra dos Pirineus (SP) (15°46′ to 15°50′S; 48°48′ to 48°53′W) (Pirenópolis, GO, Brazil), with altitude between 800 and 1385 m. Both sites are in the core of the Cerrado biome and have a gradient of physiognomies, from open grasslands to woodlands, and various soil types including Neosols with rocky outcrops savanna, known as *cerrado rupestre* (Dantas and Silva, 2013; Rizzo, 1970; Santos et al., 2012).

2.2. Plant material

Five species were collected in both study sites in rocky outcrops and surrounding open field areas (Table 1, Fig. 1). Two from the tribe Tigridieae (*Cipura paludosa* Aubl. and *C. xanthomelas* Mart. ex Klatt), two from the Trimezieae (*Trimezia cathartica* (Klatt) Niederl. and *Trimezia juncifolia* (Klatt) Benth. & Hook. f.) and one from the Sisyrinchieae (*Sisyrinchium vaginatum* Spreng.) The specimens were identified using Celis et al. (2003), Chukr and Capellari Jr (2003), Chukr and Giulietti (2008), Goldblatt et al. (1990), Goldblatt and Manning (2008) and Henrich and Goldblatt (1987). Vouchers were deposited in the Herbarium of the Universidade Federal de Goiás (UFG). All the specimens were collected in the reproductive phase, except for *S. vaginatum* Spreng in both sites, which was found only in the vegetative phase.

Underground organs from approximately four distinct individuals of each species were collected at each site. The organs were rinsed in tap water, the lignified cataphylls removed and the fresh mass determined. Approximately 1 g of each specimen was oven dried at 60 °C to constant weight, for dry mass determination (DM). These data were also used for determination of the water content (WC), expressed as percentage of total fresh mass (FM). Two grams of fresh material were used for non-structural carbohydrate extraction, except for *C. paludosa*, of which only 0.2 g was used for dry mass determination and for NSC analyses, due to the very small bulbs present in this species. All analyses were performed in triplicate.

2.3. Soluble carbohydrate analysis

Samples of fresh underground organs were sliced and boiled in aqueous ethanol (80%) for 5 min, homogenized in a mortar with a pestle and filtered. The residues were re-extracted twice with ethanol 80% at 80 °C for 15 min (modified from Pollock and Jones, 1979). The ethanolic supernatants were pooled and constituted the oligosaccharide fraction. In sequence, the residues were extracted with distilled water at 60 °C for 30 min and filtered. This procedure was repeated once, and the aqueous supernatants were pooled and constituted the soluble polysaccharide fraction. The extracts were stored at −20 °C for quantitative determinations and qualitative analysis.

The soluble carbohydrates in the oligo- and polysaccharide fractions were quantified by the phenol-sulfuric method, using glucose as standard (Dubois et al., 1956). Total fructose was determined by using a modified anthrone method, specific for ketoses (Jermyn, 1956), using fructose as standard, in order to evaluate the putative presence of fructans.

Oligosaccharide fractions were deionized in ion exchange columns containing cationic (Amberlite IRA 120) and anionic (Amberlite IRA 410) resins, and eluted in ultrapure water (18.2 MΩ). The pH was neutralized with ammonium hydroxide. Purified samples were vacuum concentrated (37 °C) to dryness, resuspended in ultrapure water, adjusted to the equivalent concentration of 400 μg fructose mL^{-1} and filtered through 0.45 μm membranes.

The neutralized extracts were analyzed by high-performance anion exchange chromatography, with pulsed amperometric detection (HPAEC-PAD) on a Dionex ICS-5000 chromatographic system, using a CarboPac PA—100 (4 × 250 mm) column. Carbohydrates were eluted in a gradient of sodium acetate in NaOH (150 mM), with the following schedule: 0–2 min, 5 mM; 2.1–8 min, 5–50 mM; 8.1–11 min, 50–150 mM; 11.1–14 min, 250 mM; 14.1–18 min 5 mM. The eluent flow was 1 $cm^3 \cdot min^{-1}$. The applied PAD potentials followed the manufacturer's recommendation. Sugars were identified by comparison with standards chromatographed under the same conditions.

Table 1
Voucher information and subterranean organ type for the species studied from Serra Dourada (SD) and Serra dos Pireneus (SP).

Tribe	Species	Underground organ	Voucher	
			SD	SP
Tigridieae	*Cipura paludosa* Aubl.	Bulb	*Sartin* 400 48924 (UFG)	*Queiroz* 90 46598 (UFG)
	Cipura xanthomelas Mart. *ex* Klatt	Bulb	*Sartin* 389 48925 (UFG)	*Queiroz* 91 46599 (UFG)
Sisyrinchieae	*Sisyrinchium vaginatum* Spreng.	Rhizome	*Sartin* 388 48926 (UFG)	*Queiroz* 87 46596 (UFG)
Trimezieae	*Trimezia cathartica* (Klatt) Niederl.	Corm	*Moraes* 131 48927 (UFG)	*Queiroz* 83 46592 (UFG)
	Trimezia juncifolia (Klatt) Benth. & Hook. f.	Corm	*Sartin* 434 48928 (UFG)	*Queiroz* 84 46593 (UFG)

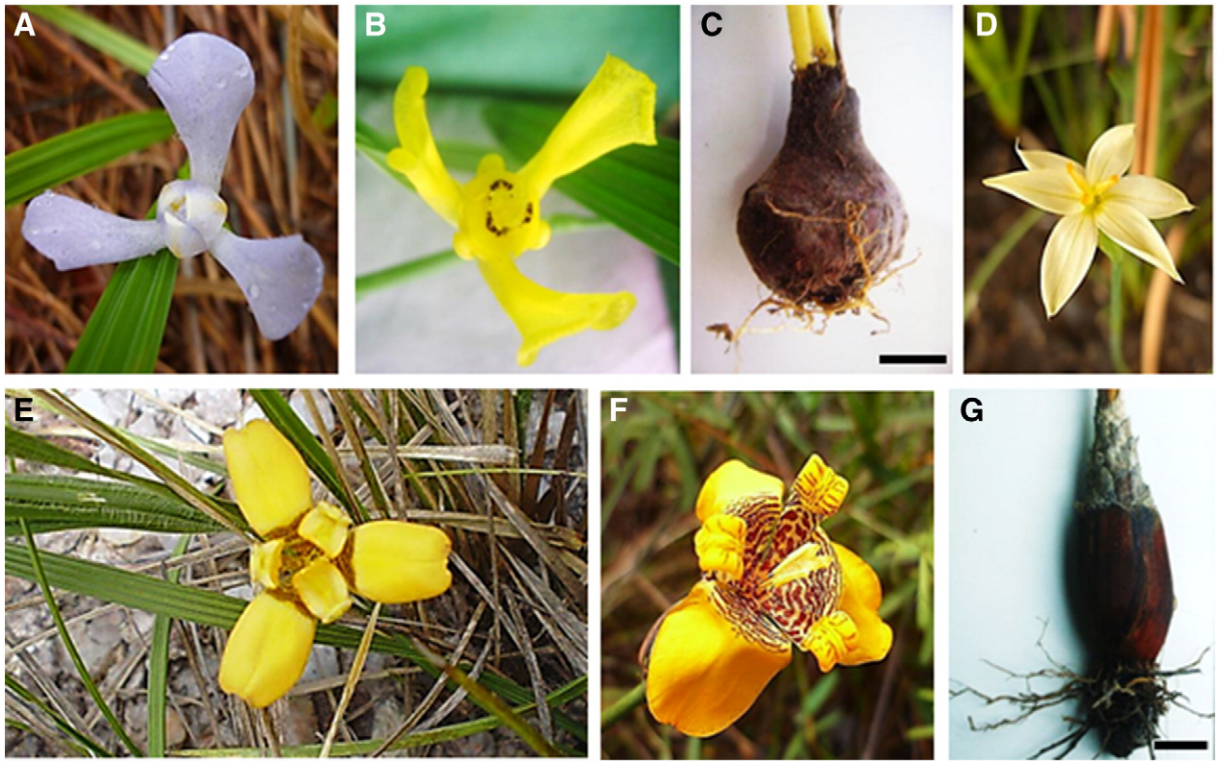

Fig. 1. Studied Iridaceae species from the Cerrado. Flowers (A, B, D, E, F) and underground organs (C, G). *Cipura paludosa* (A), *Cipura xanthomelas* (B, C), *Sisyrinchyum vaginatum* (D), *Trimezia cathartica*. Note the rocky substrate (E) and *Trimezia juncifolia* (F, G). Scale bars = 2 cm.

2.4. Starch analysis

The residues from the soluble carbohydrate extraction were oven dried at 60 °C to constant mass. In sequence, residues were ground to a fine powder. Starch analysis was performed as described by Amaral et al. (2007). Aliquots of 10 mg of the fine powder were incubated with 0.5 mL (60 U) of thermostable α-amylase from *Bacillus licheniformis* (Megazyme, Ireland), diluted in 10 mM MOPS buffer, pH 6.5 (120 U· mL^{-1}) at 75 °C for 30 min. Samples were cooled down to 50 °C and incubated with 0.5 mL (15 U) of amyloglucosidase from *Aspergillus niger* (Megazyme, Ireland) diluted in 100 mM sodium acetate buffer, pH 4.5 (30 U· mL^{-1}) at 50 °C for 30 min. Both enzymatic steps were sequentially performed twice. At the end of the incubation, 100 μL of perchloric acid (0.8 mM) was added to the reaction mixture to stop the reaction and precipitate proteins. After centrifugation, starch was quantified by glucose determination by adding 0.3 mL of a mixture containing glucose oxidase (11,000 U· mL^{-1}) and peroxidase (700 U· mL^{-1}), 4-aminoantipyrin (290 μmol· L^{-1}) and phenol (50 mM) at pH 7.5 and incubated at 37 °C for 15 min. Free glucose was determined colorimetrically at 490 nm.

2.5. Statistical analyses

Statistical analyses were performed using Bioestat 5.0 statistical software. Differences among species at each study site were evaluated using Kruskal–Wallis analysis of variance on ranks. Subsequently the Student–Neuman–Keuls (SNK) *post hoc* test ($p < 0.05$) was used for the following variables: water content of the underground organs,

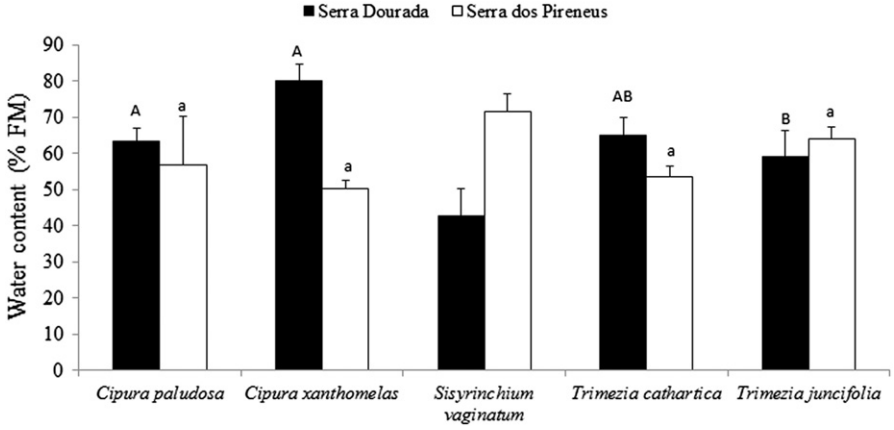

Fig. 2. Water content expressed as percentage of fresh mass in underground organs of the studied species ($n = 4$). Uppercase letters compare means of Serra Dourada and lowercase letters compare means of Serra dos Pireneus ($p < 0.05$), except for *S. vaginatum*, unavailable in the reproductive phase.

Table 2
Contents of total soluble carbohydrates, total fructose and percentage of fructose in soluble carbohydrates (% F) of the oligosaccharide fraction of subterranean organs of the studied species from Serra Dourada (SD) and Serra dos Pireneus (SP).

Species	Soluble carbohydrates ($mg \cdot g^{-1}$ DM)		Fructose ($mg \cdot g^{-1}$ DM)		% F	
	SD	SP	SD	SP	SD	SP
C. paludosa	34.1 ± 9.5[a]	39.9 ± 12.1[a]	20.3 ± 1.2[a]	23.1 ± 6.4[a]	59.6	57.8
Cipura xanthomelas	49.9 ± 19.4[a]	41.7 ± 14.4[a]	31.3 ± 14.6[a]	15.8 ± 11.4[a]	62.8	37.9
Sisyrinchium vaginatum	91.1 ± 48.8	116.0 ± 33.1	27.8 ± 14.7	25.7 ± 4.6	30.5	22.2
Trimezia cathartica	65.6 ± 38.6[a]	44.8 ± 12.4[a]	43.9 ± 19.6[a]	14.4 ± 4.1[a]	66.9	32.1
Trimezia juncifolia	36.3 ± 9.1[a]	70.1 ± 10.6[a]	29.8 ± 8.5[a]	37.2 ± 6.3[a]	82.1	53.0

Values are means ± standard deviation ($n = 4$ for SD, $n = 3$ for SP). Different letters indicate differences in each column ($p < 0.05$), except for *S. vaginatum*, unavailable in the reproductive phase.

soluble carbohydrates and fructose contents, both in oligosaccharide and polysaccharide fractions and starch. *S. vaginatum* data, were not included in the statistical analyses, since specimens were harvested in vegetative stage and in different seasons.

3. Results

3.1. Water and non-structural carbohydrate contents

The water content of the underground organs ranged from 58.9 in *T. juncifolia* to 79.9% in *C. xanthomelas* on a fresh mass basis at the Serra Dourada (SD) site and from 53.3% in *C. xanthomelas* to 71.5% in *S. vaginatum* at the Serra dos Pireneus (SP) site. *S. vaginatum* was not found in the reproductive phase in any of the studied sites. Besides, in SD, this species was found only in the dry season, presenting 42.7% of water content, in contrast to 71.52% found in plants collected in the rainy season at SP (Fig. 2). For this reason, *S. vaginatum* was excluded from the statistical analysis.

Total soluble carbohydrates (expressed as $mg \cdot g^{-1}$DM) in oligosaccharide fraction in the different species and sites were 34.1 (SD) and 39.9 (SP) in *C. paludosa*; 49.9 (SD) and 41.7 in *C. xanthomelas*; 91.1 (SD) and 116.0 (SP) in *S. vaginatum*; 65.6 (SD) and 44.8 (SP) in *T. cathartica*; 36.3 (SD) and 70.1 (SP) in *T. juncifolia* (Table 2). In oligosaccharide fraction, total fructose levels ($mg \cdot g^{-1}$DM) were 20.3 (SD) and 23.1 (SP) in *C. paludosa*; 31.3 (SD) and 15.8 in *C. xanthomelas*; 27.8 (SD) and 25.7 (SP) in *S. vaginatum*; 43.9 (SD) and 14.4 (SP) in *T. cathartica*; and 29.8 (SD) and 37.2 (SP) in *T. juncifolia* (Table 2). Total fructose ranged from 20.3 to 43.9 $mg \cdot g^{-1}$ DM in SD and from 14.4 to 37.2 $mg \cdot g^{-1}$ DM in SP and represented around 30% in *C. xanthomelas* and *T. cathartica* in SP, and in *S. vaginatum* from both sites. For the other species, the fructose percentage was always higher than 50%, reaching 82% in *T. juncifolia* from SD (Table 2).

In the polysaccharide fraction, in general, total soluble carbohydrates were lower than in the oligosaccharide fraction, except for *T. cathartica* of SP (Table 3). The levels ($mg \cdot g^{-1}$DM) found in the different species from both sites were as follows: 11.1 (SD) and 38.0 (SP) in *C. paludosa*; 33.7 (SD) and 1.6 in *C. xanthomelas*; 8.5 (SD) and 8.4 (SP) in *S. vaginatum*; 21.5 (SD) and 70.3 (SP) in *T. cathartica*; 6.9 $mg \cdot g^{-1}$DM (SD) and 5.4 (SP) in *T. juncifolia* (Table 3). Total soluble carbohydrates levels in *T. juncifolia* were lowest compared to the other species of the Trimezieae and species of Tigridieae ($p < 0.05$). Total fructose levels ($mg \cdot g^{-1}$DM) in the polysaccharide fraction of the studied species were 1.7 (SD) and 7.2 (SP) in *C. paludosa*; 2.4 (SD) and 0.7 (SP) in *C. xanthomelas*; 0.8 (SD) and 0.9 (SP) in *S. vaginatum*; 2.2 (SD) and 1.5 (SP) in *T. cathartica*; and 2.9 $mg \cdot g^{-1}$DM (SD) and 3.8 (SP) in *T. juncifolia*. Fructose represented less than 20% in the majority of species (Table 3).

Compared to the soluble carbohydrates, starch contents were notably higher in all but one species, *S. vaginatum*, in which starch was practically undetectable. However, values varied among the analyzed species; *T. juncifolia* and *Cipura* species presented the highest starch contents (approx. 80% DM) in SD (Fig. 3).

3.2. Oligosaccharide profile

Glucose, fructose and sucrose were present in the oligosaccharide fraction in all the studied species in both sites. Maltose was detected in *C. paludosa, C. xanthomelas*, *T. cathartica* and *T. juncifolia*; however, the peak area varied depending on the species and location (Fig. 4). Small peaks of raffinose and 1-kestose were found in *T. juncifolia*. *S. vaginatum* was the only species with substantial proportion of raffinose at both sites, which was confirmed by TLC analysis (data not shown). Maltose or other oligosaccharides were not detected in this species (Fig. 4).

4. Discussion

The studied species of Iridaceae showed differences regarding the nature and amount of the stored carbohydrates. Starch was the major non-structural carbohydrate in *Cipura* and *Trimezia* species, contrasting with the low contents of soluble carbohydrates found, mainly glucose, fructose, sucrose and maltose (Fig. 4). Fructose detected in the oligosaccharide fraction (Table 2) is probably free fructose and fructosyl moiety in sucrose since fructans were not detected by the chromatographic analyses.

Table 3
Contents of total soluble carbohydrates, total fructose and percentage of fructose in soluble carbohydrates (% F) of the polysaccharide fraction of subterranean organs of the studied species from Serra Dourada (SD) and Serra dos Pireneus (SP).

Species	Soluble carbohydrates ($mg \cdot g^{-1}$ DM)		Fructose ($mg \cdot g^{-1}$ DM)		% F	
	SD	SP	SD	SP	SD	SP
Cipura paludosa	11.1 ± 0.3[ab]	38.0 ± 28.3[a]	1.7 ± 0.4[a]	7.2 ± 1.7[a]	15.5	19.0
Cipura xanthomelas	33.7 ± 16.1[a]	1.6 ± 0.6[b]	2.4 ± 0.8[a]	0.7 ± 0.3[b]	7.1	44.8
Sisyrinchium vaginatum	8.5 ± 4.0	8.4 ± 6.5	0.8 ± 0.3	0.9 ± 0.1	9.7	11.3
Trimezia cathartica	21.5 ± 14.7[a]	70.3 ± 33.5[a]	2.2 ± 0.9[a]	1.5 ± 0.7[b]	10.2	2.1
Trimezia juncifolia	6.9 ± 2.1[b]	5.4 ± 2.5[ab]	2.9 ± 1.2[a]	3.8 ± 1.5[ab]	42.0	70.3

Values are means ± standard deviation ($n = 4$ for SD, $n = 3$ for SP). Different letters indicate differences in each column ($p < 0.05$), except for *S. vaginatum*, unavailable in the reproductive phase.

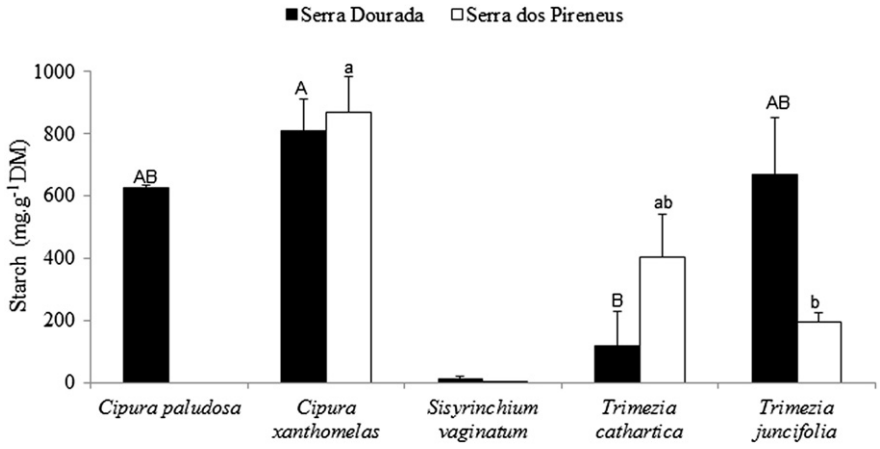

Fig. 3. Starch contents in the underground organs of the studied species ($n = 3$). Uppercase letters compare mean values of plants collected in Serra Dourada and lowercase letters compare mean values of Serra dos Pireneus ($p < 0.05$). The values for *Sisyrinchium vaginatum* are excluded from the statistical test due to unavailable plant material in the reproductive phase.

Starch is the main reserve in several geophytes, but glucose, fructose and sucrose are also common in lower amounts (Frankova et al., 2003; Ranwala and Miller, 2008; Theron and Jacobs, 1996). Starch and soluble carbohydrate contents may vary in underground organs of geophytes influenced by the specific demands of each phenological phase. In *Colchicum autumnale*, for instance, Frankova et al. (2003, 2004) reported

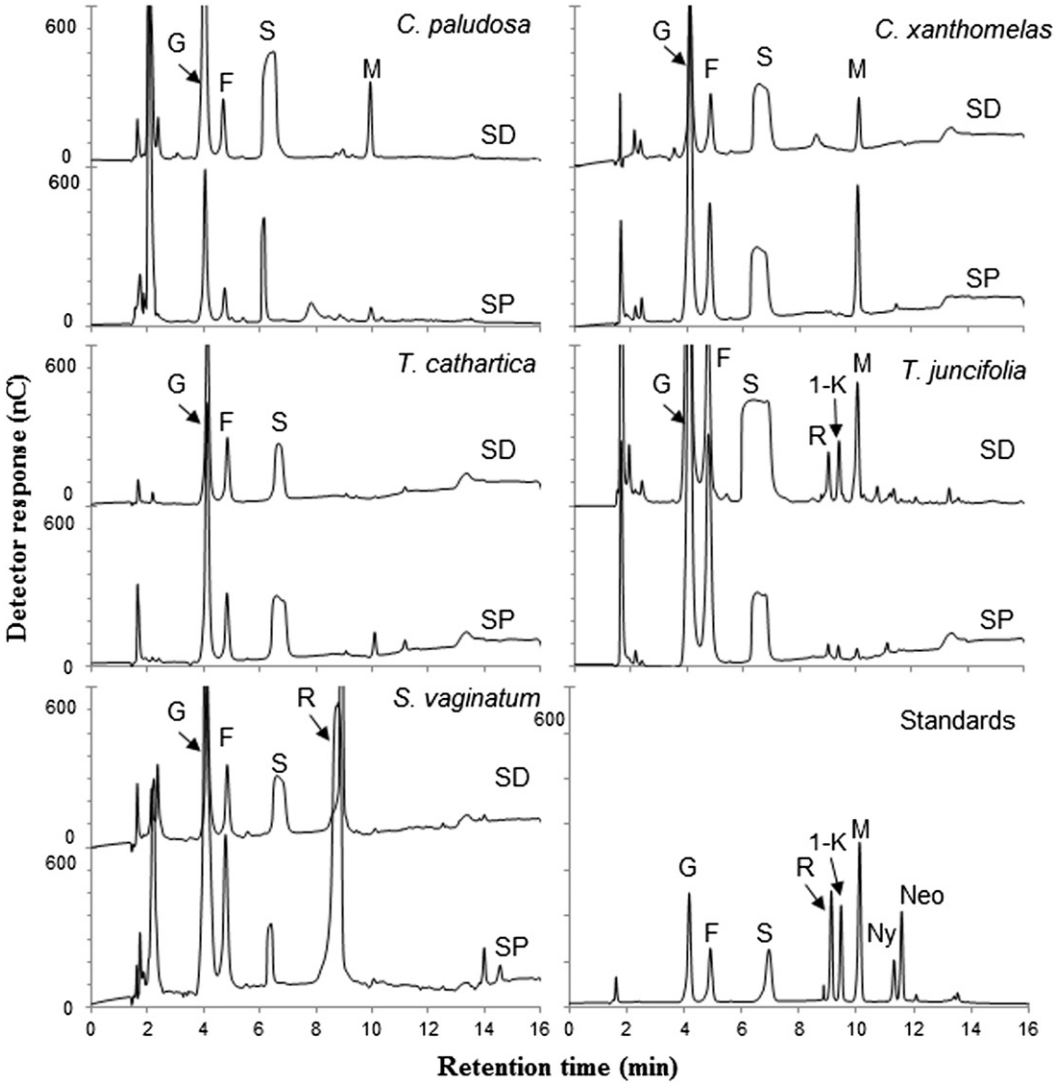

Fig. 4. HPAEC-PAD profiles of soluble oligosaccharides of the studied species collected in Serra Dourada (SD) and in Serra dos Pireneus (SP). G, glucose; F, fructose; S, sucrose; R, raffinose; M, maltose; 1-K, 1-kestose; Ny, nystose; and Neo, neokestose.

an increase in amylolytic activity and sucrose synthesis were increased to supply shoot demands. Maltose, one of the degradation products of starch may also be present in varying amounts (Stitt and Zeeman, 2012; Tetlow et al., 2004), being widespread at least in trace amounts (Lewis, 1984). This highlights the typical geophytic metabolism, with NSC turnover to meet the requirements of the alternating sinks (Frankova et al., 2003; Orthen and Wehrmeyer, 2004).

Despite the predominance of starch, *T. juncifolia* also presented 1-kestose and raffinose, but these trisaccharides were not detected in *Cipura* species or in *T. cathartica* (Fig. 4). In specimens of both *Trimezia* species collected at Serra Dourada, total fructose represented 66% and 82% of soluble carbohydrates in the oligosaccharide fraction, while in Serra dos Pireneus fructose proportions were 32% and 53% (Table 2). The higher proportions of fructose in specimens from Serra Dourada represent the prevalence of sucrose and sucrose-based oligosaccharides other than fructans in this fraction, as it was confirmed by HPAEC-PAD analyses (Fig. 4).

Fructans are the main reserve in 15% of the angiosperms and may occur in Iridaceae (Hendry, 1993). Fructans and starch can co-occur, such as in bulbs of the geophyte *Lachenalia minima* (Hyacinthaceae), which has similar amounts of both carbohydrates (Orthen, 2001). In specimens of *Trimezia* collected in Serra Dourada, total fructose represented approx. 5% of the corms in the oligosaccharide fraction (Table 2). This is similar to what was reported for tulip bulbs, in which fructans constitute 5–10% of the dry matter in the underground organs (Kamenetsky et al., 2003). When co-occurring, fructans and starch might have different functions. In *Lachenalia minima* starch is the carbon source for re-sprouting and fructans are associated with water status adjustments (Orthen, 2001). The water status in turn, is associated with the shift in phenological phase in geophytes (Kamenetsky et al., 2003; Zemah et al., 1999).

In this study, *S. vaginatum* presented distinct NSC profile compared to the other studied species. The starch content was nearly undetectable and the soluble carbohydrates prevailing were glucose, fructose, sucrose and raffinose. The presence of high amounts of raffinose has not been previously reported within Iridaceae. Similar to fructans, raffinose is a sucrose-based oligosaccharide, differing from the first ones by the galactose unit that is linked to sucrose. Therefore, the raffinose amount may be underestimated in some studies due to its inclusion in the fructan pool (Chatterton et al., 1989). Additionally, the amount of raffinose found in *S. vaginatum* might be yet higher if analyzed in other phenological phases and/or environmental conditions, factors that notably affect NSC composition and distribution in underground organs of geophytes (Frankova et al., 2003; Orthen and Wehrmeyer, 2004; Orthen, 2001; Theron and Jacobs, 1996).

Raffinose is the base trisaccharide for the synthesis of the raffinose family oligosaccharides, the RFOs (Keller and Pharr, 1996). Besides fructans, RFOs are the most notable class of soluble carbohydrates in plants (Van den Ende, 2013) being widespread in the plant kingdom. They occur mostly in seeds and storage organs as reserve compounds but also have a role in carbon transport and protection against abiotic stresses (Keller and Pharr, 1996), including prevention of oxidative damage and osmoprotection (Nishizawa et al., 2008; Van den Ende, 2013). The expressive amounts of raffinose in *S. vaginatum* indicates its role as reserve compound, in addition to the putative role in protecting plants of this species against abiotic stresses that are largely present in the Cerrado (Coutinho, 2002).

Soluble carbohydrates may reflect the relationships between plant groups. Fructans have been associated with Poaceae taxa (Smouter and Simpson, 1989) and their structural analysis can be useful in distinguishing the supertribes (Bonnett et al, 1997). In this work, we observed that species of the tribes Trimezieae and Tigridieae have similar NSC profile, with substantial accumulation of starch and the presence of glucose, fructose, sucrose and maltose. This may reflect the phylogenetic relationships between these sister groups (Goldblatt et al., 2008; Lovo et al., 2012). However, *Trimezia* species showed a difference: while *T. cathartica* followed this profile, *T. juncifoli*a additionally has raffinose and 1-kestose. Although it is necessary to expand the number of species studied in this regard, the difference in the NSC profile of the *Trimezia* species may indicate the heterogeneity in this genus, which needs taxonomic revision as pointed out by Lovo et al. (2012).

Another important aspect to highlight in this study is the predominance of soluble carbohydrates and the appreciable amount of raffinose in *S. vaginatum*, of the tribe Sisyrinchieae. Future studies aiming to confirm the association of NSC profile with the taxonomy of Iridaceae, and with the type of underground reserve organ, should include the analysis of a larger number of species with reserve organs, concerning carbohydrate fluctuation in different phenological stages and environmental conditions.

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References

Amaral, L.I.V., Gaspar, M., Costa, P.M.F., Aidar, M.P.M., Buckeridge, M.S., 2007. Novo método enzimático rápido e sensível de extração e dosagem de amido em materiais vegetais. Hoehnea 34, 425–431.

Bonnett, G.D., Sims, I.M., Simpson, R.J., Cairns, A.J., 1997. Structural diversity of fructan in relation to the taxonomy of the Poaceae. New Phytologist 136, 11–17.

Carvalho, M.A.M., Asega, A., Figueiredo-Ribeiro, R.C.L., 2007. Fructans in Asteraceae from the Brazilian cerrado. In: Shiomi, N., Benkeblia, N., Onodera, S. (Eds.), Recent Advances in Fructooligosaccharides Research. Research Signpost, Kerala, India, pp. 69–91.

Celis, M., Goldblatt, P., Betancur, J., 2003. A New Species of *Cipura* (Iridaceae) from Colombia and Venezuela. Novon 13, 419–422.

Chatterton, N.J., Harrinson, P.A., Bennett, J.H., Asay, K.H., Harrison, P.A., 1989. Carbohydrate partitioning in 185 accessions of Gramineae grown under warm and cool temperatures. Journal of Plant Physiology 134, 169–179.

Chukr, N.S. Capellari Jr. L., 2003. Iridaceae. In: Wanderley, M.G.L., Shepherd, G.J., Giulietti, A.M., Melhem, T.S. (Eds.), Flora Fanerogâmica do Estado de São Paulo. RiMa, São Paulo. v. 3, pp. 127–147.

Chukr, N.S., Giulietti, A.M., 2008. Revisão de *Trimezia* Salisb. ex Herb. (Iridaceae) para o Brasil. Sitientibus Série Ciências Biológicas 8, 15–58.

Coutinho, L.M., 2002. O bioma do cerrado. In: Klein, A.L. (Ed.), Eugen Warming e o cerrado brasileiro: um século depois. Editora UNESP, São Paulo, pp. 77–91.

da Silva, F.A.M., Assad, E.D., Evangelista, B.A., 2008. Caracterização climática do Bioma Cerrado. In: Sano, S.M., de Almeida, S.P., Ribeiro, J.F. (Eds.), Cerrado Ecologia e Flora. Embrapa Cerrados, Planaltina, pp. 69–88.

Dafni, A., Cohen, D., Noy-Meir, I., 1981. Life-cycle variation in geophytes. Annals of the Missouri Botanical Garden 68, 652–660.

Dantas, M.M., Silva, M.J., 2013. O gênero *Senna* Mill. (Leguminosae, Caesalpinioideae, Cassieae) no Parque Estadual da Serra Dourada, GO, Brasil. Hoehnea 40, 99–113.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350–356.

Eggers, L., Chukr, N., Lovo, J., Gil, A., 2010. Iridaceae. In: Forzza, R.C. (Ed.), Catálogo de Plantas e Fungos do Brasil. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro, pp. 1122–1128.

Frankova, L., Komjathyova, H., Boka, K., Gasparikova, O., Psenak, M., 2003. Biochemical and physiological aspects of developmental cycle of *Colchicum autumnale* L. Biologia Plantarum 47, 509–516.

Frankova, L., Cibirova, K., Boka, K., Gasparikova, O., Psenak, M., 2004. The role of the roots in the life strategy of *Colchicum autumnale*. Biologia (Bratislava) 59, 87–93.

Goldblatt, P., 1990. Phylogeny and classification of Iridaceae. Annals of the Missouri Botanical Garden 77, 607–627.

Goldblatt, P., Manning, J.C., 2008. The *Iris* Family: Natural History and Classification. Timber Press, Portland.

Goldblatt, P., Rudall, P.J., Henrich, J.E., 1990. The genera of the *Sisyrinchium* alliance (Iridaceae: Iridoideae): phylogeny and relationships. Systematic Botany 15, 497–510.

Goldblatt, P., Rodriguez, A., Powell, M.P., Davies, T.J., Manning, J.C., van der Bank, M., Savolainen, V., 2008. Iridaceae "Out of Australasia"? Phylogeny, biogeography, and divergence time based on plastid DNA sequences. Systematic Botany 33, 495–508.

Hendry, G.A.F., 1993. Evolutionary origins and natural functions of fructans—a climatological, biogeographic and mechanistic appraisal. New Phytologist 123, 3–14.
Henrich, J.E., Goldblatt, P., 1987. Notes on *Cipura* (Iridaceae) in South and Central America, and a new species from Venezuela. Annals of the Missouri Botanical Garden 74, 333–340.
Jermyn, M.A., 1956. A new method for the determination of ketohexoses in the presence of aldohexoses. Nature 177, 39.
Kamenetsky, R., Zemah, H., Ranwala, A.P., Vergeldt, F., Ranwala, N.K., Miller, W.B., Van As, H., Bendel, P., 2003. Water status and carbohydrate pools in tulip bulbs during dormancy release. New Phytologist 158, 109–118.
Kamenetsky, R., Peterson, R.L., Melville, L.H., Machado, C.F., Bewley, J.D., 2005. Seasonal adaptations of the tuberous roots of *Ranunculus asiaticus* to desiccation and resurrection by changes in cell structure and protein content. New Phytologist 166, 193–204.
Keller, F., Pharr, 1996. Metabolism of carbohydrates in sinks and sources: galactosyl-sucrose oligosaccharides. In: Zamski, E., Shaffer, A.A. (Eds.), Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships. Marcel Dekker, New York, pp. 157–183.
Lewis, D.H., 1984. Occurrence and distribution of storage carbohydrates in vascular plants. In: Lewis, D.H. (Ed.), Storage Carbohydrates in Vascular Plants. Cambridge University Press, Cambridge, pp. 1–52.
Lovo, J., Winkworth, R.C., Mello-Silva, R., 2012. New insights into Trimezieae (Iridaceae) phylogeny: what do molecular data tell us? Annals of Botany 110, 689–702.
Miller, W.B., 1992. A review of carbohydrate metabolism in geophytes. Acta Horticulturae 325, 239–246.
Moraes, M.G., Chatterton, N.J., Harrison, P.A., Filgueiras, T.S., Figueiredo-Ribeiro, R.C.L., 2013. Diversity of non-structural carbohydrates in grasses (Poaceae) from Brazil. Grass and Forage Science 68, 165–177.
Nishizawa, A., Yabuta, Y., Shigeoka, S., 2008. Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. Plant Physiology 147, 1251–1263.
Orthen, B., 2001. Sprouting of the fructan- and starch-storing geophyte *Lachenalia minima*: Effects on carbohydrate and water content within the bulbs. Physiologia Plantarum 113, 308–314.
Orthen, B., Wehrmeyer, A., 2004. Seasonal dynamics of non-structural carbohydrates in bulbs and shoots of the geophyte *Galanthus nivalis*. Physiologia Plantarum 120, 529–536.
Patrick, J.W., Botha, F.C., Birch, R.G., 2013. Metabolic engineering of sugars and simple sugar derivatives in plants. Plant Biotechnology Journal 11, 142–156.
Pollock, C.J., Jones, T., 1979. Seasonal patterns of fructan metabolism in forage grasses. New Phytologist 83, 9–15.
Ranwala, A.P., Miller, W.B., 2008. Analysis of nonstructural carbohydrates in storage organs of 30 ornamental geophytes by high-performance anion-exchange chromatography with pulsed amperometric detection. New Phytologist 180, 421–433.
Raunkiaer, C., 1934. The life forms of plants and statistical geography. Claredon Press, Oxford (632 pp).
Rizzo, J.A., 1970. Contribuição ao conhecimento da Flora de Goiás, Área na Serra Dourada. (Thesis) Universidade Federal de Goiás.
Rossa, B., von Willert, D., 1999. Physiological characteristics of geophytes in semi-arid Namaqualand, South Africa. Plant Ecology 142, 121–132.
Rossatto, D.R., Sternberg, L.S.L., Franco, A.C., 2013. The partitioning of water uptake between growth forms in a Neotropical savanna: do herbs exploit a third water source niche? Plant Biology 15, 84–92.
Ruiters, C., 1995. Biomass and resource allocation patterns within the bulb of the perennial geophyte *Haemanthus pubescens* L. subsp. *pubescens* (Amaryllidaceae) in a periodic arid environment of lowland fynbos, South Africa. Journal of Arid Environments 31, 311–323.
Santos, A.P.M., Fracasso, C.M., Santos, M.L., Romero, R., Sazima, M., Oliveira, P.E., 2012. Reproductive biology and species geographical distribution in the Melastomataceae: a survey based on New World taxa. Annals of Botany 110, 667–679.
Smouter, H., Simpson, R.J., 1989. Occurrence of fructans in the Gramineae (Poaceae). New Phytologist 111, 359–368.
Stitt, M., Zeeman, S.C., 2012. Starch turnover: pathways, regulation and role in growth. Current Opinion in Plant Biology 15, 282–292.
Tetlow, I.J., Morell, M.K., Emes, M.J., 2004. Recent developments in understanding the regulation of starch metabolism in higher plants. Journal of Experimental Botany 55, 2131–2145.
Theron, K.I., Jacobs, G., 1996. Changes in carbohydrate composition of the different bulb components of *Nerine bowdenii* W. Watson (Amaryllidaceae). Journal of the American Society of Horticultural Science 121, 343–346.
Valluru, R., Van den Ende, W., 2008. Plant fructans in stress environments: emerging concepts and future prospects. Journal of Experimental Botany 59, 2905–2916.
Van den Ende, W., 2013. Multifunctional fructans and raffinose family oligosaccharides. Frontiers in Plant Science 4, 1–11.
Zemah, H., Bendel, P., Rabinowitch, H.D., Kamenetsky, R., 1999. Visualization of morphological structure and water status during storage of *Allium aflatunense* bulbs by NMR imaging. Plant Science 147, 65–73.