

Anatomical plasticity and starch allocation in an invasive grass in response to fire

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Abstract

This study aimed to understand whether fire frequency in invaded areas by *Urochloa brizantha* (A.Rich.) R.D.Webster caused anatomical and physiological changes in this species. Twelve samples were collected per burn treatment in areas with different fire frequencies: fire exclusion, annual and biennial fires. Anatomical, histochemical and physiological analysis were performed on the leaves, rhizomes and roots. Our anatomical results showed that there was an increase in the thickness of the vascular bundle of *U. brizantha* leaves in the treatment of biennial burning. There were no anatomical differences in the roots. In the rhizomes, epidermis of the burned plants had greater thicknesses, the parenchyma and the vascular bundle of the biennially burned plants were more spherical than in unburned plants and in plants burned annually. This anatomical changes in grass tissues in response to fire have not yet been reported in the literature. In the physiological analyses, we found differences in the starch concentrations in relation to the different organs, the root presented more than 60% of the starch stored in the entire plant. Fire is changing the anatomy of this species, since there was an increase in the thickness of leaf tissues and also in rhizome tissues. The concentrations of starch in the root guaranteed plant to rapidly resprout after burning, which could be considered an advantage in post-fire environments to occupy rapidly the open spaces.

1 Introduction

Fire is a disturbance commonly observed in boreal forests, eucalypt woodlands, shrublands, grasslands and savanna (Bond & Keeley, 2005). Among flammable ecosystems, tropical savannas have a high fire frequency, with low to medium intensity fires (Archibald et al. 2013). Tropical savannas are located within the tropics, where average annual rainfall up to 2500 mm (Lehmann et al. 2011), are characterized by frequent fires (Staver et al. 2011) and mostly composed of C4 grasses (Bond 2008).

In the Cerrado, different species could resprout after disturbance (Pilon et al. 2021), having bud-bearing underground systems with soluble carbohydrates (Moraes et al., 2016; Pausas et al., 2018). Grasses in general have non-structural carbohydrates such as free glucose, starch and sucrose available in different concentrations in leaves and stems (Moraes et al. 2013). Moreover, they efficiently use non-structural carbohydrates such as starch stored in their roots (Souza et al. 2010). After fire, plants with underground stored root energy reserves could mobilize those reserves to facilitate aboveground growth (Bowen and Pate 1993; Bell and Ojeda 1999).

Rhizomes facilitate storage of carbohydrates (Janeček and Klimešová 2014) and their below ground protection allow for their survival after some types of disturbances (Klimešová and Klimeš 2007). Rhizomes of some monocotyledons are characterized as underground stems specialized in storing different types of carbohydrates and vegetative propagation (Appezato-da-Glória and Carmello-Guerreiro 2012), which ensures the survival of these rhizomatous species after losing their shoots (Pausas et al. 2018) by fire or grazing event. In grasses and herbaceous plants, when rhizomes were at least 2 cm deep in the soil, and buds were protected from fire, conferring an adaptive advantage (Choczynska and Johnson 2009; Clarke et al. 2013). Rhizomes are modified stems found above or below ground, which are present in several plant families including Poaceae and have lateral propagation and high number of buds (Herben and Klimešová 2020).

Some Cerrado grasses typically absorb water and nutrients from the soil surface, from 0 to 20 cm depth in the dry season and from 30 to 70 cm in the rainy season (Souza et al. 2005; Rossatto et al. 2013). Grasses have dense roots and to compete for resources with woody species seedlings (D'Antonio and Vitousek 1992).

Invasive grasses have the ability to compete with native grasses, decreasing the coverage of graminoids, native forbs and shrubs (Pivello et al. 1999b; Damasceno et al. 2018), which can cause changes in resource storage, facilitating the invasion process (D'Antonio and Vitousek 1992; Rahlao et al. 2009). They can also change fire frequency, as they modify fuel composition (Brooks et al., 2004), increase the amount of dead biomass, which can lead to more intense and severe fires (Gorgone-Barbosa et al. 2014). In addition, invasive grasses have a higher capacity to produce seeds with a high rate of germination (Pivello, et al., 1999a), which are also fire-resistant (Gorgone-Barbosa et al. 2020) and can more readily germinate in post-disturbance environments (Gorgone-Barbosa et al. 2014).

It has been estimated that in the last 35 years, around 50% of the original Cerrado area has been destroyed or converted into pasture and crops (Klink and Machado, 2005; Coutinho, 2006; Walter et al., 2008). Grasslands cover extensive areas (about 30 million km² and 25% of the world vegetation) and grasses are characterized for creating highly flammable fuels, supporting large herbivore densities and being competitors belowground (Ramankutty and Foley 1999; Bond 2008). Invasive species are considered one of the greatest threats to the Cerrado's biodiversity and conservation (Pivello et al., 1999; Durigan et al., 2007). The African genus *Urochloa* is highly representative among the grasses used for grazing. The genus is the related to invasion in natural habitats in Brazil (Zenni and Ziller 2011) and it is found in sites with low N:P ratios producing great amount of biomass (Lannes et al. 2012). It is considered a highly competitive invasive species, capable of withstanding long periods of drought (Dias-Filho, 1990), outcompeting native species and thus, changing plant communities (Damasceno et al. 2018).

Our objectives were to evaluate the starch allocation in *Urochloa brizantha* (A.Rich.) R.D.Webster by anatomical and physiological analysis of leaves, rhizomes and roots in areas under different fire frequencies (biennial, annual and fire exclusion). The anatomy and histochemistry of the three organs were analyzed for evidence of significant tissue differences in thickness when the plants were subjected to fire. We addressed the following questions as the survival of individuals of *U. brizantha* in response to fire appear to be related to an ability to resprout, probably due to the starch reserves in its rhizomes: 1) Which vegetative organ and tissue store more starch grains? (2) Are there differences in starch storage in plants from burned and unburned plots? (3) What are the anatomical differences between burned and unburned individuals? Thus, we hypothesized that recurrent fires will result in repeated resprouting and use of stored root reserves, which could lead to a depletion of starch reserves in rhizomes. On the other hand, plants in areas excluded from fire would present

higher reserves of starch. Besides, anatomical changes in plant tissues were expected in leaves: epidermis, bulliform cell, parenchyma, bigger vascular bundle and smaller vascular bundle; in rhizomes: epidermis, parenchyma and vascular bundle and in roots: epidermis, cortex and vascular cylinder.

2 Material and Methods

2.1. Study Area and Plant sample

Individuals of *Urochloa brizantha* were sampled at the Reserva Natural Serra do Tombador (RNST, 8,900 ha, Central Brazil, 47 ° 45' S; 47 ° 51' W and 13° 35' S; 13 ° 38' S; 560–1118 m a.s.l.). The climate is tropical, with wet summers and dry winters and average annual rainfall between 1300 and 1500 mm (Fundação Grupo Boticário 2011).

All experiments were carried out in campo sujo physiognomy, where many patches of *U. brizantha* can be found. The experimental area has not been burned or grazed since 2007 (Gorgone-Barbosa et al. 2014). The plots where we collected our samples were established in July 2012 and are from the fire experiment of the study Gorgone-Barbosa et al., (2014). Plots were established in invaded areas of open savannas, with different fire treatments: fire exclusion since 2007, annual and biennial fires. Each treatment contained four plots. In the middle of dry season (July, 2014) shortly after the burning, when the plants re-sprouted, three entire individuals from each plot were sampled for analysis.

2.2. Light microscopy

Samples of leaves, roots and rhizomes were collected and stored in plastic pots containing FAA 70 (Johansen, 1940). After 48 h, the plant material was stored in 70% alcohol. In the completely expanded leaf, the middle region of the leaf was analyzed at the midrib and the leaf blade area. The samples were dehydrated in ethylic series, included in hydroxy-ethyl-methacrylate (Leica Historesin) and the obtained blocks were cut in a microtome at 5–10 µm thick. The material was stained with toluidine blue 0.05% in phosphate buffer and citric acid with pH between 4.5 and 6.0 (Sakai 1973). The slides were mounted with synthetic resin "Entellan".

Histochemical tests were performed in prepared slides of leaves rhizome and roots. For starch detection it was used as lugol (Berlyn and Miksche, 1976) that blushes in black; Sudan IV was used for lipid detection (Jensen, 1962) that blushes red; for phenolics, ferric chloride (Johansen, 1940) coloring bluish-black to dark green and for pectic substances and mucilage, Ruthenium red (Johansen, 1940) that blushes purple pink. To verify the natural aspect of the tissue, the material sections were assembled only in water.

Digital photomicrographs were taken with a camera AxioCam ERC5S coupled in a microscope Zeiss Primo Star with the micrometric scales photographed and enlarged under the same optical conditions to be used. The photos of five slides per plant of the analyzed plant tissues in all treatments were submitted to the ImageJ software was used to attain biometric measurements.

2.3. Starch Extraction and Quantification

For the physiological analysis, the starch content in all the organs of three plants for each plot was quantified. So, in total 4 plots of each treatment, with 12 plants per control treatment, annual and biennial burns.

The soluble compounds were extracted from parts in a liquid-liquid extraction using methanol: chloroform: water (MCW) solution in the proportion 12:5:3 (Bialeski and Turner 1966), and the discarded extract. From the remaining precipitate, the starch was extracted twice at a 1:5 (p:v) ratio with perchloric acid at 30%, and after centrifugation the supernatants together made up the extract, according to Yemm and Willis, (1954).

The remaining extract (hydrolyzed starch) was used for total soluble sugars dosage according to Umbreit et al., (1957). For 1 mL of each sample, 2 mL of antrona reagent was added (100 mg of antrona in 2.5 mL of water + 50 mL of concentrated sulfuric acid). After shaking, the tubes were sealed and heated in water at 100°C for 3 min, then cooled to room temperature. To determine the total soluble sugars, the absorbance was measured in a spectrophotometer at 660 nm and their concentration was calculated from a standard curve obtained with glucose.

2.4. Statistical analyses

We applied general linear mixed models (GLMM) to analyze differences for each response variables (epidermis, bulliform cell, parenchyma, bigger vascular bundle, smaller vascular bundle, cortex, vascular cylinder and starch) among treatments and different parts of the plant (leaves, roots and rhizomes). We considered the treatments and parts of the plants as fixed factors and individuals as random factor. To test interactions between the treatments and between parts of plant, least square means post hoc test was conducted.

All analyses were performed in R environment (R Development Core Team, 2018) using the following packages: LME4 (Bates et al. 2015), LS means (Lenth 2016), Multcomp (Hothorn et al. 2008).

3. Results

3.1 Anatomical and Histochemical Characterization

We analyzed the epidermis, bulliform cell, parenchyma, bigger vascular bundle and smaller vascular bundle in *Urochloa brizantha* leaves (Fig. 1a-c). Of these five variables, only the bigger vascular bundle was different among treatments, indicating that biennially burned plants have increased tissue thickness. The other leaf tissues were similar among the results when comparing both burn treatments compared with the control (Table 1).

The rhizome was the organ that presented the most changes. Epidermis, parenchyma and vascular bundle showed changes in thickness in some of the fire treatments compared to the control (Fig. 1e). The epidermis increased in thickness in the two fire treatments when compared to the control (Table 1). The rhizome parenchyma thickness was greater for the biennial than the annual burn treatment, and not significant from the control (Table 1). Finally, the vascular bundle of the rhizome of biennially burned plants increased in thickness significantly compared to control and annually burned plants (Table 1).

Table 1
Mean thickness of different components of *Urochloa brizantha* leaves, roots and rhizomes (μm) at different fire frequencies: Control, biennial and annual burn treatments in invaded Cerrado areas (mean $\pm$ SD). Ep = epidermis, BC = Bulliform cell, P = parenchyma; V.b.b = Vascular Bundle bigger; V.b.s. = Vascular Bundle smaller; C = cortex; V.C. = vascular cylinder.

	Control	Biennial	Annual
Leaves			
Ep.	10.41 $\pm$ 1.71 ^a	11.04 $\pm$ 2.06 ^a	10.67 $\pm$ 1.56 ^a
B.C.	49.98 $\pm$ 8.31 ^a	53.30 $\pm$ 9.42 ^a	50.73 $\pm$ 8.05 ^a
P.	21.79 $\pm$ 5.42 ^a	20.63 $\pm$ 4.68 ^a	20.66 $\pm$ 4.55 ^a
V.b.b.	154.29 $\pm$ 18.22 ^a	166.20 $\pm$ 23.19 ^b	155.84 $\pm$ 14.70 ^a
V.b.s.	43.19 $\pm$ 6.72 ^a	45.01 $\pm$ 8.76 ^a	45.26 $\pm$ 8.60 ^a
Rhizomes			
Ep.	12.20 $\pm$ 3.53 ^a	15.51 $\pm$ 5.90 ^b	14.97 $\pm$ 5.73 ^b
P.	303.20 $\pm$ 226.39 ^{ab}	382.37 $\pm$ 218.52 ^a	238.50 $\pm$ 175.14 ^b
V.b.	143.18 $\pm$ 40.45 ^a	169.25 $\pm$ 37.47 ^b	137.62 $\pm$ 35.32 ^a
Roots			
Ep.	12.30 $\pm$ 3.79 ^a	11.73 $\pm$ 2.17 ^a	12.58 $\pm$ 3.61 ^a
C.	153.97 $\pm$ 39.91 ^a	151.54 $\pm$ 34.32 ^a	143.06 $\pm$ 28.41 ^a
V.C.	398.02 $\pm$ 92.09 ^a	409.24 $\pm$ 62.94 ^a	403.38 $\pm$ 87.04 ^a

Lower case letters represent significant differences between control and treatments ($p \leq 0.05$).

Finally, the tissues analyzed in the roots were similar among treatments (Fig. 1d). Since they are totally underground, roots may not have been affected by fire.

The histochemical tests with Lugol stained the starch that was in the sheath cells of *U. brizantha* leaf vascular bundles black (Fig. 2a). In the rhizomes, the starch was observed in parenchyma cells (Fig. 2b), and in the roots located in the cortex cells (Fig. 2c).

3.2 Starch reserve quantification

The amount of starch did not differ between burned and control treatments (Table 2). However, the values in leaves and rhizomes of individuals burned annually were lower than in control and biennial individuals. The roots presented higher amount of starch in annual fire treatments when compared to control and biennial fire treatment.

Table 2
Starch content in grams per fresh mass (mean $\pm$ SD) in leaves, rhizomes and roots of individuals of *Urochloa brizantha* at different fire frequencies: fire exclusion, biennial and annual fires in invaded open savannas in the Cerrado.

Treatments	Control	Biennial	Annual
Leaves	15.04 $\pm$ 12.20 ^a	25.10 $\pm$ 15.23 ^a	15.05 $\pm$ 11.71 ^a
Rhizomes	13.27 $\pm$ 10.91 ^b	21.59 $\pm$ 7.52 ^b	13.40 $\pm$ 10.98 ^b
Roots	52.95 $\pm$ 19.97 ^b	77.17 $\pm$ 41.42 ^b	79.27 $\pm$ 34.11 ^b

Lower case letters represent significant differences between plant organs in each treatment ($p \leq 0.05$).

4. Discussion

The vascular bundle in the C4 photosynthesis mechanism had an important role since photosynthesis occurs in segregated parts of leaf mesophyll and thus anatomical changes in vascular bundle sheath could also mean metabolic variation (Sage 2001). The chloroplasts present in photosynthetic cells in leaf mesophyll of grasses were also in the sheath cells that surround the vascular bundle (Souza et al., 2005; Miyake, 2016). The bundle sheath cells of C4 grasses comprised the decarboxylation of the four-carbon acid reaction, the release of CO₂ and its fixation in the Calvin-Benson Cycle, which later becomes a source for carbohydrates, such as starch (Taiz et al. 2017). Thus, an increase in vascular bundle thickness of *U. brizantha* could be linked to an increase in photosynthesis, enabling greater carbohydrate storage in the roots, for enhanced plant resprouting after the fire.

The rhizome parenchyma of plants burned annually decreased significantly in thickness when compared to individuals burned every two years, since the parenchyma is a tissue composed of living cells, with a primary wall and large vacuoles that contain water (Mauseth, 1988). Therefore, the decrease in parenchymatic cells may suggest that shorter periods between fires may result in less water storage in *U. brizantha* rhizome tissues. The reduction in the parenchyma of plants burned annually may be linked to water reduction in the tissue caused by the process of burning the aerial portions of the plants. Rhizomes can store great amounts of water, however, under annual fire conditions they seem not to be able to recover the water storage. Annual fire cycles do not allow the plants to regenerate their tissues between fires, but the plants burned biennially can regenerate. Besides, the thickness increase of the vascular bundles may be connected to the secondary wall thickness increase of the sclerenchyma cells, increasing general plant protection (Mauseth, 1988). Anatomical changes in grass tissues in response to fire have not yet been reported in the literature, but these anatomical changes (leaf vascular bundle and rhizome epidermis, parenchyma and vascular bundle) in *U. brizantha* may demonstrate, the adaptability of savanna species to establish themselves in disturbed environments (Nsor et al. 2019).

According to Choczynska and Johnson, (2009), fire can damage the sprouts of grasses found at 1 to 2 cm of the soil surface. It explains the anatomical changes presented by the rhizome tissues and the fact that roots have no changes for being a little deeper below ground.

As for the starch found in the vascular bundle sheath, it is important to emphasize chloroplasts that accumulate starch during the day are concentrated in the bundle sheath cells (Souza et al., 2005; Weise et al., 2011; Miyake, 2016).

The fact that the amount of starch did not differ between burned and control treatments corroborate with the initial hypothesis that fire does not cause changes in the starch storage of *U. brizantha*. The same result had been reported by Mendes et al., (2010), that observed storage of similar carbohydrates in *Axonopus affinis*, *Paspalum notatum* and *Aristida laevis* burned and unburned in the South of Brazil.

Reestablishing the aerial portions of the plant lost after fire or drought demands mobilization of carbon from storage (Moraes et al., 2016). In tropical grasses, glucose, sucrose and starch are the three major non-structural carbohydrates in leaves and stems (Moraes et al. 2013) considered as plant storage (Souza et al. 2005). In this study we found starch in different concentrations in the leaves, rhizomes and roots of *U. brizantha*, and it was observed that there is a greater amount of starch in the root organ, when compared to leaves and rhizomes. Furthermore, the fire treatment did not change the amount of starch in the organs. This supports the notion that even without the disturbance of fire, grasses are dependent on carbohydrate reserves (mainly starch) for vegetative propagation (Souza et al. 2010).

However, no significant differences occurred within burn treatments for root or rhizome reserves, although the roots showed increased starch in relation to the rhizome (Fig. 3; Table 2). This increasing value trait found in *U. brizantha* roots, although non-significant, could be related to the fact that plants with roots as their primary clonal growth organs more tolerant to hard and frequent disturbances, than plants that use rhizomes or stolons with an additional capacity of root sprouting (Klimešová et al. 2017). As observed in our study, the concentration of starch tends to increase in the roots just after fire. The presence of starch in the roots after fire allowed the resprout of the aerial portions of the plant (Bowen and Pate 1993), that was lost to the fire. According to Knox and Clarke, (2005), the increase of starch in the roots corroborates with the strategy of plant resprouting, whose strategies are to allocate starch in the roots.

In the present study, it was expected that the fire could change the anatomical characters in response to the fire in *U. brizantha*. However, the results showed that annual fire treatment did not anatomically change the plant's vegetative organs, except for the rhizome epidermis, which increased significantly, showing the organ's protection. The roots did not have anatomical changes, which was interesting because this organ has the starch storage. And in the biennial burn treatment, the vascular bundle of the leaf and the rhizome tissues (epidermis, parenchyma and vascular bundle) increased significantly, indicating full recovery due to the absence of fire. Finally, the storage organ of *U. brizantha* is not the rhizome, but the roots, since starch in large quantities was found in them.

In leaves and rhizomes of *Urochloa brizantha*, the changes that occurred in response to biennial burning could indicate plasticity after fire disturbance. The period between fires caused the increase in anatomical tissue thickness, implying in high protection after the first fire. While in plant physiological aspects, the starch storage was not affected, instead, they tend to increase after fire. The storage organ, in this case the roots, was protected by the soil. This study could contribute to the understanding of *U. brizantha* metabolism and anatomical structure in response to fire intensity, as well as support suitable management techniques aiming to manage this alien species and protecting the Cerrado's native plants.

Declarations

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The authors declare they have no Conflict of Interest.

Authors' contributions

PFR, EGB, LSC and ARM contributed to the conceptualization and study design. EGB collected material in the field. PFR performed laboratory research. PFR and EGB analysed the statistical. PFR wrote de first draft of the manuscript. EGB, LSC and ARM performed editing and revision of the manuscript. All authors approved the final version of the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

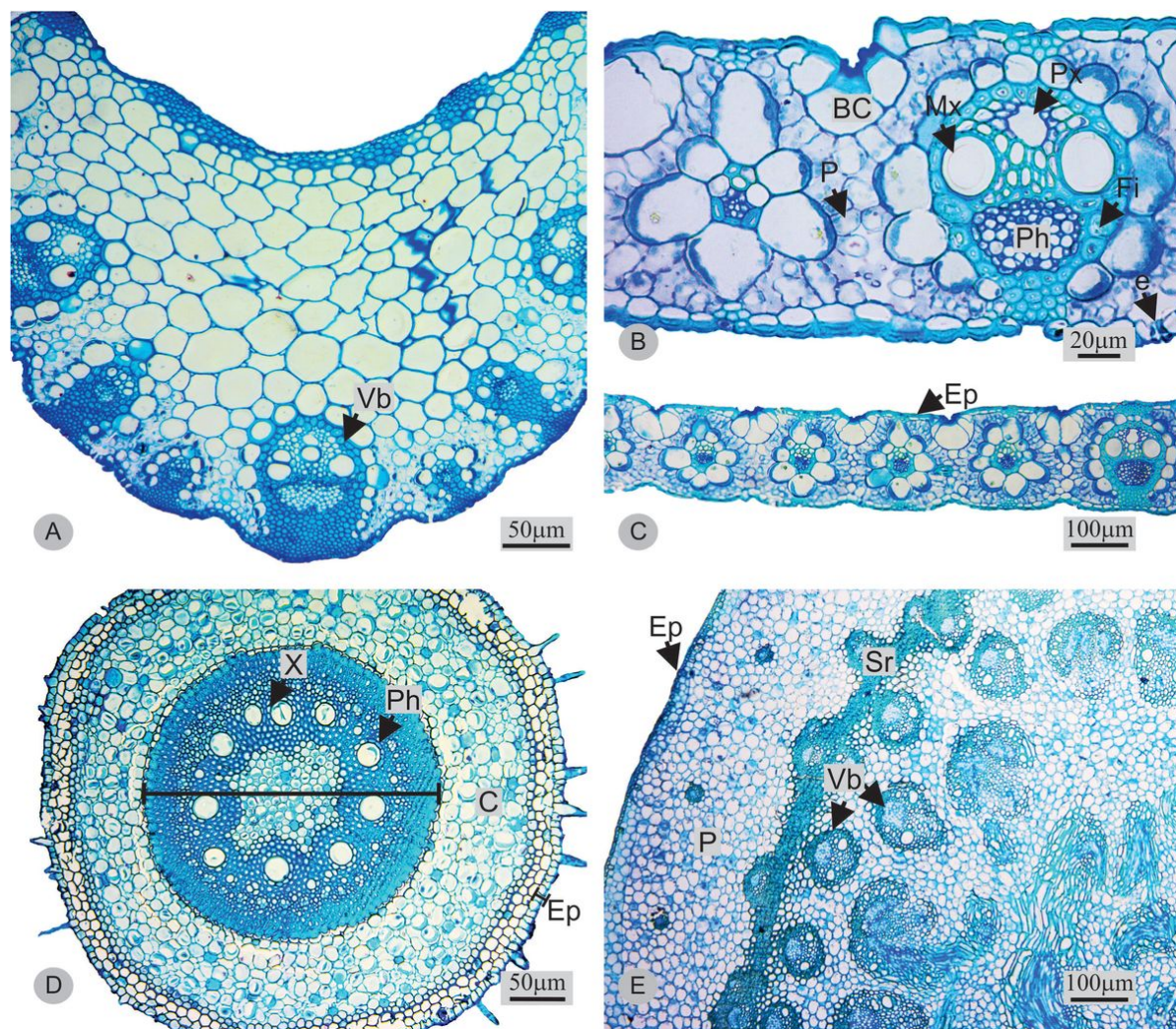


Figure 1

Cross sections of *Urochloa brizantha*. leaf. (A, B, C), root (D) and rhizomes (E) A. Midrib region. B. - C. Internervural leaf area. B. Vascular bundle. BC = Bulliform cell; e = stoma; Ep = epidermis; Ph = phloem; Fi = fibres; Mx = metaxylem; Px = protoxylem; P = parenchyma. Sr. = Sclerenchyma ring. Vb = Vascular bundle. C = cortex

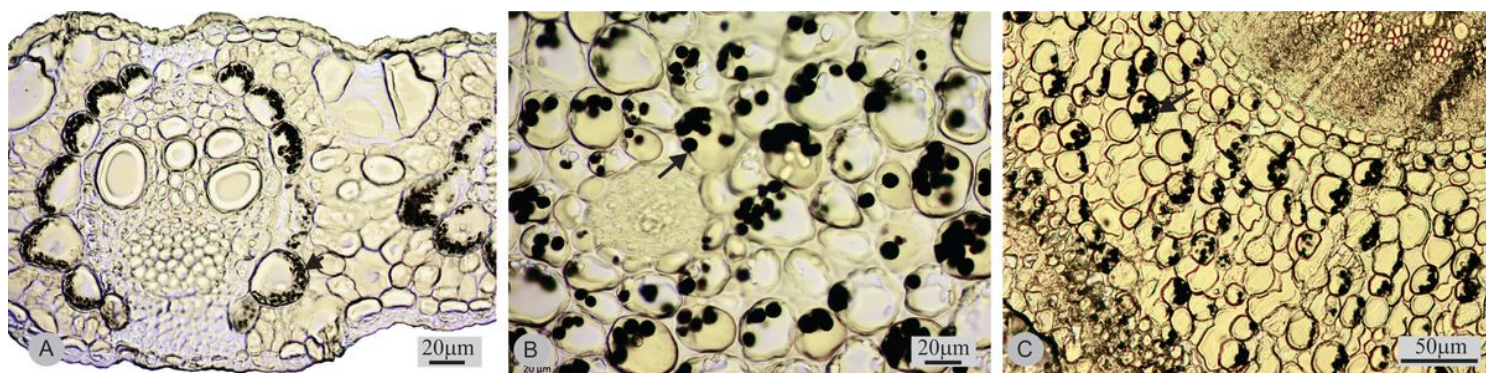


Figure 2

Cross sections of leaf, rhizomes and root of *Urochloa brizantha*. Leaf (**A**), rhizomes (**B**) and root (**C**). Histochemical tests with positive reactions for lugol (A, B, C), Arrows in A, B, and C indicate starch grains