RESEARCH ARTICLE

Seasonal changes in fructan accumulation in the underground organs of *Gomphrena marginata* Seub. (Amaranthaceae) under rock-field conditions

Fernanda Gomes da Silva[1], Lílian Mendes Borburema Cangussu[1], Sandro Lânio Abreu de Paula[1], Geraldo Aclécio Melo[1], Emerson Alves Silva[2*]

[1]General Biology Department, Universidade Estadual de Montes Claros (UNIMONTES), Montes Claros, MG, Brasil.
[2]Center of Physiology and Biochemistry, Instituto de Botânica, São Paulo, SP, Brasil.

*Corresponding author: easilva2004@yahoo.com.br
Received: March 12, 2013; Accepted: April 16, 2013

ABSTRACT: Rock field plant species present several adaptations to the climatic characteristics and seasonality of this habitat. In this study, we evaluate under field conditions the seasonal changes in non-structural carbohydrate accumulation in tuberous organs of *Gomphrena marginata*. Tuber fragments of plants of *G. marginata* were sampled monthly for a year (August 2010 to July 2011) in a rock-field area in southeastern Brazil. Quantitative analyses of total fructose, fructo-oligosaccharides, fructo-polysaccharides, reducing sugars and free proline were carried out. Qualitative analyses of carbohydrates were also performed by thin layer chromatography (TLC) and high performance anion exchange chromatography (HPAEC/PAD). Soil moisture was evaluated by gravimetry and plant water status was evaluated by measuring the relative water content (RWC) and water potential (Ψ_w) of underground organs. Throughout the analyzed period, a well-marked seasonality of fructan accumulation was observed in tuberous roots of *G. marginata* correlating significantly with seasonal changes in soil water availability. In the rainy period (October to February), the content of fructo-polysaccharides decrease while during the dry and quiescent growth period (April to September) a high accumulation of fructo-polysaccharides was observed without changes in the RWC of tuberous roots, suggesting that cell osmoregulation contributed to water status maintenance in *G. marginata*. Our study presents a second species of Amaranthaceae that accumulates fructans as its main reserve carbohydrate, which can be an important strategy for survival in an environment characterized by a long dry period.

KEYWORDS: fructans, osmoregulation, seasonality.

INTRODUCTION

The well-defined climatic seasonality of the Brazilian Cerrado is an important feature of this biome, which is characterized by rainy summers and dry winters (Coutinho 2002). Many species in this biome have adaptive strategies to survive periods of water restriction in the winter. Among these, the presence of thick underground organs that accumulate photoassimilates is well known (Mantovani and Martins 1988).

Nonetheless, changes in the composition and content of non-structural carbohydrates, such as fructans, in these underground organs have been frequently observed in some species in the cooler and drier months of the year (Carvalho and Dietrich 1993, Isejima and Figueiredo-Ribeiro 1993, Vieira and Figueiredo-Ribeiro 1993).

Fructans are polyfructose molecules that function as carbohydrate storage compounds in 15% of higher plants (Hendry

and Wallace 1993). The fructans are non-reducing carbohydrates that constitute a homologous series of oligo and polysaccharides that contain fructose residues linked to hydroxyl groups of a molecule of sucrose (Edelman and Jefford 1968, Pollock and Chatterton 1988, Pollock et al. 1996).

Changes in fructan accumulation appear to have a role in the tolerance of some plant species exposed to environmental stresses such as drought, thus constituting an adaptive strategy providing energy for the plants (Hendry and Wallace 1993, Ritsema and Smeekens 2003). Since the fructan chains can be easily depolymerized, thereby increasing the amount of hexoses, sucrose and fructo-oligosaccharides on the cell vacuole, it has been suggested that these molecules are involved in osmotic adjustment to maintain water status even under reduced water availability in the soil (Garcia et al. 2011).

It is well know that some Asteraceae species from the Cerrado accumulate inulin-type fructans in underground organs as their main reserve carbohydrate (Tertuliano and Figueiredo-Ribeiro 1993). In *Vernonia herbacea*, a native species, inulin-type fructans account for approximately 80% of its dry weight (Carvalho and Dietrich 1993). In *Gomphrena macrocephala* (Amaranthaceae), another species of the Cerrado, fructans represent about 50% of the dry weight of underground organs and seasonal variations were observed for these carbohydrates (Vieira and Figueiredo-Ribeiro 1993).

In Brazil, the rocky field habitat occurs in the Cerrado domain (Coutinho 2002) as a grassland formation characterized by herbaceous and shrubby vegetation associated with predominantly quartz lithic soils. These formations occur at altitudes above 900 m a.s.l., with sandy soils poor in nutrients (Ribeiro and Walter, 1998) and with reduced water availability over the year as a consequence of rainfall regimes with up to six months without precipitation (May to October) that comprises the dry season.

Gomphrena marginata Seub., an endemic Amaranthaceae from rock fields, presents a thickened underground organ similar to a tuberous root (Siqueira 1991). Considering the seasonality of moisture in the rock-field environment and the characteristics of *G. marginata*, this study aimed to analyze for the first time the occurrence of fructans in this species and the seasonality of its accumulation in underground organs under field conditions.

MATERIAL AND METHODS

Plant material and collection conditions: *G. marginata* Seub. plants (SP 441822) with a healthy morphological appearance were collected monthly from August 2010 to July 2011 in the Environmental Preservation Area "Conjunto Paisagístico da Serra Resplandecente", Minas Gerais State, Brazil (16°59′47″S,

43°20′01″W). Each month, nine samples of underground organs of different plants were collected, cryopreserved and ground in liquid nitrogen and stored at -20°C for biochemical analyses. All measurements and extractions were done in triplicate, each one corresponding to three plants.

Soil moisture and meteorological data: Soil moisture (H_{soil}, %) was measured by the gravimetric method (Blake, 1965). In each field expedition, 5 soil samples were collected between 10- and 20-cm depths, which correspond to the effective depth of the underground structure of *G. marginata*. Soil samples were weighed to determine their fresh weight (FW) and then dried in a stove at 70°C until they reached a constant weight. Then, they were re-weighed to determine their dry weight (DW). Humidity was determined using the formula: H_{soil} (%) = (FW – DW/DW) × 100, where FW = fresh weight of soil and DW = dry weight of soil.

During the study period, monthly data on temperature, relative humidity, and accumulated rainfall in the region were obtained from the Institute of Agricultural Sciences of the Universidade Federal de Minas Gerais.

Water potential and relative water content: The cell sap of underground structures was obtained by pressure and the water potential (Ψ_w) was determined using a vapor pressure osmometer (VAPRO 5220; Wescor, Logan, Utah, USA). This analysis was carried out from December 2010 to July 2011. Underground fragments were removed from 5 of the 9 plants collected each month to determine the relative water content (RWC). The fragments were weighed to determine fresh weight (FW) and then left in distilled water for 24 hours and weighed again to determine the turgid weight (TW). They were then dried in a stove at 70°C until they reached a constant weight and had their dry weight determined (Weatherley, 1950). The RWC was estimated using the formula: RWC (%) = (FW – DW/TW – DW) × 100, where FW is the fresh weight, DW is the dry weight, and TW is the turgid weight of the underground structure.

Extraction and fractionation of non-structural carbohydrates: The extraction of soluble carbohydrates was performed as described by Carvalho et al. (1998). These analyses were performed with the 3 monthly composite samples. For this purpose, 1 g of pulverized plant material of each composite sample was placed in 10 mL 80% ethanol and put in a water bath at 80°C for 15 min. Then the samples were centrifuged at 700 × g for 15 min at ambient temperature. The residues were re-extracted twice more in 80% ethanol with the same initial volume and the ethanolic supernatants were collected. Afterward, the residues were re-extracted twice in 10 mL of

distilled water at 60°C for 30 min and then filtered under a vacuum. The ethanolic supernatants and the aqueous filtrates were combined and the hydroethanolic extract obtained was used for quantitative analysis of reducing sugars and fructose by the specific colorimetric method.

Aliquots of the hydroethanolic extract were used for the fractionation of nonstructural carbohydrates as described below. Three milliliters of the extract were kept in a refrigerator overnight with 3 volumes of 95% ethanol. The extract was centrifuged at $5000 \times g$ for 15 min to separate the fractions of fructo-oligosaccharides (supernatant) and fructo-polysaccharides (precipitant). The latter was re-suspended and solubilized in a known volume of distilled water. Quantification of such sugars was performed by the modified anthrone method (Jermyn 1956).

Quantitative analysis of carbohydrates: Quantification of reducing sugars (RS) was performed according to the method described by Miller (1959). The concentration of such sugars was determined by a calibration standard curve of D-glucose (Sigma). The total fructose content of the samples was determined by the modified anthrone method described by Jermyn (1956). The total fructose content was determined from a standard curve of inulin (Sigma). All analyses were performed in triplicate.

Qualitative analysis of carbohydrates

Anion exchange chromatography with pulse amperometric detector (HPAEC/PAD)

Samples of the extracts of the fraction of fructo-oligosaccharides containing approximately 400 µg of fructose were deionized on cation-exchange columns (Dowex 50X8) and anion-exchange columns (Dowex 1X8) (Carvalho and Dietrich, 1993). After deionization, the samples were filtered and analyzed by a HPAEC/PAD Dionex chromatographic system model ICS-3000, using a CarboPac PA-1 column (2×250 mM). Elution of the carbohydrates was performed using a gradient of eluent A (150 mM sodium hydroxide) and eluent B (500 mM sodium acetate in 150 mM sodium hydroxide) according to the following schedule: 0–2 min, 25 mM; 2.1–8 min, 50 mM; 8.1–8.5 min, 75 mM; 8.6–10 min, 100 mM; 10.1–28 min, 450 mM; 28.1–30 min, 500 mM; and 30.1–40 min, 25 mM. The potentials applied to the pad to 0–0.4 s, 0.41–0.42 s, 0.43 s, and 0.44–1 s were 0.1, -2, 0.6, and -0.1, respectively, and the applied flow was 0.25 mL min^{-1}.

Thin layer chromatography (TLC): After deionization, the previously mentioned fractions of fructo-oligosaccharides were also analyzed by thin layer chromatography on silica-gel plates 60 F 254, 20 × 20 cm (Merck). One day before the chromatographic analysis, the standards (80 µg inulin from *Helianthus tuberosus*: 1-kestose, 6-kestose, neokestose, and levan) were applied. In addition, 80 µg equivalent extracts of fructose were applied to all samples placed about 1 cm apart. The analysis was performed using a saturated vat with a mobile phase consisting of isobutyl alcohol, 1-propanol and water in the ratio 3:12:4 (v/v/v). The vat was kept closed until the mobile phase reached approximately 1 cm before the end of the plate. The plate was withdrawn and subjected to drying under a hood by using a dryer. The next day, the analysis was repeated, and, after drying, the plate was developed using a specific reagent for fructose in free and combined forms (Wise et al. 1955). The developer was prepared in 94.4 mL of solution 1 (98.8 mL of *n*-butanol and 20 mL of distilled water) with solution 2 (5.6 mL of orthophosphoric acid and 3 g of urea dissolved in 5 mL ethanol) and applied uniformly over the plate with the aid of a sprayer. Then the plate was put in a stove at 150°C and left for 3 min until the appearance of bands. After the bands appeared, the plate was photographed.

Determination of free proline: The concentration of free proline was determined colorimetrically according to the method of Bates, Waldren and Teare (1973). The content of proline was calculated from a standard curve constructed with increasing concentrations of L-proline (Sigma), and the analyses were performed in triplicate.

Statistical analysis: Data were analyzed by simple correlations (Pearson) between all variables (environmental, biochemical and hydric relations). We used the Student's *t*-test to assess the significance of the correlations at 5% probability. The correlation analyses were performed considering the entire study period (August 2010 to July 2011) and treating the rainy (September 2010 to March 2011) and dry periods (April to July 2011 and August 2010) separately.

RESULTS

During the study period, from August 2010 to July 2011, the average monthly temperature ranged from 19.81°C to 26.86°C. The hottest months corresponded to the rainy period (October 2010 to March 2011), while mild temperatures occurred in the months that comprised the dry period (August 2010 to April – July 2011) (Figure 1A). The accumulated rainfall was significantly lower during the dry period, reaching only 1.5 mm in June and 0.5 mm in July. In August 2010, there was no precipitation.

During the rainy period, the most precipitation was observed in November 2010 with 298 mm. In February, it rained about 9 mm, characterizing a "veranico", a period of a week or up to 30 days without rainfall during the rainy season

(Figure 1B). The relative humidity (RH) ranged between 53% (October 2010) and 82% (March 2011). In general, the months with lower RH corresponded with the months with less rainfall, while months with higher rainfall had higher RH (Figure 1C). Throughout the study period, the soil moisture (H_{soil}) content ranged from approximately 0.6% in August 2010 to about 26% in March 2011. The highest levels of soil moisture were observed from November 2010 to March 2011, except February, which had slightly over 2% moisture. The months of September and October, which also included the rainy period, had low levels of soil moisture, about 0.6 and 3%, respectively (Figure 1D).

The relative water content (RWC) of underground structures remained high throughout the period with values above 87% noted for February 2011 (Figure 2A). The analysis of cell sap for water potential determination was performed only in the period from December 2010 to July 2011. During this period, higher potentials were observed in January (-0.37 MPa), March (-0.60 MPa) and June (-0.75 MPa). February 2011 had the most negative potential (-1.08 MPa) during the period analyzed. Except for February, the most negative potentials were observed in the dry period months of April, May and July 2011 (Figure 2B).

The contents of total fructose and fructo-polysaccharides were lower in August to December 2010 (Figures 3A and B) with the lowest concentration of fructo-polysaccharides in November 2010 (169 mg g^{-1} DW). From January 2011, there was a pronounced and steady increase in the concentration of these solutes, with the highest concentration observed in May 2011 (600 mg g^{-1} DW) (Figure 3B). The increase in fructo-polysaccharides occurred at the same time as the increase in total fructose content, with higher levels observed in February, about 830 mg g^{-1} DW. Fructo-oligosaccharide concentration was lower in August 2010 (64 mg g^{-1} DW) and higher in February 2011 (264 mg g^{-1} DW). In September and October 2010, high levels of these carbohydrates were also observed with 224 mg g^{-1} DW and 213 mg g^{-1} DW, respectively (Figure 3C). The fructo-oligo:fructo-polysaccharide ratio was higher in September and November 2010, and then decreased and remained relatively constant between March and July 2011 (Figure 3D). The content of reducing sugars (RS) included levels ranging from 38 mg g^{-1} DW in February 2011 to 67 mg g^{-1} DW in October 2010. Higher concentrations of these carbohydrates were found between September and December 2010 and July 2011 (Figure 4). The free proline contents observed during the study ranged from 0.83 μmol g^{-1} DW in October 2010 to 1.31 μmol g^{-1} DW in November 2011 (Figure 5).

Figure 6 presents a thin layer chromatogram of the fructo-oligosaccharide fractions. The bands containing carbohydrates of the inulin series (Ht), as well as the other standards 1-kestose (1K), 6-kestose (6K) and neokestose (N), appear clearly, while the standard levan (L) remained at the origin. Besides the standard levan, which

was retained at the origin, the origin retention of most samples was also observed in all months analyzed, indicating the presence of carbohydrates with a high degree of polymerization. In September 2010, high levels of free fructose were evidenced by the high dyeing of the band. The beginning of a new period of fructan synthesis was observed in January 2011, since more intense bands of sucrose were observed in the subsequent period from February to July 2011, along with a decrease in free fructose in the same period.

Anion-exchange (HPAEC-PAD) chromatograms showing qualitative analyses performed with the fructo-oligosaccharide fractions are presented in Figure 7. Glucose (G), fructose (F), sucrose (S) and a homologous series of fructans were detected in all months. In September, at the beginning of the rainy period, there was an increase in the peaks of the fructose and glucose monosaccharides and lower peaks of sucrose and fructans with a high degree of polymerization (DP). Conversely, in April 2011, early in the dry period, there was an increase in the
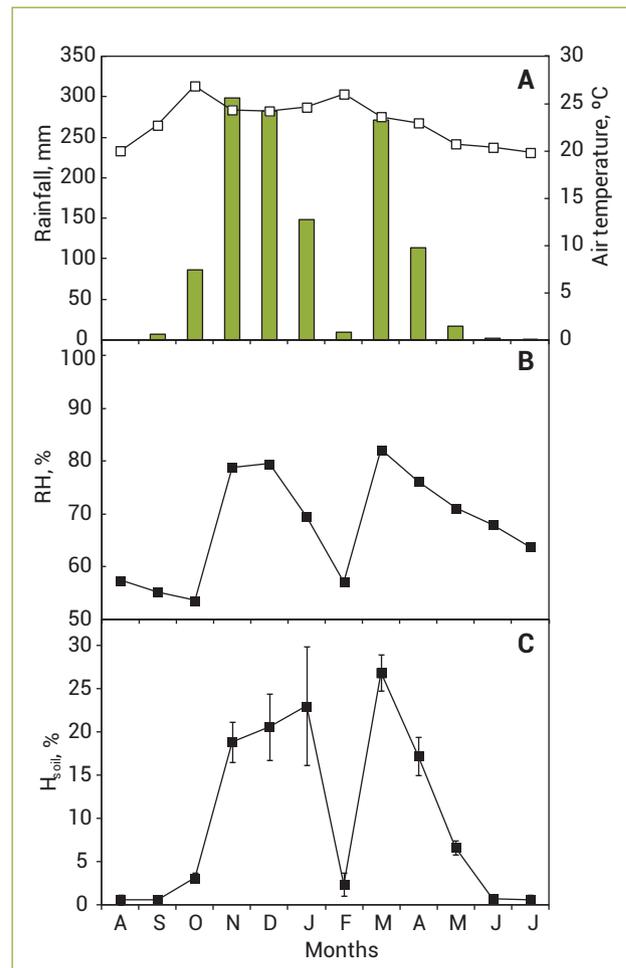
Figure 1. Mean monthly air temperature (□) and accumulated rainfall (■), (**A**) relative humidity (RH) (**B**) and soil moisture (H_{soil}) (**C**) from August 2010 to July 2011. For soil moisture, values are means±SD (n=5)
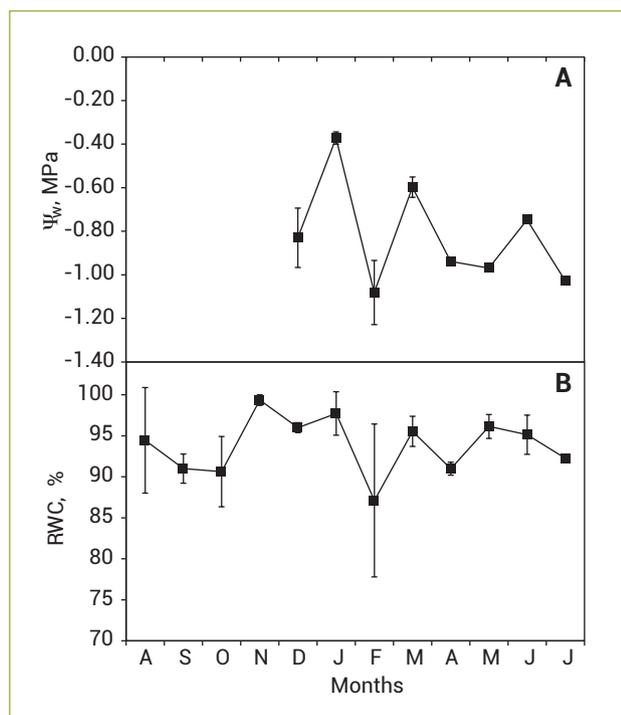
Figure 2. Water potential (Ψ_w) of the cell sap of underground structures of *G. marginata* from December 2010 to July 2011 (n=3) (**A**) and relative water content (RWC) from August 2010 to July 2011 (n=5) (**B**). Values are means±SD.

sucrose peak, coinciding with a higher intensity of the band of this sugar observed in TLC. This increase was also observed for chains with high DP fructan.

Few significant correlations were found between the biochemical (total fructose, fructo-oligosaccharides, fructo-polysaccharides, reducing sugar and free proline), environmental (air temperature, accumulated rainfall, RH and soil moisture) and plant hydric status (RWC and Ψ_w) variables when analyzed throughout the study period, August 2010 to July 2011. Soil moisture was correlated with RH (r=0.83, p=0.02) and precipitation (r=0.88, p=0.02), RH was correlated with rainfall (r=0.77, p=0.03), and total fructose was correlated with fructo-polysaccharides (r=0.93, p=0.01).

During the rainy period, the RWC was positively correlated with all environmental variables except temperature: precipitation (r=0.83, p=0.02), soil moisture (r=0.81, p=0.03), and RH (r=0.77, p=0.03). In this period, there was also an increase in the RS content and a decreasing in the contents of fructo-polysaccharides (r=-0.74, p=0.04) and total fructose (r=-0.81, p=0.03). In addition, fructo-oligosaccharide content was negatively correlated with rainfall (r=-0.93, p=0.01), soil moisture (r=-0.81, p=0.03) and RH (r=-0.88, p=0.02).

During the dry period (April to July 2011 plus August 2010), there was a simultaneous increase in the content of
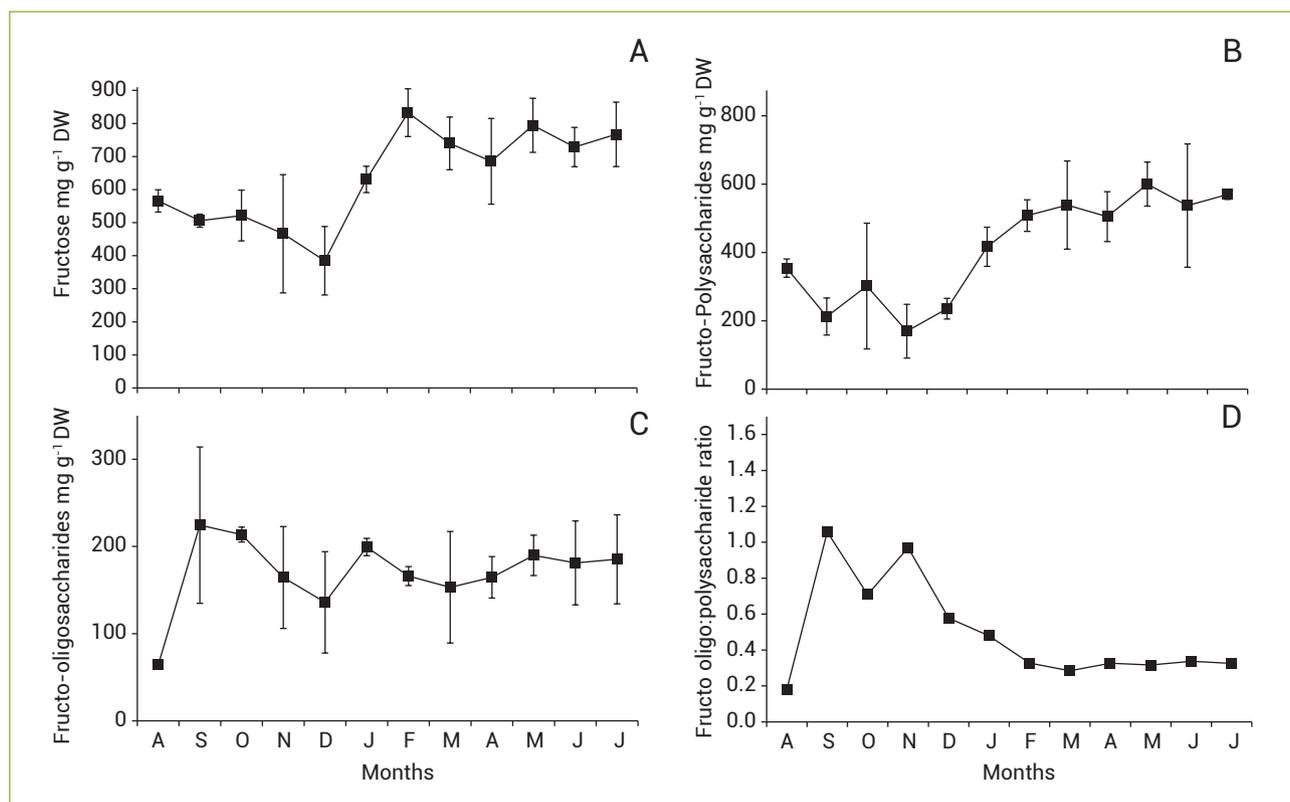
Figure 3. Contents of total fructose (**A**), fructo-polysaccharides (**B**) -oligosaccharides (**C**) and oligo:polysaccharide ratio (D) in underground structures of *G. marginata* from August 2010 to July 2011. Values are means±SD (n=3).

total fructose and fructo-oligosaccharides (r=0.95, p=0.01), and total fructose and fructo-polysaccharides (r=0.99, p=0.01). Further, the free proline content was positively correlated with the total fructose content (r=0.74, p=0.04) and fructo-polysaccharides (r=0.71, p=0.05). The accumulated rainfall, average temperature, RH and soil moisture were positively correlated with each other. When considering only the dry period that covered the sequential months

from April to July 2011, most of the same correlations were found. The values of the sieve Ψ_w of underground structures of *G. marginata* (Table 1) were included in this period.

DISCUSSION

Fluctuations in environmental variables of relative humidity, precipitation and air temperature over the study period (August 2010 to July 2011) showed a well-defined climatic seasonality for
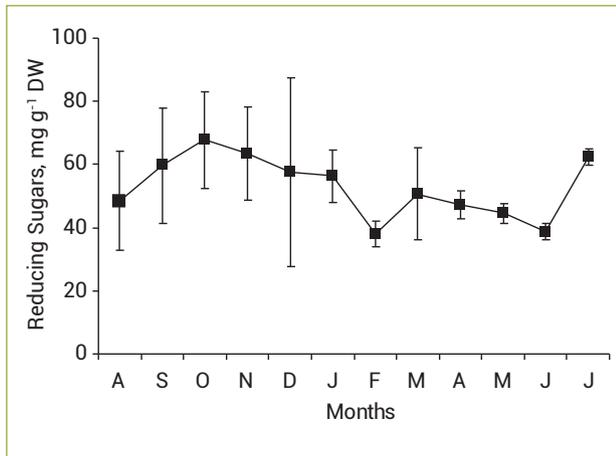
Figure 4. Content of reducing sugars (mg.g^{-1} DW) in underground structures of *G. marginata* from August 2010 to July 2011. Values are means±SD (n=3).
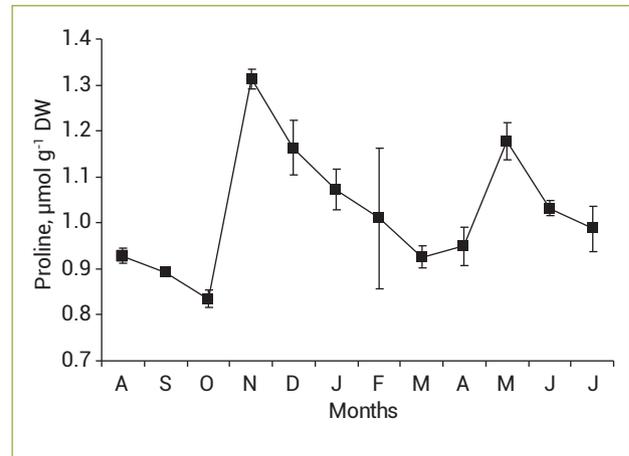
Figure 5. Free proline in the underground organs of *G. marginata* from August 2010 to July 2011. Values are means±SD (n=3)
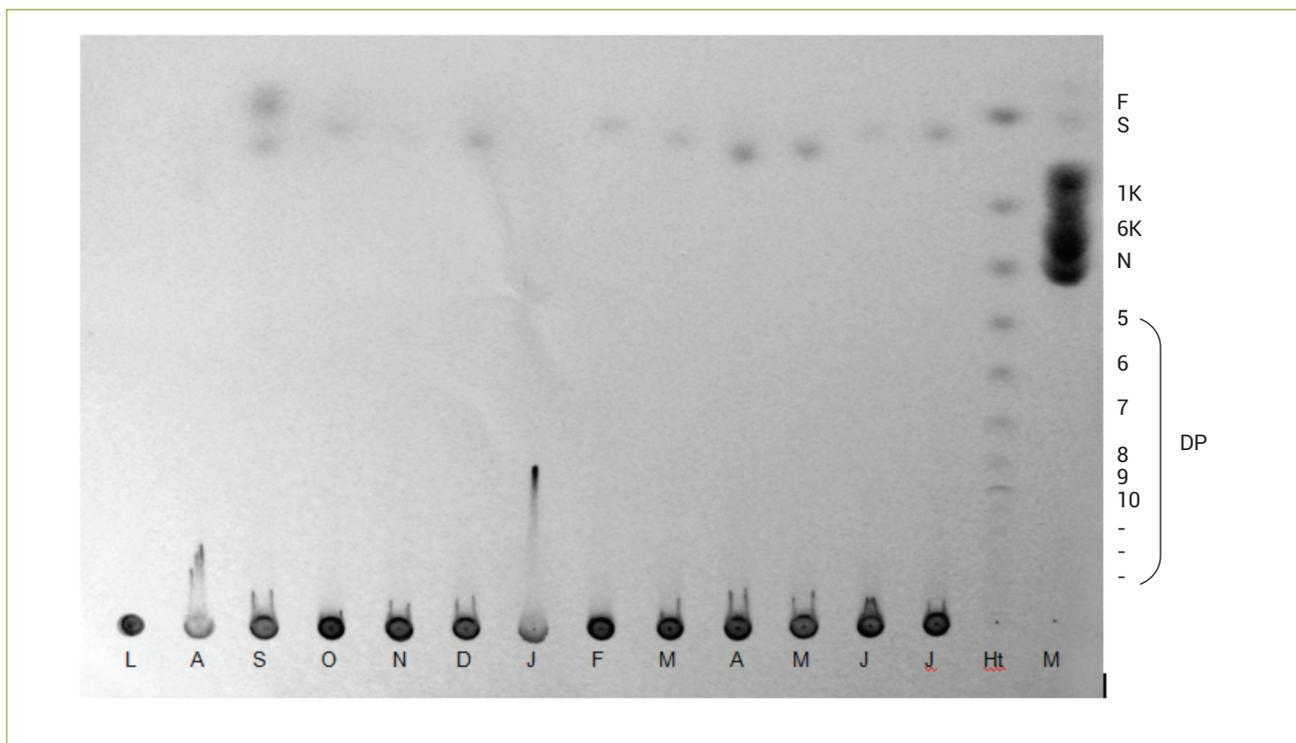
Figure 6. Thin-layer chromatography fractions of fructo-oligosaccharides from underground organs of *G. marginata* throughout the development cycle (August 2010 to July 2011). Standards levan (L), inulin extract from *Helianthus tuberosus* (Ht) and a mixture of standards: 1-kestose, 6-kestose, neokestose (M). DP: Degree of polymerization.

the studied region. Seasonality in rainfall is typical in the Cerrado regions, which are characterized by a wet summer and dry winter (Coutinho 2002). Soil moisture was positively and significantly correlated to precipitation (r=0.88, p=0.02) and relative humidity (r=0.83, p=0.03). The marked seasonality in rain distribution and the low values of soil moisture during the dry period may restrict the absorption and hydration of plant tissues, especially the underground organs that are in direct contact with the soil. In this study,

however, the relative water content of the underground organs of *G. marginata* (Figure 2A) showed only small variations throughout the year, indicating that the species has a great ability to maintain tissue hydration, even during the dry season.

The maintenance of turgor in plant tissues under hydric stress conditions as observed in this study has been associated with a reduction in osmotic potential due to the accumulation of solutes in the cells (Chaves-Filho and Stacciarini-Seraphin
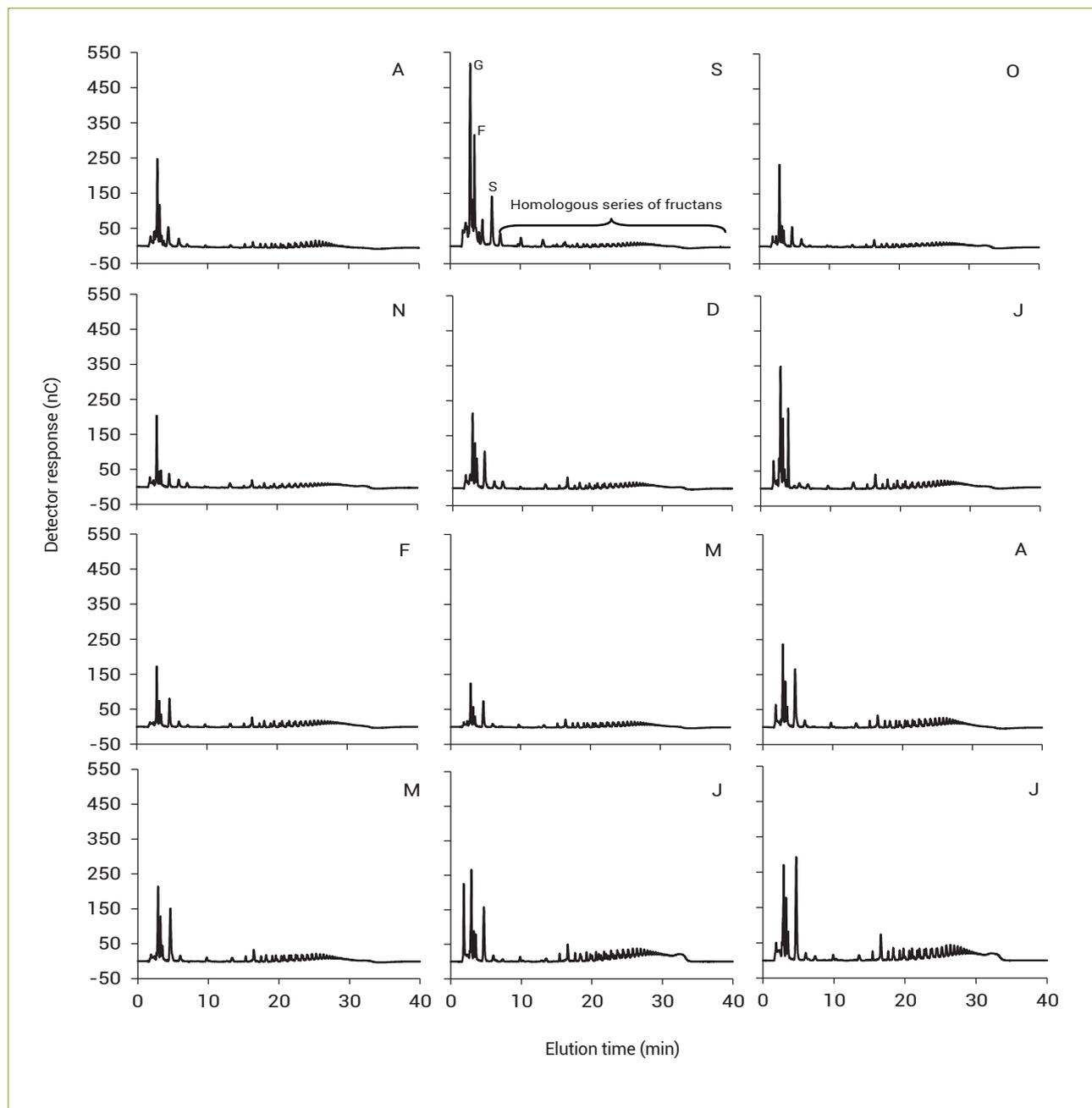
Figure 7. Anion-exchange chromatography (HPAEC/PAD) of fructo-oligosaccharides fraction of underground structures of *G. marginata*. The letters in the upper right corner correspond to the analyzed month, following the rainy (September 2010 to March 2011) and dry period (April-July 2011 and August 2010). F: fructose, G: glucose and S: sucrose.

2001, Garcia 2009). Garcia (2009) suggested that the involvement of fructans in hydric stress tolerance in *V. herbacea* was due to the increased content of fructo-oligosaccharides, reducing sugars, fructose and sucrose, indicating the occurrence of osmotic adjustment in the rhizophores of plants subjected to stress. Dias-Tagliacozzo et al. (2004) observed that plants of *V. herbacea* maintained high water content in the underground organs in the first 30 days after the suspension of irrigation. They attributed this to the reduction of water potential promoted by the accumulation of fructans. In this sense, Pollock and Jones (1979) already argued that plant fructans in temperate climates were involved in osmoregulation in plants that underwent periods of hydric restriction and/or low temperatures at certain times of the year.

During the dry period, despite the increase in reducing sugars and fructo-oligosaccharides, both involved in osmoregulatory mechanisms (Spollen and Nelson 1994), the data of Ψ_w of the cell sap did not show significant correlations. However, it is important to note that in the months of the rainy period (December 2010 to March 2011) there was less negative potential, with the exception of February, which had the most negative potential of the months (Figure 2B). This is interesting because in that month there was only 9 mm of precipitation and soil moisture decreased markedly to 2.35%. On this occasion, there was a greater content of fructo-oligosaccharides (Figure 3C). Starting in April, with the exception of June, the other months showed increased negative potentials and high reducing sugar content. Thus, the possibility that osmoregulation occurs through changes in carbohydrate metabolism in *G. marginata* cannot be excluded.

Moreover, besides osmoregulation, plants have other strategies to prevent dehydration under water stress, including stomatal closure to minimize water loss by transpiration (Paiva and Oliveira 2006), reduction in leaf area and intensification of senescence and leaf abscission (McCree and Fernández, 1989, Taiz and Zeiger, 2010). It may be that plants of *G. marginata* have these strategies to survive water stress, since signs of senescence and leaf abscission were observed during the dry months, especially June and July 2011. These features are also seen in other Cerrado species (Mantovani and Martins 1988). Dias-Tagliacozzo (1995) stated that the increase of fructo-polysaccharides in the underground organs of plants under water stress could occur due to the transport of photoassimilates from the shoot during senescence.

Fructan accumulation has been reported in species that colonize areas with seasonal drought like the Cerrado. For the family Amaranthaceae, this is the second report of fructan accumulation. The first species *G. macrocephala* is an herb from the Cerrado that accumulates levan-type fructans (Figueiredo-Ribeiro 1993). In fructan-accumulating species, the metabolism of carbohydrates has been considered important in promoting osmotic adjustment, allowing plants to survive the low soil moisture during dry periods.

Significant negative correlations between the content of reducing sugars and the levels of fructo-polysaccharides (r=-0.75, p=0.04) and total fructose (r=-0.81, p=0.03) were found in the rainy period. Especially in September to December 2010, high proportions of free fructose and sucrose indicated the occurrence of hydrolysis of fructo-polysaccharides. According to field observations, vegetative and reproductive shoots of the species grew at the beginning of the rainy period predominantly comprising September to November 2010. Thus, the depolymerization of the chains of fructo-polysaccharides in *G. marginata* appears to be linked to supplying the energy needed for plant growth.

The initiation of a new period of fructan synthesis in *G. marginata* appears to have occurred in January 2011, soon

Table 1. Correlation coefficients found between biochemical, environmental and hydric variables in *Gomphrena marginata.* Dry period (April to July 2011).

	Dry period (April to July 2011)										
	RS	Total Fructose	Fructo-Oligo	Fructo-Poly	Proline	RWC	Ψ_s	H_{soil}	Rainfall	RH	T°C
RS	1.00										
Total Fructose	0.02	1.00									
Fructo-Oligo	0.22	0.96**	1.00								
Fructo-Poly	-0.06	1.00***	0.94**	1.00							
Proline	-0.45	0.78*	0.75*	0.82*	1.00						
RWC	-0.14	0.66*	0.75*	0.67*	0.89**	1.00					
Ψ_w	-0.20	0.38	0.15	0.40	0.02	-0.38	1.00				
H_{soil}	-0.75*	-0.60	-0.78*	-0.54	-0.26	-0.51	0.20	1.00			
Rainfall	-0.58	-0.76*	-0.90**	-0.70*	-0.47	-0.65	0.13	0.97**	1.00		
RH	-0.84*	-0.56	-0.69*	-0.49	-0.04	-0.21	-0.08	0.93*	0.87*	1.00	
T°C	-0.66	-0.74*	-0.87*	-0.68	-0.36	-0.52	0.01	0.98**	0.98**	0.94*	1.00

*$p \leq 0.05$, **$p \leq 0.02$ and ***$p \leq 0.01$. RS: reducing sugars, fruct-oligo: fructo-oligosaccharides, fructo-poly: fructo-polysaccharides, RWC: relative water content, Ψ_w: water potential, H_{soil}: soil humidity, RH: relative humidity, T °C: average temperature.

after flowering. Likewise, the increase in fructan contents in plants of *V. herbacea* after flowering (at the end of) summer indicated the onset of the synthesis of these carbohydrates in this species (Carvalho and Dietrich 1993). Depolymerization of long-chain fructans during aboveground growth, as described here for *G. marginata*, and the synthesis and storage of fructo-polysaccharides at the end of the cycle of vegetative growth in Cerrado species has been widely documented by Carvalho et al. (1997) and Carvalho and Dietrich (1993). Vieira and Figueiredo-Ribeiro (1993) found seasonal variations in the content and composition of fructans when studying *G. macrocephala*. During sprouting and vegetative growth phases, there was a predominance of low molecular weight fructans, with an increase in the molecular weight of fructo-polysaccharides and reduction of levels of fructo-oligosaccharides in the stages of flowering/fruiting and early dormancy.

The accumulation of fructans in *G. marginata* seems to follow the pattern observed for the above-mentioned species from the Cerrado because in the dry period there was a significant increase in the concentration of fructo-polysaccharides. For the sequential months from April to July 2011, the increase in reducing sugar content correlated significantly and negatively with relative humidity ($r=-0.84$, $p=0.02$) and soil moisture ($r=-0.75$, $p=0.04$). Changes in the levels of fructo-oligosaccharides during the dry period correlated significantly with fluctuations in prevailing environmental conditions. These carbohydrates increased their concentrations when there were lower values for environmental variables such as air temperature ($r=-0.87$, $p=0.02$), precipitation ($r=-0.90$, $p=0.02$), RH ($r=-0.69$, $p=0.05$) and soil moisture ($r=-0.78$, $p=0.03$). These carbohydrates were also associated positively with the relative water content ($r=0.75$, $p=0.04$).

Significant positive correlations were found between proline and fructo-polysaccharides and proline and fructo-oligosaccharides during the dry period. In addition, this amino acid was positively correlated with the relative water content. Proline accumulation in plants exposed to abiotic stress can promote the stabilization of proteins (Sharma and Dubey 2005), the removal of oxygen-reactive species and osmotic adjustment together with increased levels of other osmolytes (Valliyodan and Nguyen 2006), and can also promote cell signaling by activating multiple responses to stress (Maggio et al. 2002). However, the accumulation of this amino acid may occur in other physiological events throughout the development cycle of the plant. In this study, higher free proline contents were observed in November 2010 (the end of the flowering season of *G. marginata*) and in May 2011 (Figure 5). In November, the accumulation of this amino acid may have occurred due to the formation of pollen (Phang, 1985), since the oxidation of a proline molecule can generate 30 molecules of ATP (Hu et al. 1996). It may also have promoted signaling for enzymatic activity. During the dry period, significant positive correlations of free proline and fructans with the relative water content suggest the involvement of this amino acid in protection against environmental water stress. Garcia (2009) suggested that proline was involved as an osmoregulator and flags other responses in *V. herbacea*. After 22 days under hydric stress the plants had 25 μmol g^{-1} DW-proline in their rhizophores. Despite the low-proline (≤ 1.31 μmol g^{-1}) in *G. marginata* it is possible that this amino acid and other solutes promoted the maintenance of the relative water content.

This study documented that *G. marginata* synthesizes and accumulates fructan-type carbohydrates. The variation of these sugars followed the phenological cycle of the species and the climatic seasonality. The accumulation of fructo-polysaccharides in the underground organs of *G. marginata* occurred during the dry period, while the depolymerization of carbohydrates, as evidenced by the reduction in the total fructan content, occurred during the rainy period, which coincided with the growing season of the plant. The accumulation and mobilization of these carbohydrates, besides being related to growth, may be involved in drought tolerance since this species occurs in an environment marked by the occurrence of seasonal drought.

ACKNOWLEDGMENTS

REFERENCES

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39:205-207.

Blake GR (1965) Bulk density. In: Black CA, Evans DD, White JL, Ensminger LE, Clark FE (eds.). Methods of soil analysis, p. 374-390. American Society of Agronomy, Madison.

Carvalho MAM, Dietrich SMC (1993) Variation in fructan content in the undergound organs of *Vernonia herbacea* (Vell.) Rusby at different phenological phases. New Phytologist 123:735-740.

Carvalho MAM, Pinto MM, Figueiredo-Ribeiro RCL (1998) Inulin production by *Vernonia herbacea* as influenced by mineral fertilization and time of harvest. Revista Brasileira de Botânica 21:281-285.

Carvalho MAM, Zaidan LBP, Dietrich SMC (1997) Growth and fructan contents in plants of *Vernonia herbacea* (Asteraceae) regenerated from rhizophores. New Phytologist 136:153-161.

Chaves-Filho JT, Stacciarini-Seraphin E (2001) Alteração no potencial osmótico e teor de carboidratos solúveis em plantas

jovens de lobeira (*Solanum lycocarpum* St.-Hil.) em resposta ao estresse hídrico. Revista Brasileira de Botânica 24:199-204.

Coutinho LM (2002) Eugen Warming e o Cerrado brasileiro: um século depois. In: Klein AL (org), p. 77-91. UNESP, Imprensa Oficial do Estado de São Paulo, São Paulo.

Dias-Tagliacozzo GM (1995) Frutanos de *Vernonia herbaceae* (Vell) Rusby: Variações em função da temperatura e da disponibilidade de água. Tese de doutorado. Universidade Estadual de Campinas, Campinas.

Dias-Tagliacozzo GM, Itaya NM, Carvalho MAM, Figueiredo-Ribeiro RCL, Dietrich SMC (2004) Fructans and water suppression on intact and fragmented rhizophores of *Vernonia herbacea*. Brazilian Archives of Biology and Technology 47:363-373.

Edelman J, Jefford TG (1968) The mechanism of fructosan metabolism in higher plants as exemplified in *Helianthus tuberosus*. New Phytologist 67:517-531.

Figueiredo-Ribeiro RCL (1993) Distribuição, aspectos estruturais e funcionais dos frutanos, com ênfase em plantas herbáceas do cerrado. Revista Brasileira de Fisiologia Vegetal 5:203-208.

Garcia PMA (2009) Variações no metabolismo de frutanos em *Vernonia herbacea* (Vell) Rusby (Asteraceae) em resposta ao déficit hídrico e sua relação com a tolerância à seca. Dissertação de mestrado. Instituto de Botânica da Secretaria do Meio Ambiente de São Paulo, São Paulo.

Garcia PMA, Asega AF, Silva EA, Carvalho, MAM (2011) Effect of drought and re-watering on fructan metabolism in Vernonia herbaceae (Vell.) Rusby. Plant Physiology and Biochemistry 49:664-670.

Hendry GAF, Wallace RK (1993) The origin, distribution and evolutionary significance of fructans. In: Suzuki M, Chatterton JN (eds). Science and Technology of Fructan, CRC Press, Boca Raton.

Hu CA, Lin WW, Valle D (1996) Cloning, characterization and expression of cDNAs encoding human D1-pyrroline-5-carboxylate dehydrogenase. The Journal of Biological Chemistry. 271:9795-9800.

Isejima EM, Figueiredo-Ribeiro RCL (1993) Fructan variations in tuberous roots of *Viguiera discolor* Baker (Asteraceae): the influence of phenology. Plant and Cell Physiology 34:723-727.

Jermyn MA (1956) A new method for determination of ketohexoses in presence of aldohexoses. Nature 177:38-39.

Maggio A, Miyazaki S, Veronese P, Fujita T, Ibeas JI, Damsz B, Narasimhan ML, Hasegawa PM, Joly RJ, Bressan RA (2002) Does proline accumulation play a active role in stress-induced growth reduction? The Plant Journal 31:699-712.

Mantovani W, Martins FR (1988) Variações fenológicas das espécies do cerrado da Reserva Biológica de Moji-Guaçú, Estado de São Paulo. Revista Brasileira de Botânica 11:101-112.

McCree KJ, Fernández CJ (1989) Simulation model for studying physiological water stress responses of whole plants. Crop Science 29:353-360.

Miller, GL (1959) Use of dinitrosalicylle acid for determination of reducing sugar. Analytcal Chemistry 11:426-428.

Paiva R, Oliveira LM (2006) Fisiologia e Produção Vegetal. UFLA, Lavras.

Phang JM (1985) The regulatory functions of proline and pyrroline-5-carboxilic acid. Current Topics in Cellular Regulation 25:92-132.

Pollock CJ, Cairns AJ, Sims IM, Housley TL (1996) Fructans as reserve carbohydrates in crop plants. In: Zamski E, Shaffer AA (eds) Photoassimilate distribution in plants and crops: source − sink relationships, p. 97-113. Marcel Dekker, New York.

Pollock CJ, Chatterton NJ (1988) Fructans. In: Preiss J (ed) The biochemistry of plants: a comprehensive treatise, p.109-140. Academic Press, New York.

Pollock CJ, Jones T (1979) Seasonal patterns of fructan metabolism in forage grasses. New Phytologist 83:9-15.

Ribeiro JF, Walter BMT (1988) Fitofisionomias do Bioma Cerrado. In: Sano SM, Almeida SP (org.). Cerrado: ambiente e flora. Planaltina, Embrapa-CPAC, p.89-166.

Ritsema T, Smeekens S (2003) Engineering fructan metabolism in plants. Journal of Plant Physiology 160:811-820.

Sharma P, Dubey RS (2005) Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. Journal of Plant Physiology 162:854-862.

Siqueira JC (1991) O gênero Gomphrena L. (Amaranthaceae) no Brasil. Tese de doutorado. Universidade Estadual de Campinas, Campinas-SP.

Spollen WG, Nelson CJ (1994) Response of fructan to water deficit in growing leaves of tall fescue. Plant Physiology 106:329-336.

Taiz L, Zeiger E (2010) Plant Physiol, 5th edn. Sinauer Associates, Sunderland.

Tertuliano MF, Figueiredo-Ribeiro RCL (1993) Distribution of fructose polymers in herbaceous species of Asteraceae from the cerrado. New Phytologist 123:741-749.

Valliyodan B, Nguyen HT (2006) Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Current Opinion in Plant Biology 9:1-7.

Vieira CC, Figueiredo-Ribeiro RCL (1993) Fructose-containing carbohydrates in the tuberous root of *Gomphrena macrocephala* St. Hil. (Amaranthaceae) at different phenological phases. Plant, Cell and Environment 16:919-928.

Weatherley PE (1950) Studies in the water relations of cotton plant. I. The field measurement of water deficits in leaves. New Phytologist 49:81-97.

Wise CS, Dimler RJ, Davis HA, Rist CE (1955) Determination of easily hydrolysable fructose units in dextran preparation. Analytical Chemistry 27:33-36.