

# Morphoanatomic characterization of *Gomphrena perennis* and *Gomphrena pulchella* leaves

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**Abstract:** **Background:** *Gomphrena perennis* (Gpe) and *G. pulchella* (Gpu) are perennial glyphosate-tolerant amaranthaceous weeds of central and northern Argentina where glyphosate-resistant soybeans are grown with no-till. The study of weeds leaves morphoanatomy is important to understand environmental adaptation and could be used to explain herbicide absorption. Leaf surfaces of *Gomphrena* species were previously described for phylogenetic studies, and morphoanatomy of two *Gomphrena perennis* populations was characterized for glyphosate tolerance. There are no reports on comparative studies of Gpe and Gpu. We hypothesize that these two species differ in their morphoanatomical characteristics.

**Objective:** The aim of this research was to characterize and compare their leaf morphoanatomy throughout their life cycle.

**Keywords:** Lámina total; Epidermis; Mesófilo; Estomas; Tricomas

**Methods:** Fully developed leaves of the upper middle third were extracted from plants grown in pots in a greenhouse at three phenological stages: eight true leaves (S1), beginning of branching (S2), and full flowering (S3). In both leaf sides, densities of epidermal cells, stomata and trichomes, and ostiole lengths were quantified. In main and secondary bundle anatomic parameters were measured: thickness of total blade, upper and lower epidermis, and mesophyll, and sheath length.

**Results:** Both species are amphistomatic. Gpu had higher stomata densities in lower epidermis than Gpe at S1. Gpu had higher trichome densities than Gpe, whereas the latter presented higher values in anatomic variables mainly in secondary bundle (total blade and total mesophyll) at all stages.

**Conclusions:** There were no marked differences in the morphoanatomy between the two species throughout their life cycle.

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## 1. Introduction

The genus *Gomphrena* is one of the largest in the family Amaranthaceae, including approximately 120 species (Bena, 2017), distributed mainly in South America (Mussury et al., 2006), Australia and the Indo-Malayan region (Oliveira, 2018) with predominance in the Central Andes of Argentina and Bolivia, and in the Cerrado habitats in Brazil (Bena, 2017). According to Borsch et al. (2001) cit. by Carvalho (2005), the Amaranthaceae family is dominant in arid and semiarid ecosystems due to morphological and anatomical characteristics that allow them to survive in unfavorable environments: C4 photosynthetic metabolisms (Oliveira, 2018; Carvalho et al., 2015), specialized roots systems like xylopodium, dense pubescence in aerial parts, stems and leaves senescence in dry seasons, and thick cuticles (Carvalho, 2015).

In Argentina, 22 species of *Gomphrena* grow in temperate and warm subtropical areas, ranging from the northern part of the country to northern Patagonia, with a great diversity of habitats (Acosta, 2012). *Gomphrena perennis* L. var. *perennis* and *G. pulchella* Mart. ssp. *albisericea* (E. Holzh.) Pedersen, are two perennial species, considered glyphosate tolerant weeds whose increase in central and northern Argentina is attributed to the use of glyphosate resistant soybean cultivars in direct sowing (Rainero, 2008; Olea, 2013). *G. perennis* L. is an erect plant up to 1 m high. It has cylindrical stems somewhat thickened at the nodes, striated, and pubescent. Leaves are opposite, lanceolate, ovate-lanceolate, with acute apex and short petiole, few hairs in upper surface and very pubescent on the underside. It has globose inflorescences, single or branched, with small, yellowish-white hermaphrodite flowers. The fruit is a small utricle containing one seed that serves as a propagation organ (Burkart, 1987; Acosta et al., 2018).

*G. pulchella* Mart. is an erect plant, with deep taproot, stems 30-50 cm high, hairy, and with a woody base. Leaves are opposite, lanceolate, mucronate, with short petiole, and densely pubescent at the back. Inflorescences in dense round clusters of pink-purple colored flowers on long peduncles. The fruit is a one-seeded utricle (Carrizo e Isasmendi, 1998).

Leaves anatomical characteristics are useful to infer adaptations to a specific environment, due to their relationship with functional parameters like photosynthesis

(Carvalho et al., 2015). Presence of stomata within a species can be modified by light intensity, leaf thickness, photosynthetic capacity, and stomatal conductance. Stomata in abaxial leaf side favor plants growing in habitats with low relative humidity and high temperatures, by reducing water vapor losses, because of lower temperatures. On the other hand, stomata in both sides allow easier CO<sub>2</sub> diffusion, and this is characteristic of plants with high photosynthetic capacity growing in environments with elevated luminosity (Carvalho et al., 2015). Furthermore, morphological and anatomical characteristics of leaves can affect herbicide absorption, by exclusion from the leaf area, depending on number and size of stomata and trichomes in the leaves (Carbone, 2015). Under water deficit conditions, an increase in cuticle thickness can difficult herbicides absorption (Dall'Armellina, Zimdahl, 1989).

Morphoanatomical characteristics of epidermis, wax deposits, and trichome and stomata densities behave as physical or mechanical barriers for the absorption and penetration of glyphosate (Acosta et al., 2018). Hence, knowledge of morpho anatomical characteristics of leaves is a useful tool to evaluate plant adaptability to environments and for weed management in crops.

The aim of this research was to characterize the morpho anatomy of the leaves of these two weed species at three phenological stages.

## 2. Materials and Methods

The trial was conducted in the locality of El Zanjón (27°46'60"S and 64°16'00" W). Seeds *G. pulchella* were collected at Quimili, *Departamento Moreno* (27°38'46" S 62°24'47" W), and seeds of *G. perennis* were collected at Bandera, *Departamento Belgrano* (28°54'10" S and 62°16'00" W). Seeds of both species were collected in 2015. Seeds were sown in multicell trays and at the stage of two true leaves were transplanted to pots with soil (sandy loam textured soil, pH: 7.1; %MO: 2.2; CE 0.28 dSm<sup>-1</sup>). Twenty pots were established for each sampling of the following phenological stages: S1 (early vegetative-eight true leaves), S2 (late vegetative-beginning branching), and S3 (reproductive-full flowering). The minimum and maximum air temperatures were registered with data logger. For photoperiod, data from National Institute

of Agricultural Technology – INTA nearest experiment station were used. At each stage, a pool of fully developed leaves from the middle third of the plant was sampled (Catan et al., 2017). After collection, samples were fixed with a solution of ethanol and acetic acid in the ratio 3:1 (Carnoy). Dates of sowing and sample collection, data on temperatures and daylength are included in Table 1.

To obtain upper and lower epidermis, leaf blades were digested in aqueous 50% sodium hypochlorite, then washed repeatedly with distilled water, and clarified with chloral hydrate 5% aqueous solution (Johansen, 1940). A total of 5 samples of each upper and lower epidermis of individual leaves were mounted with a solution of gelatin-glycerin in slides and coverslips (Johansen, 1940). From each holder glass, 6 random fields were selected for observations, readings, and counting. A total of 30 microscopic fields were read for each epidermis and treatment. The following parameters were observed and quantified: epidermal cell densities (EpCed), stomatal densities (Sd), trichomes densities (Td), and ostioles lengths (OL). All observations and measurements were made with a microscope with image digitizer Motic BA210 Digital (Motic China Group., Ltd. (China).

To observe cross-sections of Blades, the Dizeo de Strittmatter (2000) technique was followed, to prepare 5 slides, 5 paraffin blocks from each species and stage were obtained. Cuttings were made with a rotary microtome at 12 mm thickness. Sections were stained with safranin-fast green. A total of 30 fields were read for each species and stage. Observations and quantifications were made on the following parameters: thickness of total blade (BT), upper and lower epidermis (UEp and LEp), mesophyll (MT), sheath length of secondary bundle (SB2), and collenchyma (C). All data were analyzed with a t-test for independent samples (Di Rienzo et al., 2017).

## 3. Results and Discussion

### a. - Epidermic variables

Values of morphological characteristics in adaxial and abaxial leaf sides at different phenological stages, can be seen in Table 2. The only difference in epidermal cell densities, was found in upper epidermis at S1, where Gpe had higher values than Gpu (359.51 and 295.80 cells mm<sup>-2</sup>).

**Table 1** - Dates of sowing, emergence, and leaves collection in *G. perennis* (Gpe) and *G. pulchella* (Gpu). Temperatures and photoperiod at sampling dates

|             | Sowing     | Emergence  | S1: 8 true leaves | S2: beginning branching | S3: full flowering |
|-------------|------------|------------|-------------------|-------------------------|--------------------|
|             | 01/15/2018 | 01/17/2018 | 02/06/2018        | 02/20/2018              | 03/28/2018         |
| T° max      | 31.8       | 28.8       | 40                | 36.4                    | 31.9               |
| T° min      | 16.3       | 20.7       | 19.4              | 18.3                    | 10.6               |
| T° mean     | 24.1       | 24.8       | 29.7              | 27.4                    | 21.3               |
| Photoperiod | 13.59      | 13.56      | 13.17             | 12.83                   | 11.82              |

**Table 2** - Upper and lower epidermis (UEp, LEp) epidermal cells densities (Ced), stomatal densities (Sd), ostiole lengths (OL), and trichome densities (Td) evaluated at stages S1, S2, and S3 in *G. perennis* (Gpe) and *G. pulchella* (Gpu). Densities and length values expressed in mm<sup>-2</sup> and µm, respectively

| Variable | S1      |         | S2      |         | S3      |         |
|----------|---------|---------|---------|---------|---------|---------|
|          | Gpe     | Gpu     | Gpe     | Gpu     | Gpe     | Gpu     |
| UEp-Ced  | 359.51a | 295.80b | 237.53a | 246.42a | 279.01a | 317.04a |
| LEp-Ced  | 208.89a | 380.74a | 232.59a | 246.91a | 275.56a | 289.88a |
| UEp-Sd   | 106.67a | 105.68a | 75.56a  | 73.09a  | 97.78a  | 101.73a |
| LEp-Sd   | 64.20a  | 112.59b | 66.17a  | 72.59a  | 76.54a  | 80.00a  |
| UEpOL    | 11.71a  | 12.30a  | 14.17a  | 15.53b  | 13.08a  | 12.77a  |
| LEpOL    | 12.71a  | 12.50a  | 14.67a  | 15.70b  | 12.55a  | 12.37a  |
| UEpTd    | 5.05a   | 9.27b   | 5.66a   | 7.03b   | 9.08a   | 9.33a   |
| LEp-Td   | 14.86a  | 15.08a  | 8.84a   | 9.14a   | 5.96a   | 10.95b  |

For each variable and phenological stages, means followed by different letters, differ by the t-test at  $p \leq 0.05$ .

Gpu had higher stomata densities in lower epidermis than Gpe at S1 (112.59 and 64.20 stomata mm<sup>-2</sup>, respectively). Both species are amphiestomatic (having stomata in the two leaf sides), like other species of *Gomphrena* such as *G. arborescens* (Carvalho, Ribeiro, 2005), *G. pohlii*, *G. virgate*, and *G. globosa* (Carvalho et al., 2010).

Stomatal densities (stomata mm<sup>-2</sup>) in both epidermises to Gpu and Gpe were higher than those reported by Carvalho and Ribeiro (2005) in *G. arborescens* (44.47 and 40.31 in upper and lower epidermis, respectively). However, Frank de Carvalho et al. (2010) studying *Gomphrena* adult species from Cerrado in Brazil determined higher stomatal densities in *G. arborescens*, *G. pohlii* and *G. globosa* in upper epidermis (138, 136, and 137 stomata mm<sup>-2</sup>) and lower epidermis (140, 126 and 113 stomata mm<sup>-2</sup>). In *G. virgate* they found lower values for upper epidermis (32 stomata mm<sup>-2</sup>) and lower epidermis (45 stomata mm<sup>-2</sup>), due to higher stomata sizes. Large stomata in low densities is usually associated to adaptation to drought and high luminosity conditions (Carvalho, Ribeiro, 2005).

Species from Cerrado have amphiestomatic leaves, intermediate leaf thickness (100-500 µm), and similar stomatal densities in both leaf surfaces. These are considered an adaptation to increase stomatal conductance, associated to C4 species with high photosynthetic rates (Carvalho et al., 2010). In our trials, where plants were grown without water restrictions, stomatal densities were higher (Table 2). Stomatal density values were similar for both species, and did not show differences in UEp-Sd in the stages evaluated; and were generally lower in LEp, except for Gpu in S1. Values are intermediate between those determined by Carvalho et al. (2010) and those of Carbone (2015), who, studying stomatal densities of both leaf sides in biotypes of Gpe growing in field conditions, found lower values in plants in Santiago del Estero (40 and 38 stomata mm<sup>-2</sup>) as compared to those in Córdoba

(54 and 36 stomata mm<sup>-2</sup>). Carbone (2015) concluded that this was an adaptive response to avoid water losses.

Acosta et al. (2018) state that lower stomatal density in adaxial side determines the low sensitivity of *G. perennis* to glyphosate. Likewise, Procopio et al. (2003) in weed species like *Galinsoga parviflora*, *Conyza bonaerensis* and *Ipomoea cairica*, considered a potential barrier to penetration of herbicides applied to the canopy, the low stomatal densities in the adaxial side, that were of (48, 42 and 47 stomata mm<sup>-2</sup>) in the adaxial side and of (156, 162 and 143 stomata mm<sup>-2</sup>) in the abaxial side.

Ulguin et al. (2017), in studies on *Eleusine indica*, found that characteristics of surface and leaf anatomy may affect the effectiveness of glyphosate absorption by weeds. Features such as composition of the cuticle, number of stomata, and trichomes interfere with the foliar absorption of herbicides and may indicate auxiliary resistance mechanisms. Thus, weeds anatomy characteristics may influence resistance to herbicides, and therefore their determination is important.

Weeds in general are amphiestomatic and, in most of them, higher stomatal densities occur in the abaxial leaf side (Ferreira et al., 2002). Amphiestomatic leaves are considered an adaptation to increase stomatal conductance, which associated with high photosynthetic rates favor accumulation of CO<sub>2</sub> in periods of water availability. Besides, it allows them to successfully establish in environments with high solar radiation (Carvalho, Ribeiro, 2005).

Gpu had higher ostioles lengths than Gpe in both leaf sides at S2 (15.53 µm UEpOL, and 15.70 µm LEpOL).

Also, the two species under study had trichomes at each leaf side, and this could be a barrier to herbicide penetration, according to Carbone (2015). Trichome densities were higher than Gpe in upper epidermis for Gpu at S1 and S2 (9.27 and 7.03 trichomes mm<sup>-2</sup>, respectively), and higher in lower epidermis at S3 (10.95 trichomes mm<sup>-2</sup>) (Figure 1a and 1b). Presence of trichomes

in both leaf sides would indicate adaptation to dry environments and may prevent herbicides from reaching epidermis (Ferreira et al., 2002). Trichome densities

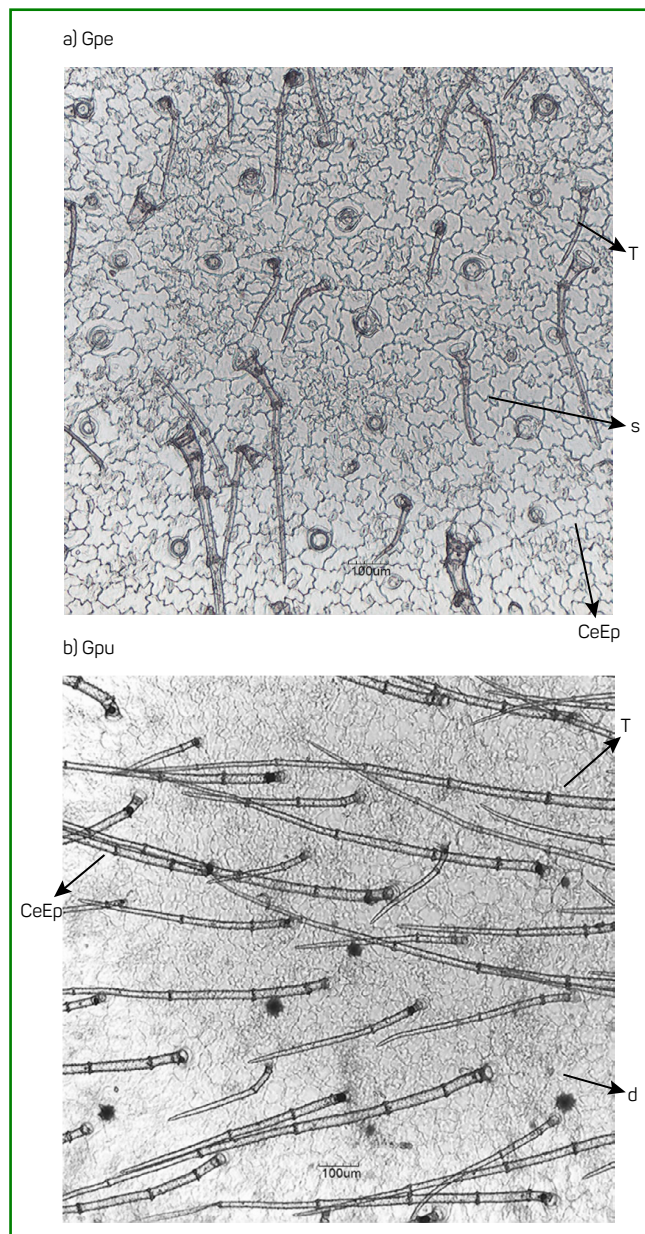
(trichomes  $\text{mm}^{-2}$ ) found at S3 in Gpe (9.08 and 5.96 trichomes  $\text{mm}^{-2}$ ) and Gpu (9.33 and 10.95 trichomes  $\text{mm}^{-2}$ ), were similar to densities in *G. arborescens* (5 and 5 trichomes  $\text{mm}^{-2}$ ) and *G. pohlii* (5.9 and 6.22 trichomes  $\text{mm}^{-2}$ ), lower than densities in *G. globosa* (16.5 and 28.05 trichomes  $\text{mm}^{-2}$ ), and higher than those of *G. virgata* (2.6 and 1.4 trichomes  $\text{mm}^{-2}$ ), in adaxial and abaxial sides, respectively (Carvalho et al., 2010). Carbone (2015), working with *G. perennis* biotypes, determined densities in upper and lower epidermis of 23.9-31.9 and 40-50 trichomes  $\text{mm}^{-2}$ , respectively, in plants collected in rainfed in Santiago del Estero, whereas in those collected in Córdoba, values were of 7.5-11.5 and 8.3-11 trichomes  $\text{mm}^{-2}$ . Acosta et al. (2018) and Carbone et al. (2021), state that in Gpe, presence in abundance of trichomes on both leaf sides associated to teicodes, constitute a barrier to chemical control of the species. There are no data on trichome density on Gpu under field conditions, where growing environment could modify trichome number, and thus affect the efficacy of its control.

#### b. - Anatomic variables in main bundle

Thickness values of anatomic variables in main bundle are shown in Table 3. There were significant differences in upper epidermis, with higher values for Gpu at S2 (20.56  $\mu\text{m}$ ), and for Gpe at S3 (28.83  $\mu\text{m}$ ). Lower epidermis values were higher for Gpu at S2 (18.61  $\mu\text{m}$ ). Gpe showed higher values for leaf blade at S1 (572.75  $\mu\text{m}$ ) and S2 (642.30  $\mu\text{m}$ ). Mean total blade thickness in Amaranthaceae ranges between 100-500  $\mu\text{m}$ . However, Gpe at S1 and S2 was above this range. Both, Gpe and Gpu showed higher thickness than other weed species of the family, such as *Amaranthus deflexus* (149.50  $\mu\text{m}$ ), *A. spinosus* (264.34  $\mu\text{m}$ ), *Alternanthera tenella* (252.39  $\mu\text{m}$ ) (Ferreira et al., 2003), *Amaranthus hybridus* (220  $\mu\text{m}$ ) (Silva et al., 2018), and weeds of other families (Ferreira et al., 2002a; 2002b). Upper and Lower Epidermis thickness values in general were similar in the two species studied, as it was observed in *A. spinosus*. However, in *A. deflexus* and *A. tenella* upper epidermis thickness values were higher (17.93  $\mu\text{m}$  and 18.80  $\mu\text{m}$ ) than lower epidermis values (13.91  $\mu\text{m}$  and 7.92  $\mu\text{m}$ ) (Ferreira et al., 2003).

#### c. - Anatomic variables in secondary bundle

Thickness values of anatomic variables in SB2 are presented in Table 4. Gpe had higher values than Gpu, for lower epidermis at S2 (27.35  $\mu\text{m}$ ), for BT (190.22 to 264.27



**Figure 1** - Lower epidermis 4X of a) Gpe and b) Gpu at S3, CeEp (epidermal cells), T (trichomes), s (stomata) and d (druses).

**Table 3** - Upper and lower epidermis (UEp and LEp) and thickness of total blade (BT), at stages S1, S2, and S3 in *G. perennis* (Gpe) y *G. pulchella* (Gpu) in main bundle. Values expressed in  $\mu\text{m}$

| Variable | S1      |         | S2      |         | S3      |         |
|----------|---------|---------|---------|---------|---------|---------|
|          | Gpe     | Gpu     | Gpe     | Gpu     | Gpe     | Gpu     |
| UEp      | 15.36a  | 16.91a  | 14.75a  | 20.56b  | 28.83a  | 16.68b  |
| LEp      | 15.31a  | 13.62a  | 15.12a  | 18.61b  | 16.13a  | 14.29a  |
| BT       | 572.75a | 454.72b | 642.30a | 484.54b | 417.51a | 408.65a |

For each variable and phenological stages, means followed by different letters, differ by the t-test at  $p \leq 0.05$

**Table 4** - Upper and lower epidermis (UEp and LEp), thickness of total blade (BT), sheath of secondary bundle (SB2) and total mesophyll (MT) evaluated at stages S1, S2 and S3 in *G. perennis* (Gpe) and *G. pulchella* (Gpu). Values expressed in  $\mu\text{m}$ 

| Variable | S1      |         | S2      |         | S3      |         |
|----------|---------|---------|---------|---------|---------|---------|
|          | Gpe     | Gpu     | Gpe     | Gpu     | Gpe     | Gpu     |
| UEp      | 31.49a  | 30.27a  | 26.93a  | 24.80a  | 24.25a  | 24.40a  |
| LEp      | 29.33a  | 25.50a  | 27.35a  | 22.41b  | 21.66a  | 23.94a  |
| BT       | 245.94a | 198.71b | 264.27a | 177.29b | 190.22a | 181.55b |
| SB2      | 90.19a  | 82.74a  | 110.27a | 72.07b  | 88.85a  | 77.79b  |
| MT       | 230.97a | 143.38b | 208.72a | 132.95b | 145.23a | 134.76b |

For each variable and phenological stages, means followed by different letters, differ by the t-test at  $p \leq 0.05$

$\mu\text{m}$ ) MT (145.23 to 230.97  $\mu\text{m}$ ) at all stages, and for leaf sheath at S2 (110.27  $\mu\text{m}$ ) and S3 (88.85  $\mu\text{m}$ ) (Figure 2a). All values of MT were lower than those of *G. arborescens* (384.40  $\mu\text{m}$ ) (Carvalho et al., 2005). MT values of Gpe at S1 (230.97  $\mu\text{m}$ ) and S2 (208.72  $\mu\text{m}$ ) were higher than those determined by Silva et al. (2018) in *A. hybridus* (160  $\mu\text{m}$ ). Gpu had lower values than Gpe at all stages (Table 4 and Figure 2b).

The study of weeds leaf anatomy is useful to identify and discover structures that could affect herbicide absorption (Procopio et al., 2003). Weed management in arid regions poses higher difficulties due to thickening of epidermis, which confers a barrier to herbicide penetration (Dall'Armellina, Zimdahl, 1989). Santos et al. (2014) postulated that higher thickness of anatomic variables in adaxial leaf side, coupled with higher trichome densities and lower stomatal densities, would be barriers to herbicide absorption.

#### 4. Conclusions

Under the conditions of the experiment (without water stress) we found no marked differences in the morphoanatomy of both species, except for higher trichome densities in upper epidermis in *G. pulchella* at S1 and S2. Gpu had higher stomata densities in lower epidermis than Gpe at S1. Also Gpu had higher ostioles lengths in both leaf sides at S2.

Thickness of mesophyll and total blade were significantly higher in *G. perennis*.

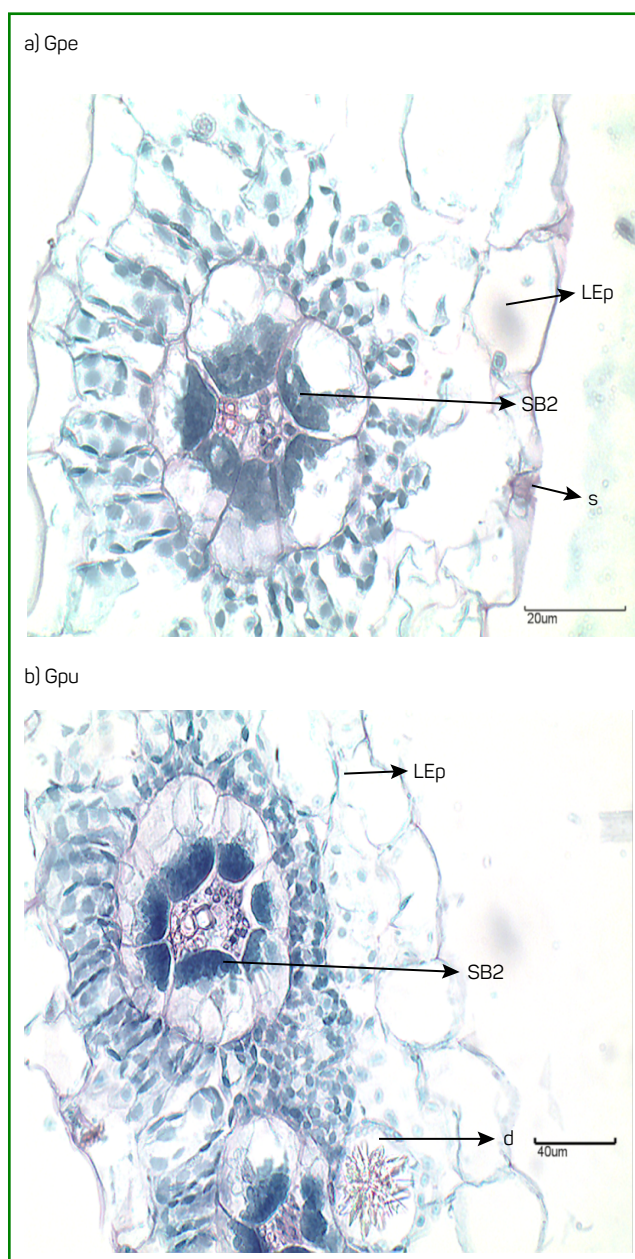
Hairiness in Gpu at early stages could mean a barrier to herbicides

Absence of marked differences between both species would suggest similar behavior in the efficacy of foliar herbicides applications.

It would be necessary to study morphoanatomical characteristics of the two species under field conditions to determine implications on their management.

#### Authors' contributions

MCO, RC and SC: conceptualization of the manuscript and development of the methodology. MCO, AC, GT, and AF: data collection and data analysis. MCO and SC: data interpretation. MCO, RC, and SC: writing the original



**Figure 2** - cross-sections 40X of a) Gpe and b) Gpu at S2, LEp (lower epidermis), s (stomata), d (druse), SB2 (sheath of secondary bundle)

draft of the manuscript. MCO, RC, and GT: writing, review and editing. AC: reading and measurement of anatomic variables in *G. pulchella*. MGT: reading and measurement of anatomic variables in *G. perennis*. AF: preparation and cuttings for laboratory measurements.

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