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Original Paper

The specialized parenchyma in the *Paspalum vaginatum* stem as a strategy to water deficit and salinity

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Abstract

Paspalum vaginatum is a halophyte plant found along coastal plains, which presents cells with atypically thickened walls in the ground tissue of the stem stele (GTS). The tolerance of this species to high salinity and water stress led us to investigate whether the thickened walls could be related to adaptation to the coastal environment. Thus, we sought to characterize the cell walls that make up the GTS of *P. vaginatum*, describe the tissue, and verify the influence of the water resource on the thickening of the walls and a possible function related to the reserve of substances. For this, analyses were carried out using light microscopy, transmission electronic microscopy, and histochemical tests. The samples were collected in the field during low and high rainfall periods. *Paspalum vaginatum* GTS cells have pectic-cellulosic primary walls. In most basal internodes, these cells presented thickened walls formed in two to three layers. Statistical analysis demonstrated that the level of precipitation is directly related to cell wall thickening. The data suggest the storage and mobilization of substances through the cell wall of the specialized parenchyma.

Key words: coastal environment, collenchymatous tissue, grass anatomy, seashore paspalum, storage cell wall.

Resumo

Paspalum vaginatum é uma planta halófita encontrada ao longo de planícies litorâneas, que apresenta células com paredes atipicamente espessadas no tecido fundamental do estelo caulinar (TFE). A tolerância dessa espécie a alta salinidade e déficit hídrico nos levou a investigar se as paredes espessadas poderiam estar relacionadas à adaptação ao ambiente costeiro. Dessa forma, buscou-se caracterizar as paredes celulares que constituem o TFE de *P. vaginatum*, descrever o tecido, assim como verificar a influência do recurso hídrico no espessamento das paredes e uma possível função relacionada à reserva de substâncias. Para isso, foram realizadas análises em microscopia de luz, microscopia eletrônica de transmissão e testes histoquímicos. As amostras foram coletadas a campo em período de baixa e alta pluviosidade. As células do TFE de *P. vaginatum* possuem paredes primárias péctico-celulósicas. Nos entrenós mais basais, essas células apresentaram paredes espessadas formadas por duas a três camadas distintas. A análise estatística demonstrou que o nível de precipitação está diretamente relacionado ao espessamento da parede celular. Os dados sugerem o armazenamento e mobilização de substâncias através da parede celular do parênquima especializado.

Palavras-chave: ambiente costeiro, tecido colenquimatoso, anatomia de gramínea, seashore paspalum, parede de reserva.

Introduction

Paspalum vaginatum Sw. (Poaceae) or "seashore paspalum", is a halophyte plant tolerant to high salinity and water stress (Duncan & Carrow 1999; Lee *et al.* 2008a). These characteristics make it the target of research to understand the mechanisms that allow its growth in saline soils (Lee *et al.* 2005, 2008a) and the functional characterization

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of genes for the genetic improvement of plants (Liu *et al.* 1995; Endo *et al.* 2005; Wang *et al.* 2006; Neibaur *et al.* 2008). The species is found along coastal plains composing the upper beach vegetative community (Bona *et al.* 2020). The cells of the ground tissue of the stem stele (GTS) of *P. vaginatum* show an unusual thickening of the cell wall, similar to gelatinous fibers and collenchyma cells, which may be related to the tolerance to water and salt stress of this species (De-Paula-Machado 2022).

Coastal environments are subject to a higher frequency of seasonal disturbances (Assis *et al.* 2011), saline spray, low water retention, and other conditions that play a robust selective role for vegetation (Hesp 1991; Marques *et al.* 2015). Plants in this region have a set of structural and physiological adaptations to remain functional (Maun 2009). The juiciness or presence of mucilaginous content, for example, helps in the plant's accumulation of water and hydration (Ghanem *et al.* 2010; Hameed *et al.* 2013). Another mechanism halophyte plants use is synthesizing and accumulating of compatible osmolytes in the cytoplasm (Lee *et al.* 2008a). The main compatible osmolytes observed in *P. vaginatum* were proline, glycine-betaine, and trigonelline, which help in osmoprotection and act in preventing water loss under stress (Tramontano *et al.* 1997; Lee *et al.* 2008a). Plants under high salt content and water stress often show changes in the molecular mechanisms involved in the expression of proteins and biosynthesis or degradation of polysaccharides (Touchette 2007; Riadh *et al.* 2010; Shuang *et al.* 2020). These can occur in the cytoplasmic content (Lee *et al.* 2008b) and in the chemical and structural composition of cell walls (Cesarino 2019).

The cell wall functions as a plant-environment sensory interface and undergoes changes in response to salinity and water stress (Le Gall *et al.* 2015). The cell wall can change in terms of its thickness and in terms of the biosynthesis and degradation of structural components such as pectin, phenolic substances, cellulose, hemicelluloses, and other non-cellulosic polysaccharides that can cause changes in the physical properties of the cell wall (De Lima *et al.* 2014; Haghighi *et al.* 2014; Oliveira *et al.* 2020). Gelatinous fibers ("G-fibers"), for example, have a secondary cell wall with a thick, translucent inner layer consisting mainly of crystalline cellulose and rhamnogalacturonan I (Bowling & Vaughn 2008). This modification in the cell wall of "G-fibers" is related to tension and associated with water deficit due to the change in the thickness of this wall layer in more or less humid regions (Piva *et al.* 2019, 2020) and the absorption capacity of water (Evert 2006). The potential for water absorption and protection against drought is also seen in cells with polysaccharide reserve walls, such as xyloglucans and galactomannans (Buckeridge 2000).

To understand the nature of the cell wall of the cells that make up the *P. vaginatum* GTS, we sought to characterize the ultrastructure of the cell wall and answer the following questions: i) Are the cell walls of the GTS of a primary or secondary nature? ii) Do periods of high and low average rainfall influence the cell wall thickness of GTS cells? iii) Would the GTS be considered parenchyma with a storage function in the cell walls?

Materials and Methods

Collection of samples

The individuals of *Paspalum vaginatum* Sw. were collected in the coastal region of Pontal do Sul (Paraná, Brazil), along the beach line, in the locus described as the upper beach by Bona *et al.* (2020), within a radius of 200 m from the geographical coordinate 25°34'26.9"S/ 48°20'57.4"W. In southern Brazil the climate is subtropical and the coastal plain is separated by the Serra do Mar mountain. The coastal lowland vegetation is characterized by a mosaic of herbaceous, shrubby and arboreal species associated with the Atlantic Forest Biome. In the upper beach, the vegetation is herbaceous and predominantly creeping.

Fieldwork was carried out in February and July 2020, covering a period of higher average rainfall (290 mm) and lower average rainfall (100 mm), respectively (Vanhoni & Mendonça 2008). Samples for anatomical analysis were collected in three populations distant from each other, about 20 m. Stem portions - third to the ninth internode - of four individuals of the *P. vaginatum* species were selected for each population, in February and July.

Light microscopy

The samples were fixed in FAA 70 (Johansen 1940) at the time of collection and processed to semi-permanent slides. The fixed pieces were cross-sectioned in the median region of the internode, freehand with disposable blades, were stained with astra blue and safranin (Bukatsch 1972, modified by Kraus & Arduin 1997) or toluidine blue

(O'Brien *et al.* 1964) and subsequently mounted on semi-permanent slides using glycerinated gelatin (Kaiser 1880). The slides were analyzed under light microscopy and used to measure the thickness of the GTS cell wall. Measurements were performed using the ImageJ software, based on photographic records made using an Olympus BX41 photomicroscope (Olympus, Japan) with an image capture camera (SC30) attached.

Statistical analysis

The Kruskall-Wallis test (Kruskall & Wallis 1952) was performed to verify whether there is a statistical difference in the thickness of the GTS cell wall between each population, followed by Dunn's post-hoc test, with adjustment of the p-value by the Bonferroni method. The Mann-Whitney test (Mann & Whitney 1947) was also made to verify whether the thickness of the cell wall differs statistically between population groups, with the period of precipitation as a predictor variable. The tests presented a null hypothesis H0 = no statistical difference between the groups. For this, cell walls were measured (median region of the internode) of 20 cells per individual in each population. Statistical analyzes were performed using the RStudio software. The Kruskall-Wallis test (Kruskall & Wallis 1952) was performed to verify whether there is a statistical difference in the thickness of the GTS cell wall between populations, followed by Dunn's post-hoc test, with adjustment of the p-value by the Bonferroni method. The Mann-Whitney test (Mann & Whitney 1947) was also made to verify whether the thickness of the cell wall differs statistically between population groups, with the period of precipitation as a predictor variable. The tests presented a null hypothesis H0 = no statistical difference between the groups. For this, cell walls were measured (median region of the internode) of 20 cells per individual in each population. Statistical analyzes were performed using the RStudio software.

Histochemical tests

To detect wall compounds in GTS cells, the following histochemical tests were performed -in freehand sections-: Periodic acid reaction - Schiff reagent /PAS (McManus 1948) for general characterization of polysaccharides, linked to the presence from the vic-glycol group (-CHOH-CHOH) or related groups (does not react with cellulose and callose); ruthenium red for the detection of pectins (Johansen 1940) and mucilages (Gregory & Baas 1989); alcian blue and neutral red (Ruzin 1999) for the presence of acid mucopolysaccharides; alcian blue and PAS (Ruzin 1999) to detect acid mucopolysaccharides and general polysaccharides; acid phloroglucine (Johansen 1940) to detect lignin; Sudan III (Johansen 1940) to stain fats, and Lugol to verify amyloplasts in the cytoplasm (Johansen 1940).

Transmission electron microscopy

For the analysis of the ultrastructure of the GTS cell walls, the samples were fixed in glutaraldehyde (2.5%) and paraformaldehyde (1%) (Karnovsky 1965, modified by Kraus & Arduin 1997), washed in 0.1M phosphate buffer (pH 7.2), post-fixed in 1% osmium tetroxide (OsO4) and washed in distilled water. Subsequently, the material was dehydrated in a total ethanol series and included in LR White® resin, following the manufacturer's instructions.

Semi-thin sections (1 µm) were made on a Leica hand-held rotating microtome using glass knives, stained with toluidine blue (O'Brien *et al.* 1964), and mounted on semi-permanent slides. Ultrathin sections (70–100 nm) were performed on a Leica Ultracut UCT ultramicrotome (Vienna, Austria) with a Diatome ultra 45º diamond knife (Pennsylvania, United States), deposited on 200-mesh copper grids, counterstained in a 2% uranyl acetate solution and 10% lead citrate solution (Reynolds 1993). The analysis used a JEOL Transmission Electron Microscope - 1200 EX II (Tokyo, Japan).

Results

Anatomical analysis

The stem epidermis of *Paspalum vaginatum* is unistratified, composed of isodiametric cells and lignified walls. The cortex is made up of about five to nine layers of cells (Fig. 1a). The hypodermis, with one or more layers, has a suberin lamella external to the secondary wall, thickened in a U shape and lignified (see arrowhead tip Fig. 1b). The remaining cells of the cortex are parenchymatic with thin walls or, less frequently, thickened walls like those of the medulla (Fig. 1b). The stem stele is delimited by a sheath of sclerenchyma and diffusely dispersed vascular bundles (atactostele) in the ground tissue (Fig. 1a). The ground tissue of the stele (GTS), in the third internode, is composed of parenchyma with small air gaps and thin primary cell walls (Fig. 1c). At the sixth and

ninth internodes, in several specimens, the GTS cell walls are thickened uniformly (Fig. 1d-e). These walls present vast fields of pits in the contiguous areas, being more evident in the thickened regions (circles in Fig. 2a-b). The thickened wall shows two to three layers of different constitution: the first layer (outermost) is thin, homogeneous and reacts to astra blue and toluidine blue O (Fig. 2c-d layer L1); the second layer often does not respond homogeneously to the stains used and can be smooth (Fig. 2c arrowhead) or porous in the same region of the stem (Fig. 2d layer L2; Fig. 2f-g arrowhead); the third layer is the innermost and, when present, does not react to staining for cellulose, hemicellulose and lignin compounds, presenting a pearly appearance (Fig. 2d layer L3; Fig. 2g arrow).

Histochemical tests

Histochemical tests (Tab. 1) applied to transverse sections of the *P. vaginatum* stem, showed that some positive-reacted components

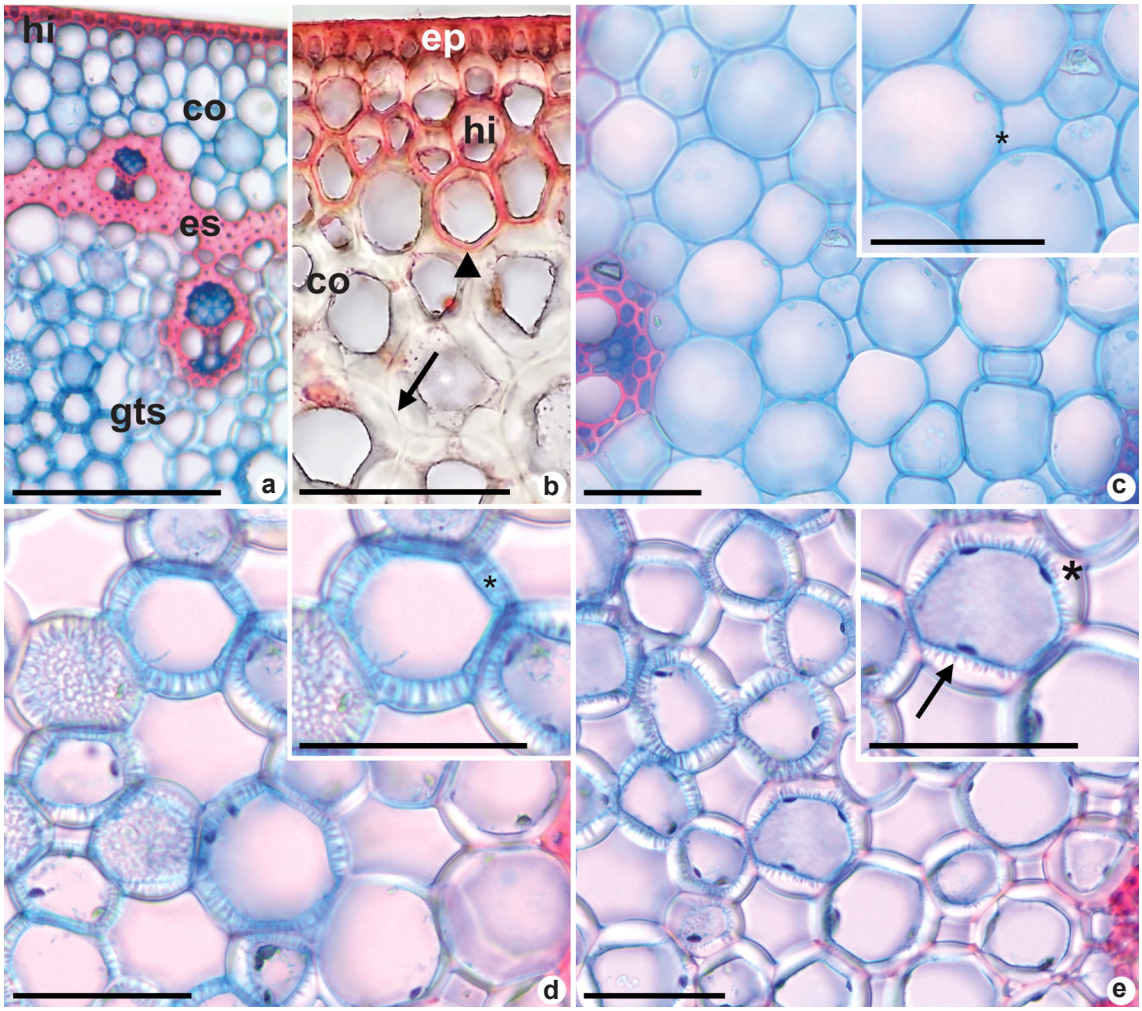

Figure 1 – a-e. Cross-sections of the stem of *Paspalum vaginatum* stained with astra blue and safranin – a. cortical region (co) with thin-walled cells and ground tissue of the stele (GTS) with thick-walled cells, delimited by a sclerenchyma sheath (es); b. cortical region (co) with epidermis (ep), hypodermis (hi) with suberin lamella (arrowhead) and cells with thickened walls (arrow) in the cortex; c. third internode with GTS cells showing thin primary wall in blue; d. GTS cells in the sixth internode, with thickened walls (*) and astra blue stain on most of the wall; e. cell walls with accentuated thickening in the GTS of the ninth internode, showing differences stained with astra blue, staing blue in the porous regions (arrow). Scales bars: a = 100 µm; b-e = 50 µm.

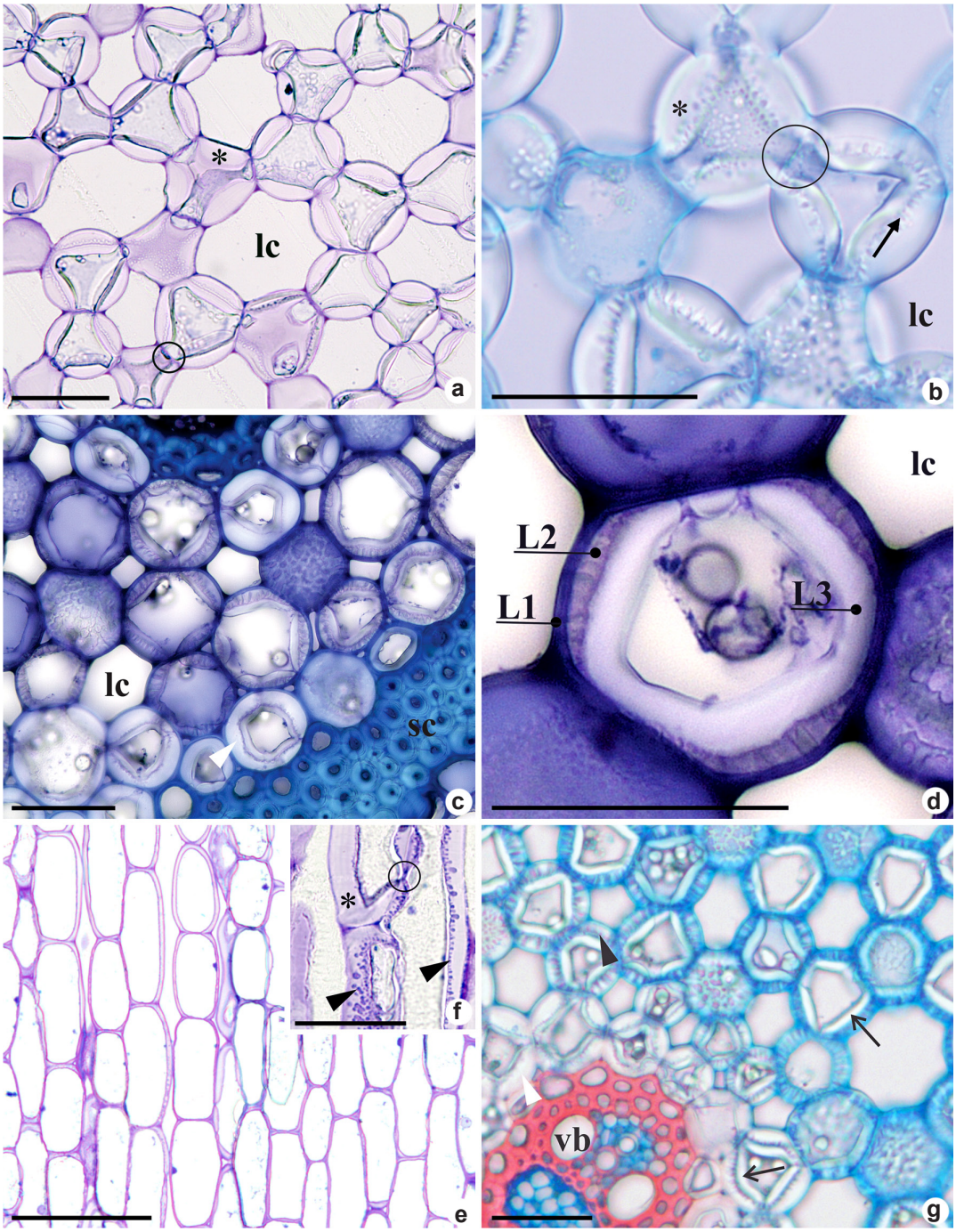

Figure 2 – a-g. Transverse sections of the stem of *Paspalum vaginatum* (a-d, g) and longitudinal (e-f), stained with toluidine blue (a, c, d, e) and astra blue and safranin (b, g) – a. GTS cells with thickened walls (*), large intercellular spaces (lc) and wide pit fields (circle); b. detail of the pit fields (circle) and porosity in layer 2 (arrow); c. GTS cell wall with accentuated thickening, showing two to three distinct layers, (arrow) smooth layer 2; d. detail of the thickened wall containing three layers, the first being the thinnest and outer layer that stains intens purple (L1), the second layer, porous, which stain irregularly (L2) and the third layer which does not respond to the dyes used and has a pearly appearance (L3); e-f. GTS cells with thickened walls (*), with porous regions in the second layer (arrowhead) and pitted fields (circle); g. GTS cells with thickened walls showing three layers and porous areas in the second layer (arrowhead) and smooth regions in the third layer (arrows), stained with astra blue and safranin. Scales bars: a-d, detail in e = 50 µm; e, g. 100 µm.

Table 1 – Histochemical tests on the cell wall of the ground tissue of the stem stele (GTS) of *Paspalum vaginatum*.

Substance detected	Test	Positive / negative reaction		
		L1	L2	L3
Lignin	Acid phloroglucin	-	-	-
Polysaccharides	Schiff's reagent (PAS)	+	+	+
Acid mucopolysaccharides	Alcian blue and neutral red	-	-	-
Acid mucopolysaccharides	Alcian blue and PAS	-	-	-
Pectin	Ruthenium red	+	+	+
Mucilage	Ruthenium red	-	-	-
Mucilage	Tannic acid and ferric chloride	-	-	-

L1, L2, L3 = layers of the cell wall.

investigated, such as polysaccharides and pectins, presented homogeneous distribution in the cell walls of GTS cells without distinction among the L1, L2 and L3, such as polysaccharides and pectins (Fig. 3). Lugol solution which reacts with starch showed the presence of starch grains in the cytoplasm content of GTS cells (Fig. 3a). The phloroglucinol-HCl test, specific for lignins, reacts with the cell walls of the epidermis and sclerenchyma and does not react in the cell walls layers of the GTS (Fig. 3b). Acid mucopolysaccharides recognized by the alcian blue tests performed are absent in all layers (Fig. 3c). Alcian Blue/PAS confirms the negative reaction to the acid mucopolysaccharides, whereas general polysaccharides are observed with PAS reaction in the wall of cortex cells and GTS cells with magenta colored walls in all layers (Fig. 3e). This reaction is stronger in the middle lamella (Fig. 3e, inset). Detection of pectin in the GTS cell wall is positive in all layers when performed with ruthenium red test. The reaction is stronger in the outer layers and middle lamella of the GTS cell walls (Fig. 3f). Ruthenium red/lead citrate and Tannic acid/ferric chloride test shows negative reaction to mucilage (Fig. 3g-h). The reaction for lipids is also negative (Fig. 1b).

Transmission electron microscopy

The GTS cells' ultrastructure analysis showed that the three cell wall layers have different contrast and are distinct from each other (Fig. 4a-b). The cell wall's first (outermost) layer (L1) is more electron-dense and narrow, it is the initial wall deposited before thickening. This layer stains more intensely with toluidine blue and astra blue (Fig. 2d, g). The second layer (L2) is heterogeneous with more or less electron-dense regions and has, in general, the most significant thickness among the layers. It is the first thickened layer that contains, in addition to the initial primary wall, the deposition of pectin and polysaccharides (Fig. 3e-f). The third layer (L3) is the most variable in terms of thickness and is best defined because it is homogeneous and less electron dense, as it is the layer that does not react with toluidine blue, astra blue and lignin (Fig. 2d, g). The cells present living protoplast with numerous mitochondria and dense content near the pit field (Fig. 4c). The membrane has irregularities (ripples) along the thickened walls (Fig. 4a), with vesicles nearby (Fig. 4d) that seem to fuse with the plasma membrane (Fig. 4e), leaving it wavy (Fig. 4f).

Statistical analysis

The Kruskall-Wallis test resulted in a statistical difference regarding the thickness of GTS cell walls between the analyzed populations [$x^2(2)=307.83$, $p<0.001$]. Dunn's Post-hoc showed that populations 1, 2, and 3 from the low precipitation period do not differ among themselves but differ pretty significantly from populations 4, 5, and 6 from the high precipitation period (Tab. 2).

Using the Mann-Whitney test, it can be seen that the median cell wall thickness of the GTS of the February population set (high precipitation) is different from the median cell wall thickness of the GTS of the July population set (low rainfall) ($W=54606$, $p<0.001$). The February populations (13.3 and 3.19, median and IQR) had a higher median than the July ones (9.15 and 2.11) (Fig. 5).

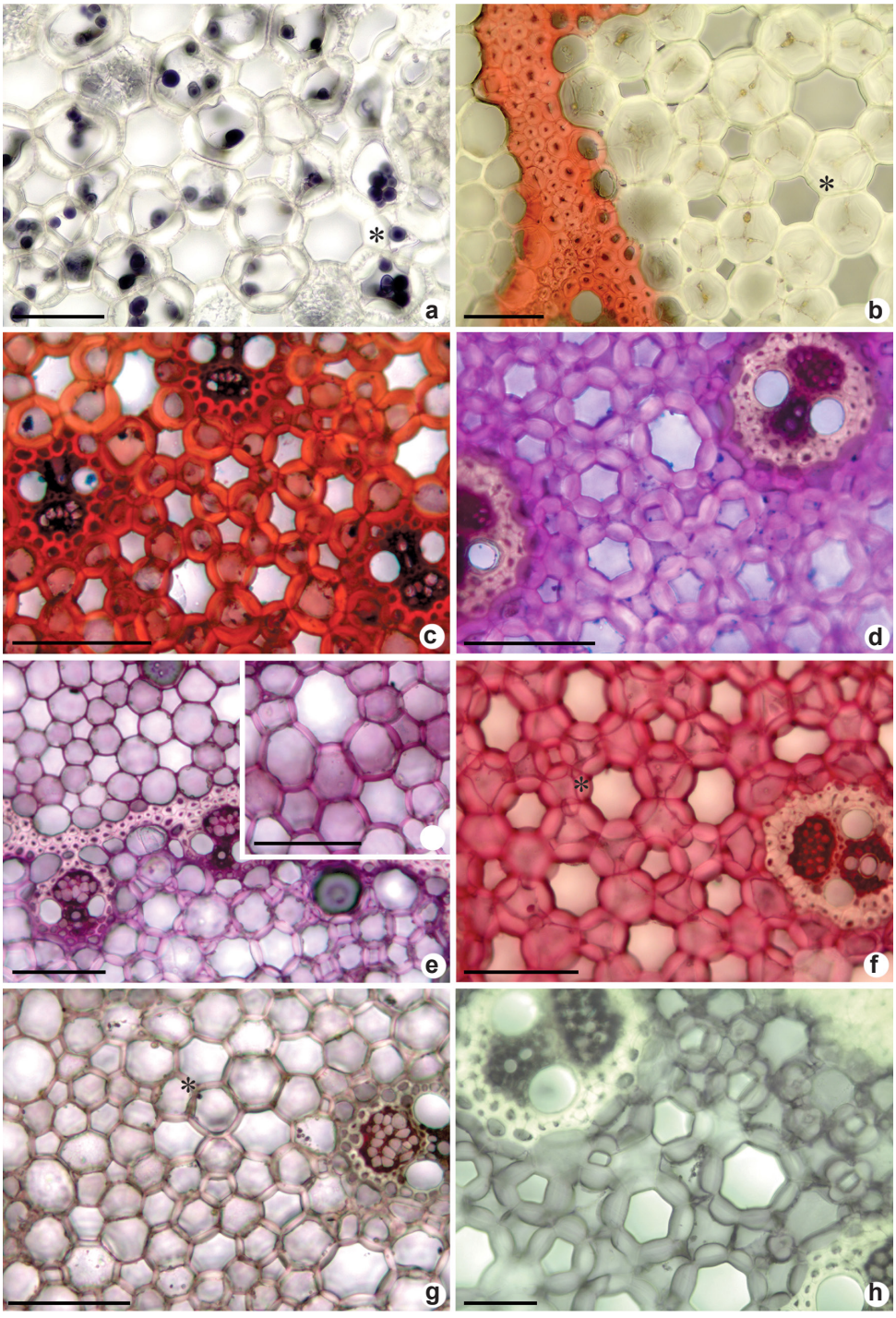

Figure 3 – a-h. Histochemical tests on cross-sections of the stem of *P. vaginatum* showing the ground tissue of the stele (GTS) – a. lugol, showing amyloplasts in the cytoplasm (dark blue); b. acid phloroglucine, detecting lignin (orange) in sclerenchyma cells and the absence of lignin in GTS (*); c. alcian blue and neutral red (contrasting) with negative reaction to acidic mucopolysaccharides; d. alcinane blue/PAS with negative reaction to acidic mucopolysaccharides and positive to general polysaccharides (magenta); e. periodic acid reaction - Schiff reagent (PAS) with positive reaction to general polysaccharides (magenta); f. ruthenium red (Johansen 1940), detecting pectin in the cell wall (intense pink to red); g. ruthenium red solution (Gregory & Baas 1989) with negative reaction to mucilages; h. tannic acid/ferric chloride with negative reaction to mucilage. Caption: * = thickened cell wall. Scales bars: a-b, h = 50 µm; c-g and inset (e) = 100 µm.

Discussion

The pattern found in the ground tissue of the stem stele (GTS) of the individuals of *Paspalum vaginatum* analyzed is similar to the tissue observed in the stolon of *Paspalum distichum* L. and *Hemarthria altissima* Poir. in floodplains in China (Yang *et al.* 2011, 2013). When describing the tissue observed in the stolon of *P. distichum* and *H. altissima*, Yang *et al.* (2011, 2013) refer to thickened wall cells using the terms "collenchymatous thickening" and "collenchymatic cells". These terms are often used to refer to cells or tissues that present characteristics of collenchyma, such as walls that are atypically thickened in parenchyma cells, or when there is no clarity as to the definition

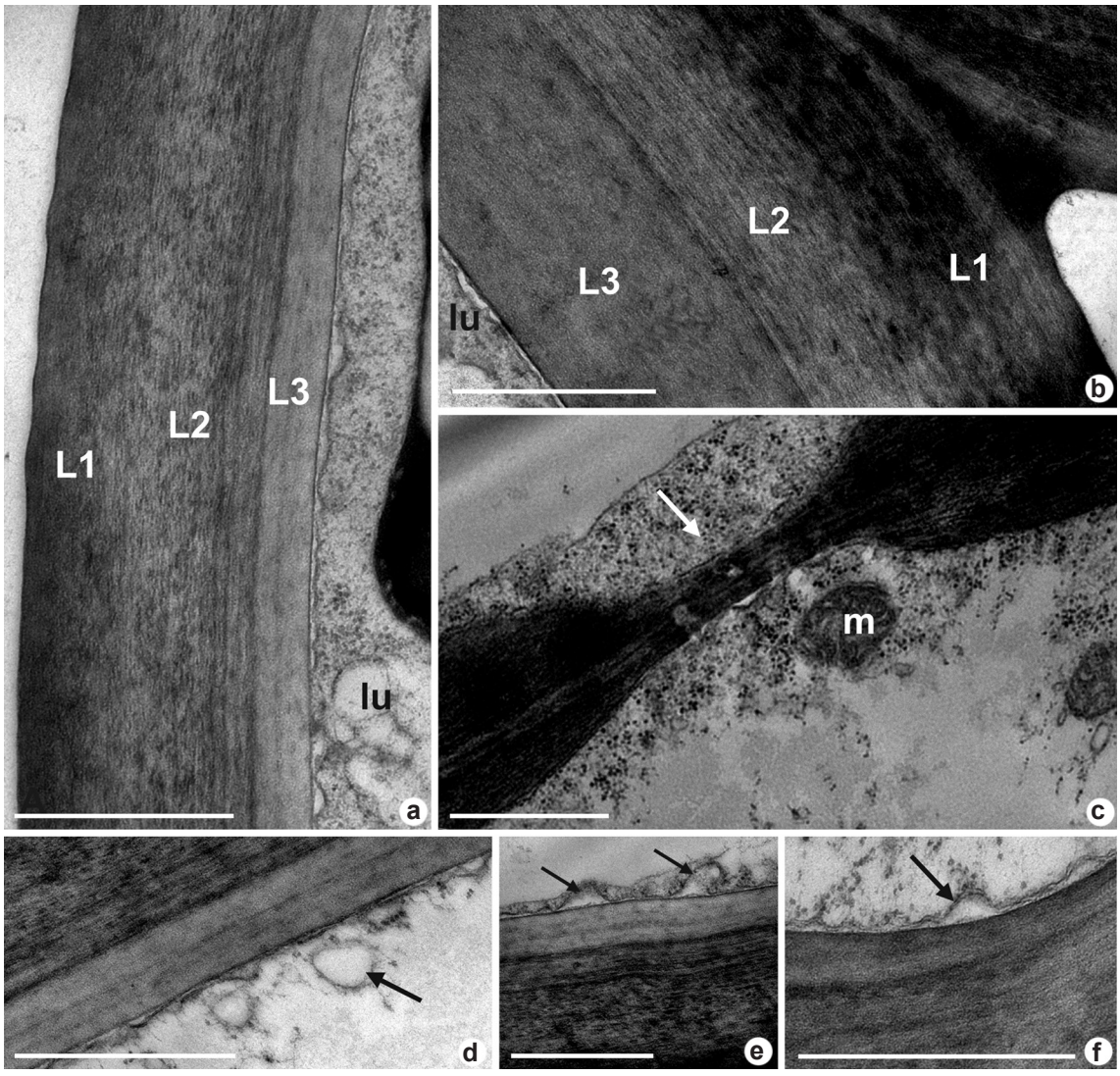

Figure 4 – a-f. Ultrastructure of the thickened cell walls of the fundamental tissue of the stem stele of *Paspalum vaginatum* in Transmission Electron Microscopy – a-b. cell wall with three distinct layers, in which the outermost layer is thinner and more electron-dense than the others (L1), the second layer presents less or more electron-dense regions in a heterogeneous way (L2), and the third deposited layer is uniform and less electron-dense (L3); c. adjacent cells with cell wall thickening interrupted by a wide pit field (arrow), showing a region with mitochondria (m) and dense cytoplasmic content; d-f. detail the presence of vesicles close to the cell wall (d) and their fusion to the plasmatic membrane (arrows in e, f). Scales bars = 1 µm.

Table 2 – Kruskall Wallis test with adjustment of the p-value by the Bonferroni method for cell wall thickness of the ground tissue of the stem stele (GTS) of *Paspalum vaginatum*. Test between each population collected in the periods of higher (1, 2, and 3) and lower (4, 5, and 6) average rainfall.

Population		p-value
p.1	p.2	0.02
p.1	p.3	0.73
p.1	p.4	< 0.001
p.1	p.5	< 0.001
p.1	p.6	< 0.001
p.2	p.3	1
p.2	p.4	< 0.001
p.2	p.5	< 0.001
p.2	p.6	< 0.001
p.3	p.4	< 0.001
p.3	p.5	< 0.001
p.3	p.6	< 0.001
p.4	p.5	1
p.4	p.6	0.05
p.5	p.6	1

of the tissue (Leroux 2012; Kuhn *et al.* 2020; Scatena & Scremin-Dias 2022). Parenchyma and collenchyma share morphological and structural characteristics, and observing plants with regions with intermediate characteristics to these two tissues is not uncommon (Leroux 2012). The shape and size of the cells that make up the collenchyma vary, but they are generally described as fusiform cells (Madjumdar & Preston 1941; Scatena & Scremin-Dias 2022), short or long with tapered ends, similar to fibers (Paiva & Machado 2003; Leroux 2012). In addition, another common feature of collenchyma is primary walls with uneven deposition and, consequently, irregular thickenings (Scatena & Scremin-Dias 2022). In this sense, the GTS cells in *P. vaginatum* resemble parenchyma, both for the rectangular cells in the longitudinal direction and for the uniform deposition in the cell wall, which is common in this tissue. Although the parenchyma commonly has a thin primary wall, it can also develop secondary walls impregnated with lignin, and this thickening is sometimes exacerbated, as in bamboo culm (He *et al.* 2002; Lian *et al.* 2020). Parenchyma cells with thickened walls also occur in the endosperm of *Acrocomia aculeata* (Jacq.) Lodd. *ex* Mart. (Moura *et al.* 2010) and cotyledons of *Hymenaea courbaril* L. (Buckeridge *et al.* 2000), functioning as a polysaccharide storage wall.

The GTS of *P. vaginatum* has to have more of a reserve function, as exemplified above, than one of support since the stele and the vascular bundles are surrounded by a wide sheath of sclerenchyma, which would perform the support function. The reserve function is also reinforced by the abundant presence of vesicles that fuse with the plasmatic membrane in a process similar to exocytosis. In addition, the morphological variations in the same layer suggest the removal of previously stored substances. The presence of the hypodermis with suberin lamellae and thickened walls, which act as a barrier to water loss, also reinforces the storage function of the GTS.

In the youngest internodes (third internode) of the stem of *P. vaginatum*, the GTS cells present a thin primary wall that reacts positively to cellulosic compounds and polysaccharides. In this portion of the stem, the cell wall behaves as expected since about 50% of the

polysaccharides that make up primary cell walls in grasses are cellulosic (Sakurai 1991). The other polysaccharides found in large amounts in the primary walls of grasses are arabinoxylans and glucuronide arabinoxylans, while pectins, xyloglucans, and glucomannans are found in smaller amounts (Sakurai 1991; Carpita 1996; Leucci *et al.* 2008). During stem elongation and plant development, the chemical structure of the cell wall may be altered through the synthesis, degradation, and distribution of polysaccharides and other wall components that can cause changes in the physical properties of the cell wall (Sakurai 1991). Along the stem, *P. vaginatum* GTS cells show alterations in cell wall thickness and composition. The remarkably thickened walls of the GTS, in the oldest regions of the stem (sixth to ninth internodes) of *P. vaginatum*, react positively to cellulosic compounds and pectin and negatively to lignin, characterizing the primary nature of walls (Evert 2013).

The storage of pectin and polysaccharides in the cell wall, which makes up the GTS of *P. vaginatum* is directly related to the environmental conditions in which individuals of the species develop, that is, under water and saline stress. It is frequent for plant species exposed to stressful abiotic factors to show changes in the cell wall in response to adversity (Le Gall *et al.* 2015; Shafi & Zahoor 2019). Changes in pectin, cellulose and xyloglucan, which make up the cell wall matrix, occur in response to salinity and water scarcity

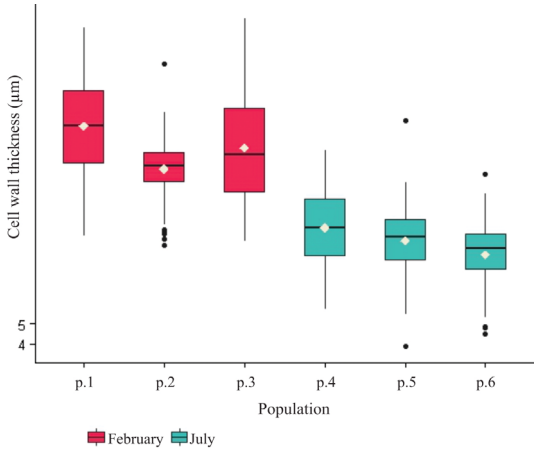

Figure 5 – Cell wall thickness (µm) of the ground tissue cells of the stem stele of six *Paspalum vaginatum* populations collected in periods of high precipitation (February) and low rainfall (July).

(Piro *et al.* 2003; Leucci *et al.* 2008; De Lima *et al.* 2014; Shafi & Zahoor 2019; Oliveira *et al.* 2020). The pectin:cellulose ratio varies among species and can change in response to abiotic factors (Cosgrove 2005), playing a key role against desiccation and water loss during drought stress (Willats *et al.* 2001; Leucci 2008; Le Gall *et al.* 2015) and decreasing the permeability of the cell wall to salt (De Lima *et al.* 2015). Pectins are directly related to the most notable ability to absorb and store water by cell walls (De Lima *et al.* 2014; Le Gall *et al.* 2015). The increase in pectin and the reduction in cellulose accelerate the rate of water absorption being commonly recorded in drought-tolerant plants (Piro *et al.* 2003; Leucci *et al.* 2008; Boanares *et al.* 2018). On the other hand, polysaccharides, mainly hemicelluloses, act as carbon reserves in vegetative organs (Hoch 2007). In *P. vaginatum*, the thickest walls were recorded in individuals with greater water resources. It is interesting to point out that in the coastal plains, in addition to dealing with less water availability at certain times of the year; the plants consequently transition from a higher salt concentration in the soil caused by the lack of precipitation. Exposure to periods of greater and lesser rainfall significantly influenced the thickness of the GTS cell wall.

The accentuated thickening in the wall of the GTS cells of *P. vaginatum* is similar to the parenchyma cells of the endosperm with a carbohydrate reserve wall (Buckeridge *et al.* 2000; Tiné *et al.* 2000; Moura *et al.* 2010). The reserve walls have greater thickening due to the deposition of polysaccharides in the primary walls of the parenchymal tissue and remain that way until these polysaccharides are mobilized as a source of carbon and energy for the plants (Buckeridge *et al.* 2000). Walls with reserve polysaccharides commonly occur in endosperms and cotyledons (Buckeridge *et al.* 2010; Mouta Trafford *et al.* 2013), playing an essential role in germination and initial growth of plants, including secondary functions such as hardness, water imbibition, and xeroprotection (Buckeridge *et al.* 2000). However, Hoch (2007) discussed the possibility that structural polysaccharides in the cell walls of mature and non-reproductive tissues can be stored and consequently mobilized when necessary. (1–3; 1–4)-β-D-glucans, for example, have a structural role in grass cell walls and can function as a quickly mobilized reserve compound (Christensen *et al.* 2010; Trafford *et al.* 2013).

We can conclude that GTS cells are parenchymatous, composed of thickened primary walls, which resemble collenchyma and some seeds storage tissues. Since the walls of the GTS in *P. vaginatum* increase in thickness in the favorable period and decrease under water and salt stress, we conclude that the GTS cells primarily function as water and polysaccharide reserves. The reserves of substances would occur in the favorable period, increasing the thickness, and the reabsorption in the unfavorable period, reducing the thickness and presenting the registered characteristic porosity. However, for more accurate conclusions, biochemical studies must be performed.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that there is no data sharing of this manuscript.

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