

Full Length Research Paper

The first anatomical and histochemical study of tough lovegrass (*Eragrostis plana* Nees, Poaceae)

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Received 11 September, 2014; Accepted 16 July, 2015

***Eragrostis plana* Nees (Poaceae)**, known commonly as tough lovegrass, is the most abundant invasive plant found in the rangelands of Southern Brazil. This study is the first to document the anatomical and histochemical features of the leaves and roots of this grass. Five leaf blades and five roots were collected, fixed in formaldehyde, glacial acetic acid, ethanol 70° (FAA 70), sectioned, stained, and photographed under a light microscope. Anatomical analysis of the leaves was supplemented with observations under a scanning electron microscope. Manual cuts were made into fresh material, which was subjected to specific reagents to determine the lipid, phenol, tannin, lignin, alkaloid, and starch presence. The analyses revealed that the leaves are amphistomatic with paracytic stomata, and the epidermis has lignified cells, a Kranz structure, collateral vascular bundles of two sizes, tectors, unicellular and crystalline trichomes. The roots are polyarc with air gaps on the cortical parenchyma, U-thickened endodermal cell walls and a parenchymatic pith with starch storage cells. Leaves and roots contain lipids, phenols, lignins, and alkaloids; starch is only present in the roots. Anatomical and histochemical traits of tough lovegrass suggest that the plant has adapted to survive under biotic and abiotic stress, which enhances its performance in relation to native plants.

Key words: Anatomy, histochemistry, invasive.

INTRODUCTION

Tough lovegrass (*Eragrostis plana* Nees, Poaceae) is a South-African grass, which is the most abundant invasive plant in the rangelands of Southern Brazil. It is a densely tufted and perennial grass. The basal leaf sheaths overlap and are distinctly flattened. Culms are erect, up to 100 cm tall, and the inflorescence is a panicle with a long narrow, contracted tip. Spikelets have toothed margins and are slightly coarse and olive-green edged

with purple (Cope, 1998).

E. plana was accidentally introduced to the state of Rio Grande do Sul in the 1950s as a seed contaminant in *Chloris gayana* Kunth seed shipments (Zenni and Ziller, 2011). The species is currently established on more than two million hectares of mostly degraded or overgrazed steppes in Rio Grande do Sul, and also occurs to a lesser extent in Santa Catarina, Paraná, São Paulo,

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Mato Grosso, Mato Grosso do Sul, Bahia, Tocantins, Pará, and Distrito Federal, as well as in Uruguay (Medeiros and Focht, 2007). The allelopathy has been suggested as a factor of the success of this grass in Biome Pampa (Coelho, 1986; Medeiros and Focht, 2007; Favaretto et al., 2011; Ferreira et al., 2008). In addition, the tough lovegrass has a strong root system, high resistance to pests and diseases and low acceptability by animals, which contributes to its expansion.

Eragrostis Wolf is one of the most diverse genera of the Poaceae family; it lacks subgeneric classification that would facilitate its study (Sanchez and Koch, 1998). Since grasses are belonging to this genus, they are morphologically similar; there is confusion in the identification and differentiation of species based on external morphological description so other descriptors, such as anatomy, are commonly used (Sanchez and Koch, 1998). The leaf anatomy of about 70 species of *Eragrostis* has been characterized by Ingram (2010) and Sanchez and Koch (1998); however, the anatomy of *E. plana* has not been addressed.

In addition to corroborating taxonomic characterization, plant anatomy provides an important contribution to the elucidation of ecological relationships among plants (Silva and Potiguara, 2009). As such, it can be used to clarify the adaptations of tough lovegrass to adverse weather and soil conditions, as well as its rejection by animals. Histochemical analysis can help identify the structure of the plant and the composition of primary and secondary metabolic compounds (Costa and Proença da Cunha, 2000), which can prove useful for screening purposes to target the main bioaccumulation sites of these compounds (Campos et al., 2014). For tough lovegrass, which is a known allelopathic plant, such studies can be useful in the localization and identification of allelochemicals (Rodrigues et al., 2009). This study is the first to report detailed analysis of the anatomical and histochemical features of the leaves and roots of tough lovegrass.

MATERIALS AND METHODS

Five individual plants of *E. plana* were collected in Passo Fundo (28° 15' S, 53° 24' W), Rio Grande do Sul, Brazil, in October 2012. The specimens were then deposited in the Herbarium of Universidade de Passo Fundo (RSPF 11832). The fresh plants were divided into aerial parts and roots and then fixed in FAA 70 (formaldehyde, glacial acetic acid, ethanol 70° GL; 5:5:90, v/v) for 48 h, with later storage in ethanol 70% (Johansen, 1940). The last fully expanded leaf and the longest root of each plant were isolated. Manual transverse cuts were made in the medial portion of these parts and the sections were used to prepare semi-permanent slides. The sections were cleared with a commercial solution of sodium hypochlorite diluted to 20%, washed in distilled water and stained with basic fuchsin and alcian blue to highlight lignified and cellulosic walls, respectively. The slides were mounted in glycerin 50% and analyzed under an optical microscope (Olympus CX31).

Anatomical analysis of the leaves was supplemented by observation under a scanning electron microscope using the technique of Robards (1978). For this, samples were fixed in 2.5%

glutaraldehyde, 4.0% paraformaldehyde, and a 0.05 M sodium cacodylate buffer (pH 7.2); they were post-fixed in 1% osmium tetroxide in the same buffer for one h, and dehydrated in an alcoholic series. The material was subsequently submitted to the critical point of CO₂ and covered with a 3 nm layer of gold. The material was observed under a scanning electron microscope (VEGA 3 SEN).

Histochemical tests were performed on fresh material that was sectioned manually, using the central part of the last totally expanded leaf and the longest root. The sections were then stained with the following compounds to determine qualitatively the composition of roots and leaves: Sudan III to indicate the presence of total lipids, phloroglucinol for lignin, ferric chloride for phenolic compounds, lugol for starch (Johansen, 1940), Dragendorff for alkaloids (Effland, 1977), and vanilin 5% for tannins (Mace and Howell, 1974). Histochemical reactions were analyzed and photographed under a light microscope (Olympus CX31).

RESULTS AND DISCUSSION

Anatomical study revealed that the leaf blade has a smooth cuticle and a uniseriate epidermis, whose cells have thick, lignified outer periclinal walls (Figure 1). This thickening and lignification limits digestion of the cell wall by herbivores (Akin, 1989), and is one of the reasons for the tough lovegrass's low nutritional quality and rejection by animals (Zenni and Ziller, 2011), contributing to its persistence in pastures. Additionally, the lignified epidermal cells, which are hard and impermeable, give the leaves high tensile strength making it more tolerant to drought and mechanical damage (Balsamo and Orkwiszewski, 2008).

Tough lovegrass has rigid leaves, which fold in the middle along the midrib. This characteristic is an adaptation of the Eragrostideae tribe to stressful conditions, because the leaf, when folded or wrapped, keeps the abaxial epidermis exposed, while the adaxial epidermis entirely protects the stomata, reducing excessive transpiration (Renvoise, 1983). Other species of *Eragrostis* vary in their tolerance to water deficit: *E. curvula* is tolerant, *E. tef* (Zucc.) Trotter is moderately tolerant and *E. capensis* (Thumb.) Trin. is non-tolerant. Balsamo et al. (2006) suggested that there is a positive correlation between leaf tensile properties and drought tolerance, and that increased tensile strength in drought-tolerant species correlates with aspects of tissue architecture and cell wall chemistry. Tough lovegrass leaves are amphistomatic. In grasses it is common for stomata to be present on both surfaces of the leaf blade, generally in two rows on the abaxial and adaxial surface, alternating with the interstomatal cells on the abaxial surface. The stomata are paracytic (Figure 2), as verified in *E. tremula* (S.W. Beauv.) (Ogie-Odia et al., 2010), and elliptical, as verified in *E. glomerata* (Walt.) L.H. Dewey, *E. sessilis* (Buckley) and *E. obtusiflora* (E. Fourn.) Scribn. by Sanchez and Kock (1998). According to these authors, the shape of the stomata was one of the relevant anatomical characteristics for grouping these species of *Eragrostis*.

The adaxial surface of the leaf has furrows along its

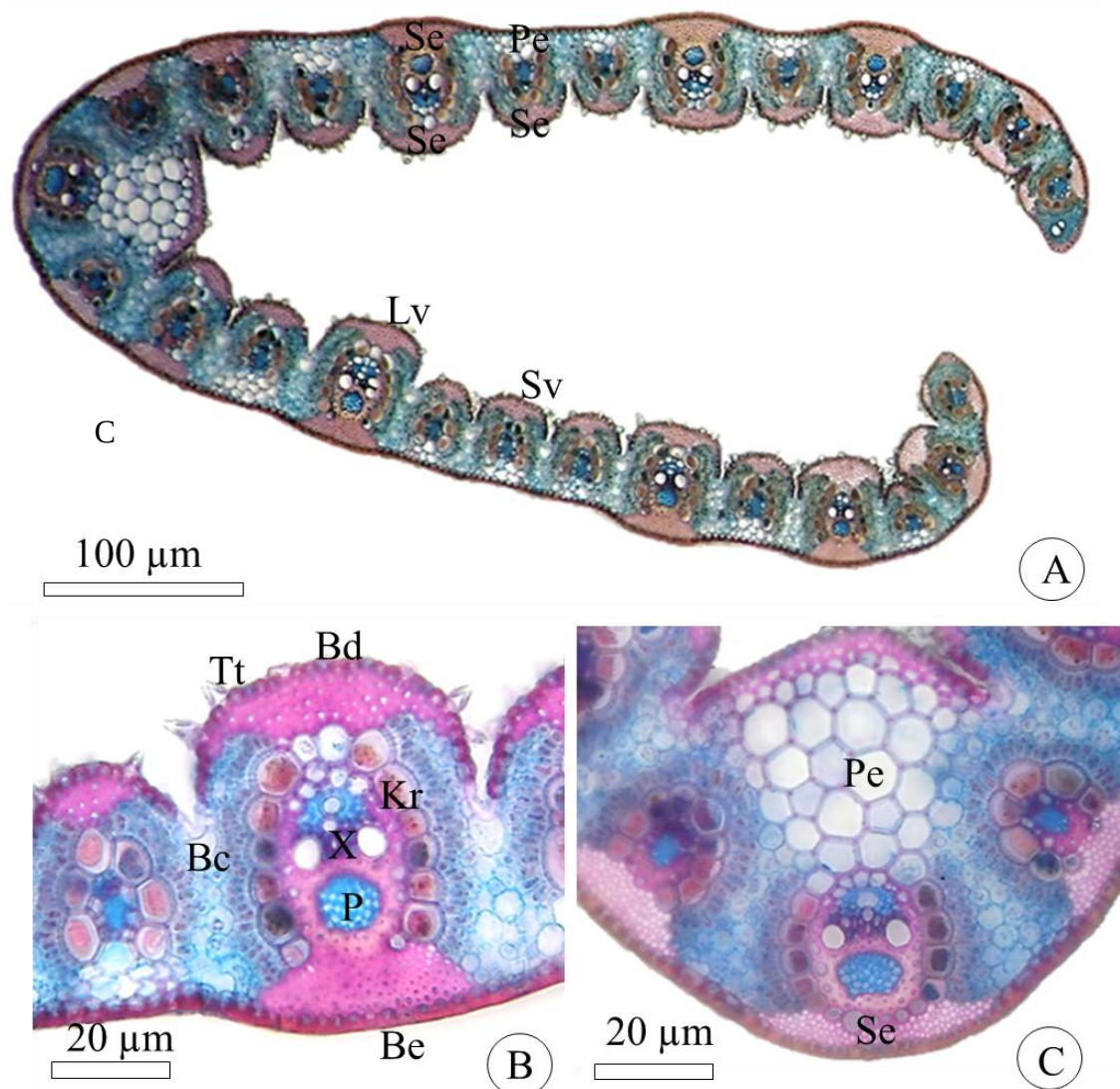


Figure 1. Transverse section of the whole leaf of *E. plana* (A). A vascular bundle showing Kranz anatomy (B). The keel (C). Bc - bulliform cells; Be - abaxial epidermis; Bd - adaxial epidermis; C - cuticle; Kr - Kranz structure; Lv - large vascular bundles; P - phloem; Pe - parenchymatic extension; Se - sclerenchymatic extension; Sv - small vascular bundles; Tt - tector trichomes.

length (Figure 1A) where the bulliform cells and stomata are located (Figure 1B). In the genus *Eragrostis* the bulliform cells can be shield-shaped, but in many species, such as *E. tenella* (L.) Beauv. and *E. intermedia* Hitchc., as well as tough lovegrass, the bulliform group is fan-shaped (Ingram, 2010). The bulliform cells are responsible for leaf enrolment and in unfavorable hydric conditions they reduce the transpiration area. In *E. capensis*, a species which is non-tolerant to hydric deficiency, the bulliform cells are found in several thick strata and are one of the biggest components of the leaf blade, compared to plants that are more tolerant to dryness (*E. curvula* and *E. tef*) (Balsamo et al., 2006).

The presence of furrows on the adaxial surface, and

sometimes on the abaxial surface, with stomata, microtrichomes, papillae, and sharp trichomes was described by Renvoize (1983) in the *Eragrostideae* tribe. Tough lovegrass trichomes are tectors located only on the adaxial surface of the leaves, and are unicellular and crystalline (Figures 1B, 2B and C). The presence of trichomes can be interpreted as protection for the stomata and the mesophyll against excess heat, as well as being important in the isolation and reflection of light (Fahn, 1990).

The species presents Kranz anatomy (Figure 1B), which is common for the *Eragrostis* genus (Ingram, 2010). This type of structure limits consumption by animals, since it is difficult for ruminal microorganisms to

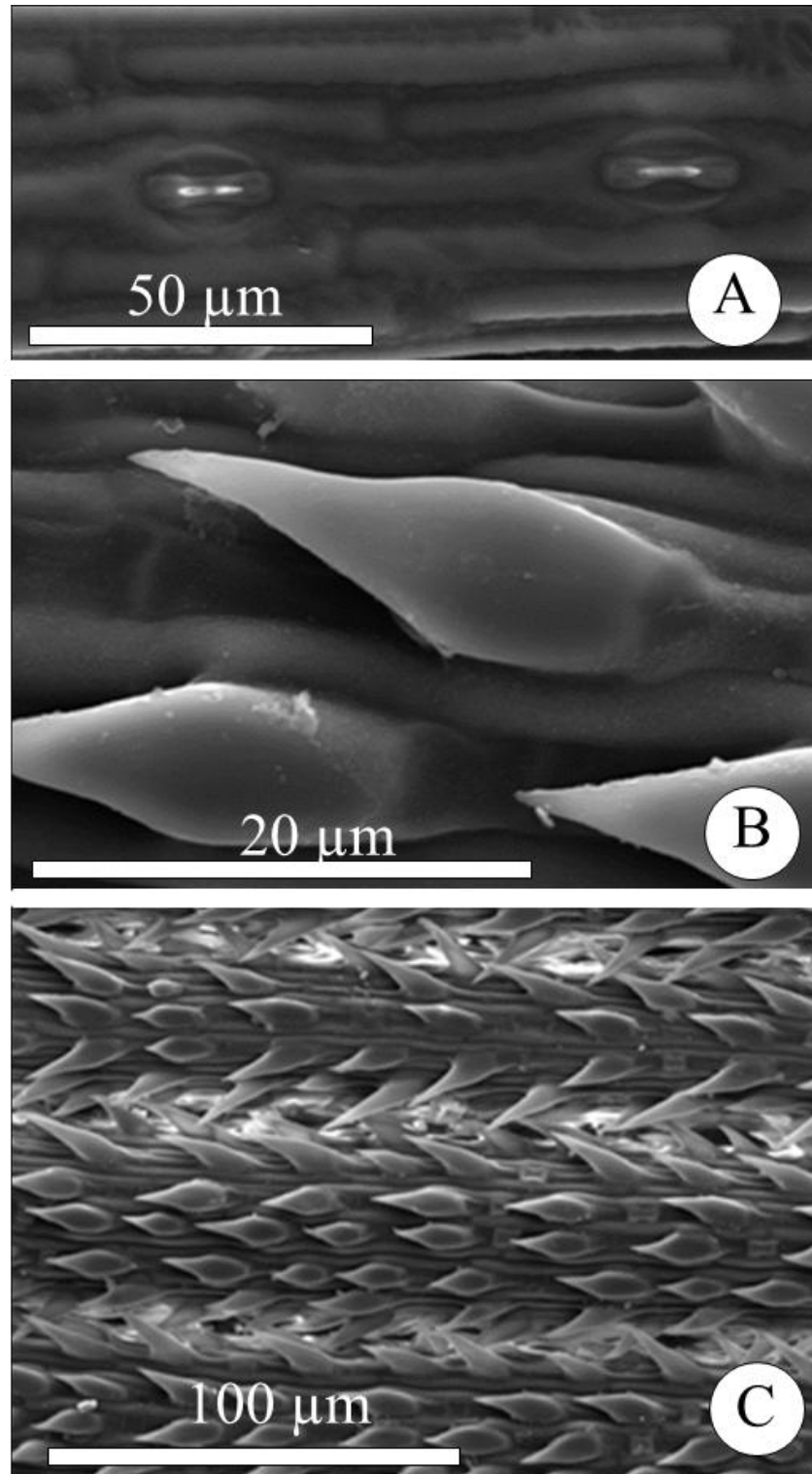


Figure 2. Stomata of the leaves of *Eragrostis plana* (A). Tector trichomes (B). Front view of the adaxial epidermis under scanning electron microscopy, emphasizing the high density of trichomes (C).

digest the thick cell wall of the bundles to access the nutrients in the inner cells, which contain more than 50%

of the carbohydrates and proteins in leaf reserves (Wilson, 1994). There are two distinct sizes of these

collateral vascular bundles (Figure 1A). In tropical grasses, the epidermis is tightly adhered to the rest of the leaf by a girder like structure, formed by portions of sclerenchyma, the bundle sheath cells, and by the vascular bundle itself. This structure can be classified as an "I"- or a "T"-shaped sclerenchyma girder (Wilson, 1994). In tough lovegrass, the large vascular bundles and the two last vascular bundles from each side of the wing have an "I"-shaped sclerenchyma girder, that is, they have a sclerenchymatic sheath that extends to the abaxial surface, as well as to the adaxial one. The small bundles, on the other hand, have a "T"- shaped sclerenchyma girder, that is, the sclerenchymatic sheath extends just to the adaxial surface (Figure 1A). The structure of the "T" sclerenchyma girder was checked by Ingram (2010) in *E. dielsii* Pilg., *E. japonica* (Thunb.) Trin., and *E. sessilispica* Buckl., and was used as one of the criteria to differentiate them taxonomically from the other species in this genus.

The girder structure makes it difficult to detach the epidermis from the rest of the leaf, resulting in a higher resistance to mechanical and chemical damage during digestion by ruminants, due to the thickening and lignification of the epidemical cells (Paciullo, 2002); it also reduces the size of the middle lamella space. Digestion resistance is higher in species which have an "I"-shaped sclerenchyma girder, compared to those with a "T"-shaped girder (Wilson, 1994). The sclerenchyma girder in the Eragrostideae tribe is an adaptation to extreme climatic conditions (Renvoize, 1983). In the keel of the tough lovegrass, between 3 and 12 vascular bundles were observed. Only the central bundle showed parenchymatic extension of the cell on the adaxial surface and the others had sclerenchymatic extension of the sheath on the abaxial surface (Figure 1C).

The root of tough lovegrass is polyarc (Figure 3A), with a uniseriate epidermis, lignified exodermis, and U-thickened endodermal cell walls; the cortical parenchyma has large areas for storage of air, forming the aerenchyma (Figure 3B and D). The parenchymatic pith has cells with plastids which store starch (Figure 3C). Aerenchyma is the term given to plant tissues containing enlarged gas spaces exceeding those commonly found as intracellular spaces. It is formed in the roots and shoots of wet land species and in some dry land species in adverse conditions, either constitutively or because of abiotic stress. While usually associated with hypoxia resulting from water logging, it may also be caused by other forms of stress, including high temperatures, drought, and nutrient deficiency (Evans, 2003). The aerenchyma provides not only an internal pathway for O₂ transfer, but also simultaneously reduces the number of O₂⁻ consuming cells, a feature that might assist in low O₂ environments (Drew et al., 2000). Several reports confirmed the formation of aerenchyma in grasses. The corn (*Zea mays* L.) forms this tissue in response to soil compaction (Bergamin et al., 2010); in *Cynodon dactylon*

(L.) Pers. it is an anatomical adaptation to salinity (Hameed et al., 2010), whereas in the wheat (*Triticum aestivum* L.), aerenchyma formation occurs under flooding conditions (Malik et al., 2003). The tough lovegrass plants used in this study were not found in flooded or compacted soil. Although the presence of aerenchyma in tough lovegrass is likely to be an adaptation to soil conditions, the developmental mechanism and associated factors remain unknown for this species.

The cortex is the principal storage region for starch in monocotyledonous roots; species of the genus *Glyceria*, for example, have cortex cells which store starch in abundance (Soper, 1959). In tough lovegrass starch was found in the pith cells. The presence of pith in the roots of grasses is common (e.g., in *Molinia caerulea* (L.) Moench (Jefferies, 1916), *Saccharum munja* Roxb. (Rahar et al., 2011), *Spartina densiflora* Brong. (Perazzolo and Pinheiro, 1991), and *C. dactylon* (Hameed et al., 2010)). However, starch presence in this structure is unusual. Vetiver grass (*Vetiveria zizanioides* L. Nash, Poaceae) has a root system similar to tough lovegrass, with aerenchyma and starch storage in the roots (Delistoianov and Toledo, 1960), and is able to withstand extreme climate conditions: dryness, long periods of flooding, temperatures ranging from -10 to 48°C, soil acidity, and alkalinity (Mickovski and Van Beek, 2009). In the amyloplasts of the non-photosynthetic tissues, like the roots, starch is accumulated over long periods and is mobilized when necessary for growth (Ong et al., 1994). Reserve carbohydrates are used to support respiration and growth when leaf area and photosynthesis are low; these are then reconverted into sucrose and translocated to meristematic areas for growth (Nelson, 1995). Overall, the root anatomical structure of tough lovegrass, with aerenchyma and starch reserves, is one of the features which confirms its adaptation to poor and compacted soils, as well as its tolerance to frost and herbivory.

Histochemical tests indicated the presence of total lipids in the leaf cuticle. In the root, lipids were observed in the exodermis and endodermis. From the phenolic compounds evaluated, phenol and lignin were found in the leaf and in the root, but the results were negative for tannin. In the leaf, phenols were observed in the bundle sheath cells and in the palisade parenchyma, while lignin was detected in the fibers of the vascular bundles and the vessel elements. In the root, phenol was present in the intercellular spaces and lignin was identified in the vascular bundles (Table 1). Phenolic compounds are known to be related to plant protection from desiccation and herbivory (Daniel et al., 1999). The phenols act to protect the cellular structure against excessive ultraviolet radiation and maintain protoplast integrity in stressful hydric situations (Taiz and Zeiger, 2013). Lignin, a phenolic compound, is resistant to pathogens and reduces the digestibility of dry matter (Silva et al., 2005).

The reaction for alkaloid detection was positive in the

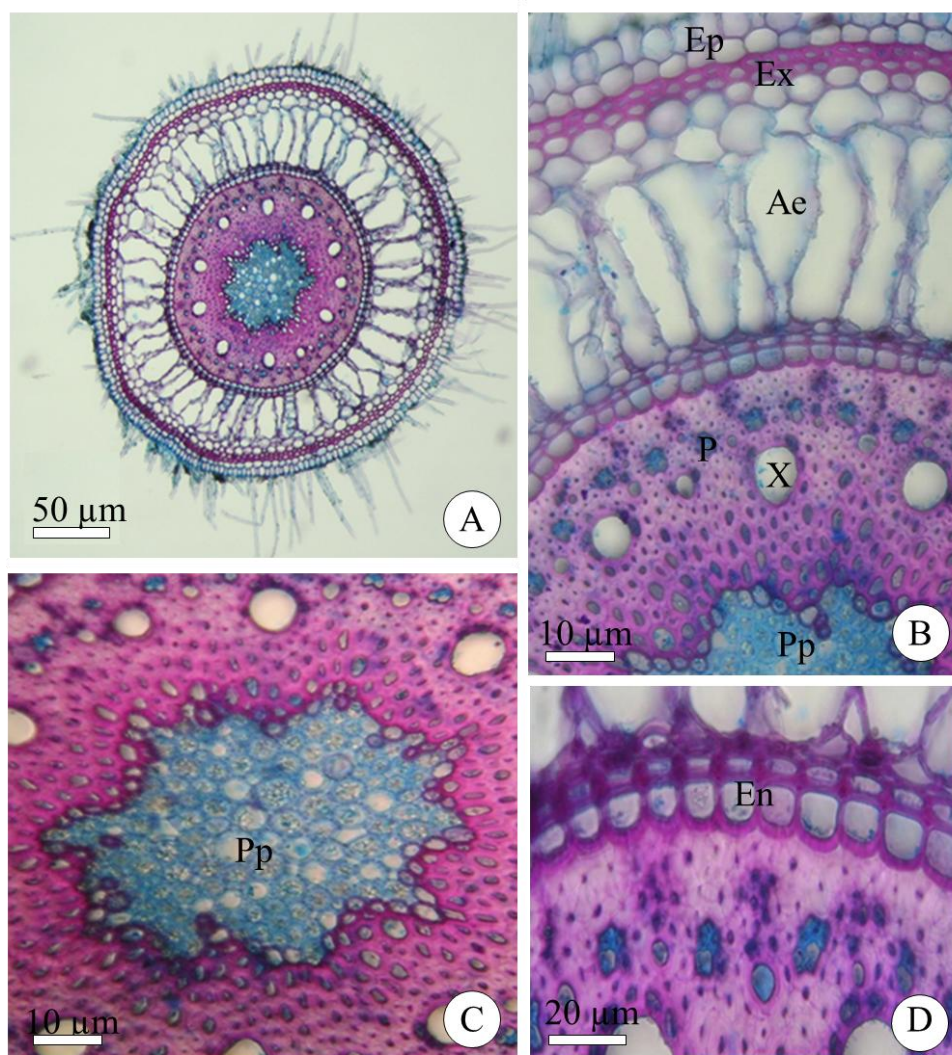


Figure 3. Transverse section of the whole root of *Eragrostis plana* (A). Parenchymatic pith (C). Endodermis (D). Ae - aerenchyma; En - endodermis; Ep - epidermis; Ex - exodermis; P - phloem; Pp - parenchymatic pith; X - xylem (B).

leaf and in the root, showing the presence of these compounds along the vascular bundles in both organs (Table 1). In *E. tremula* the presence of alkaloids was also verified (Ogie-Odia et al., 2010). Most alkaloids are believed to function as defense against predators (Taiz and Zeiger, 2013), besides their allelopathic potential (King and Ambika, 2002). The reaction to detect starch was negative in the leaf, but positive in the root in the pith (Table 1), confirming the results of anatomical analysis described above. With the exception of starch, which was absent in the leaf and present in the root, histochemical analysis did not detect any difference between the two organs. The presence of phenols and alkaloids in tough lovegrass can be associated with its allelopathic effect. Continued studies into the phytochemical aspects of this grass are important in order to determine which chemical

groups are related to the defense mechanisms of the plant, taking into consideration the fact that field observations have shown no attacks by predators or other natural enemies.

Conclusion

Anatomical analyses show that the tough lovegrass leaves are amphistomatic with paracytic stomata, and the epidermis has lignified cells, a Kranz structure, collateral vascular bundles of two sizes, tectors, unicellular and crystalline trichomes. The roots are polyarc with air gaps on the cortical parenchyma, U-thickened endodermal cell walls and a parenchymatic pith with starch storage cells.

Tough lovegrass presents phenols, alkaloids and lignin

Table 1. Results of histochemical tests performed on the leaves and roots of *E. plana*.

Metabolic group	Color observed	Organ	Result	Tissue/anatomical structure
Total lipids	Red	Leaf	+	Cuticle
	Red	Root	+	Exodermis, endodermis
Phenols	Black	Leaf	++	Bundle sheath cells, palisade parenchyma
	Black	Root	+	intercellular spaces
Tannins	---	Leaf	-	---
	---	Root	-	---
Lignin	Red	Leaf	+	Vascular bundles, vessel elements
	Red	Root	+	Vascular bundles
Alkaloids	Brown	Leaf	++	Vascular bundles
	Brown	Root	++	Vascular bundles
Starch	Violet	Leaf	-	---
	Violet	Root	+	Pith

Negative reaction (-); positive reaction (+); strongly positive reaction (++); not observed (---).

in the leaves, aerenchyma and starch in the parenchymatic pith of roots. This anatomical and histochemical traits suggest adaptive features that can explain its persistence under biotic and abiotic stress.

Conflict of Interest

The authors have not declared any conflict of interests.

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