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Micromorphological Diversity and Taxonomic Significance of the Leaf Epidermis in *Cymbopogon* Spreng. (Poaceae)

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Abstract

The genus *Cymbopogon* (Poaceae: Andropogoneae) comprises a medicinally and ethnobotanically important group of grasses with a wide global distribution, predominantly across tropical and subtropical regions. The present study conducts a comparative micromorphological analysis of the leaf epidermis in five species, *C. citratus*, *C. martini*, *C. nardus*, *C. polyneuros*, and *C. iwarancusa*, to evaluate their taxonomic significance. Detailed examination of foliar traits, including stomatal size, density, index, and shape, as well as epidermal cell number, subsidiary cells, silica bodies, trichomes, papillae, and prickles, was performed on both adaxial and abaxial surfaces. Marked interspecific variations were recorded in stomatal dimensions, density, and distribution patterns. *C. polyneuros* exhibited the largest stomata (82.43 ± 1.79 µm × 51.44 ± 1.33 µm) with a stomatal index of 28.57, whereas *C. iwarancusa* showed the smallest stomata (16.78 ± 0.37 µm × 13.39 ± 0.30 µm) and a stomatal index of 35.08. Distinct variations in silica body morphology, trichome types, and papilla distribution further differentiated the species. The observed micromorphological attributes serve as reliable diagnostic characters of high taxonomic value and also indicate adaptive responses to diverse ecological conditions.

Keywords: *Cymbopogon*; Leaf micromorphology; Poaceae; Andropogoneae

Introduction:

The genus *Cymbopogon* (family Poaceae) comprises more than 55 species natives to tropical and subtropical regions of Asia and is also distributed across South and Central America, Africa, and other tropical countries. These are citrus-scented, tufted, perennial C4 grasses characterized by multiple stiff culms emerging from a short rhizomatous base (Weiss, 1997). The plant parts can be dried and powdered or consumed raw. The generic name *Cymbopogon* is derived from two Greek words, "Kymbe," meaning "boat," and "Pogon," meaning "beard." The term "boat" refers to the boat-shaped spatheoles of the inflorescence, while "beard" refers to the awns of the spikelets (Shah et al., 2011). These grasses thrive in warm and humid environments and typically grow erect in dense clumps.According to Bor (1960), 34 species are recognized within this genus, which belongs to the tribe Andropogoneae in the grass flora. In addition to morphological investigations of the genus (Kabeer & Nair, 2009; Sreekumar & Nair, 1991), most previous studies have emphasized molecular (Shah et al., 2011), genetic (Baruah et al., 2016; Kumar et al., 2009), and anatomical aspects of Poaceae members. Metcalfe (1960) identified distinctive anatomical features such as epidermal cell types, stomatal complexes, and the arrangement of sclerenchymatous tissues around the vascular bundles of leaves. Ellis (1979) developed a standardized approach for comparative leaf anatomical studies in Poaceae, introducing a unified method for describing grass leaf epidermal anatomy. His system provided descriptive keys, definitions, and illustrations for approximately 340 micromorphological characters visible in surface view, facilitating standardization and simplification of epidermal anatomy descriptions.Franks and Beerling (2009) examined variations in stomatal size and density over geological timescales and demonstrated their influence on maximum leaf conductance under changing CO_2 concentrations. Their findings highlighted the functional significance of stomatal dimensions, showing that leaves with higher stomatal densities can achieve greater CO_2 uptake rates due to spatial constraints governing stomatal packing.Chauhan et al. (2011) investigated the diversity, distribution, and frequency characteristics of *Arundo donax* phytoliths. Their study revealed a wide variety of opal phytolith morphotypes differing in size, shape, and anatomical position within leaves and stems. Distinctive phytolith forms such as bilobate, trapezoid, prickly microhairs, long microhairs, epidermal long cells, stomata, bulliform, parallelepipedal, and knobbed spine types serve as valuable taxonomic markers for grasses.Previous studies have reported that silica bodies in *Cymbopogon* are predominantly cross- or dumbbell-shaped (Folorunso & Oyetunji, 2007; Krishnan et al., 1999). However, despite these earlier investigations, comparative analyses of micromorphological features across *Cymbopogon* species remain limited. The present study addresses this gap by documenting and comparing the diagnostic micromorphological traits of leaf epidermis among *Cymbopogon* taxa occurring in Tamil Nadu and provides an identification key for the species examined.Previous research revealed that *Cymbopogon*'s silica bodies are predominantly cross-to dumbbell-shaped (Folorunsa & Oyetunji, 2007; Krishnan *et al.,* 1999). Despite all the prior research, much has not been done to compare the micromorphological characteristics of the genus *Cymbopogon*. The identification key for the taxa is also provided in the current study, which exposes the diagnostic micromorphological traits of the leaves of the *Cymbopogon* taxa found in Tamil Nadu.

Materials and Methods:

For light microscopy research, the six grass species and floral components were collected from various locations in Tamil Nadu between May and March 2021. The obtained plant specimens were pressed, recorded, dried, identified, and mounted on typical herbarium sheets. (Provide all the details of geo coordinates, voucher specimen numbers etc. Table 5).

Leaf Epidermal Studies:

The modified Clark, 1960 methodology was used to prepare the leaf samples. To prevent drying, fresh leaves were removed from plants that were actively growing and submerged in water for two hours. These leaves' upper and lower epidermis had epidermal strips that could be removed by simple peeling, applying nail polish, or scraping techniques. Sometimes the leaves with thick venations were fixed in a 1:1 mixture of nitric acid and chromic acid before being promptly preserved in 70% alcohol. Slides for the microscopic examination were made for both the adaxial and abaxial surfaces. For each species, five samples of the adaxial and abaxial surfaces were prepared, and several parameters were evaluated. The epidermal peelings were stained with Toluidine blue 'O' method and Safranin before being mounted in glycerine. Microphotographs were shot with a polarising camera while the peels and imprints were directly examined under a light microscope (Nikon 80i Fluorescence microscope). At various magnifications, various epidermal peel and impression features were examined (4X, 10X, 20X, and 40X objectives). Using the image one program Nis-Elements-BR, micrograph calibration and analysis were performed.

Preparation of stains:

Safranin: 0.5 g (500mg) of Safranin dissolved in 100 ml of absolute ethanol (Ruzin 1999).

Toluidine blue O method: This stain was prepared by dissolving 0.05% of TBO in benzoate buffer (benzoic acid 0.25g and sodium benzoate 0.29g in 200ml of dissolved water at a pH of 4.4) Epidermal peeling was treated with the stain for 2-5 minutes and the excess stain was removed by flooding the peelings in distilled water till the ideal stain is achieved (O'Brien, *et al.,* 1964).

Statistical analysis:

Statistical analysis (Descriptive statistics, ANOVA and Multiple Comparison Tukey test) of lower epidermis stomatal length and width and Upper epidermis stomatal length and width were performed using SPSS software comparison among means were done considering statistically significant differences is $P \leq 0.05$ (Hirotsu, 2017).

Stomatal index SI = S/E+S×100 (Poole & Kurschner, 1999)

SI = Stomatal index

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S = Number of Stomata present per unit area (Stomatal density).
E = Number of Epidermal cells present per unit area (Epidermal density).

Mean $M = \Sigma(X)/N$

ΣX = Sum of observations
N = Number of observations

Standard deviation $Sd = \sqrt{S^2}$

S^2 = Variance of observations

Variance $S^2 = \Sigma(X-M)^2/n-1$

n = Number of observations

Standard Error $SE = Sd/\sqrt{n}$

Sd = Standard deviation
n = Number of observations

Table 1: Abaxial leaf epidermal features of *Cymbopogon* species

Epidermal surface characters		*Cymbopogon polyneuros*	*Cymbopogon citratus*	*Cymbopogon iwarancusa*	*Cymbopogon nardus*	*Cymbopogon martini*
Abaxial	Stomatal Length average of 50 cells	82.4 µm	17.68 µm	16.77 µm	17.69 µm	54. 03 µm
	Width average of 50 cells	51.43 µm	13.18 µm	13. 39 µm	14.37 µm	33. 81 µm
	Stomatal type & Shape	Paracytic Dome	Paracytic Dome	Paracytic Dome	Paracytic Dome	Paracytic Dome
	Number of Stomata present in per square millimeter (5 samples)	228 ± 2.54	357 ± 2.73	380 ± 2.12	470 ± 1.58	450 ± 1.58
	Stomatal index	28.57	30.18	35.08	37.96	38.46
	Number of stomatal rows 2 veins	1 band	4 banded between the veins	2-3 banded Between the veins	3 banded between the veins	2 banded between the veins
	Number of epidermal cells present in per square millimeter (5 samples)	570 ± 2.23	826 ± 3.67	703 ± 2.73	768 ± 2.12	720 ± 2.12
	Subsidiary cell shape	Parallel	Parallel	Parallel	Parallel	Parallel
	Guard cells shape	Dumbbell shape	Dumbbell shape	Dumbbell shape	Dumbbell shape	Dumbbell shape
	Silica body shape	Bilobate Short cell	Bilobate Short cell and trilobate	Bilobate Short cell and trilobate	Bilobate Short cell	Bilobate Short cell
Trichomes	Micro hairs	Present	Present	Absent	Present	Present
	Papillae	Present	Present	Present	Absent	Present
	Prickle	Present	Present	Present	Present	Absent

Table 2: Adaxial leaf epidermal features of *Cymbopogon* species

Epidermal Surface Characters		*Cymbopogon polyneuros*	*Cymbopogon citratus*	*Cymbopogon iwarancusa*	*Cymbopogon nardus*	*Cymbopogon martini*
Adaxial	Stomatal Length average of 50 cells	76.12 µm	28.54 µm	30.43 µm	23.3 µm	26.04 µm
	Width average of 50 cells	56.39 µm	24.23 µm	25.53 µm	22.91 µm	22.39 µm
	Stomatal type	Paracytic Dome	Paracytic Dome	Paracytic Dome	Paracytic Dome	Paracytic Dome
	Number of Stomata present in per square millimeter number (5 samples)	30 ± 1.58	104 ± 1.22	60 ± 1.58	56 ± 1.58	58 ± 1.22
	Stomatal index	13.04	22.46	11.67	10.72	15.38
	Number of stomatal rows between 2 veins	1 band	2 banded between the veins	vein margin 2 banded	2 banded between the veins	vein margin 2 banded
	Number of epidermal cells present in per square millimeter (5 samples)	200 ±2.12	359 ± 2.23	454 ± 2.23	466 ± 2.34	319 ± 1.58
	Subsidiary cell shape	Parallel	Parallel	Parallel	Parallel	Parallel
	Guard cells shape	Dumbbell shape	Dumbbell shape	Dumbbell shape	Dumbbell shape	Dumbbell shape
	Silica body shape	Bilobate Short cell	Bilobate Short cell	Bilobate Short cell	Bilobate Short cell	Bilobate Short cell
Tricho Mes	Micro hairs	Absent	Present	Absent	Present	Absent
	Papillae	Present	Present	Absent	Absent	Present
	Prickle	Present	Present	Present	Present	Present

Table 3. Stomata Size – from abaxial and adaxial epidermis surfaces of *Cymbopogon* Leaves.

Species	Traits	Mean length ± SE µm	Mean width ± SE µm
Cymbopogon polyneuros	Abaxial epidermis Length & width	82.43 ± 1.79	51.44 ± 1.33
	Adaxial epidermis length & width	76.12 ± 1.49	56.39 ± 1.48
Cymbopogon citratus	Abaxial epidermis Length & width	17.68 ±0.37	13.18 ± 0.36
	Adaxial epidermis length & width	28.55 ± 0.64	24.24 ± 0.67
Cymbopogon iwarancusa	Abaxial epidermis Length & width	16.78 ± 0.37	13.39 ± 0.30
	Adaxial epidermis length & width	30.44 ± 0.66	25.54 ± 0.80
Cymbopogon martini	Abaxial epidermis Length & width	54.04 ± 1.23	33.84 ± 1.11
	Adaxial epidermis length & width	26.04 ± 0.66	22.39 ± 0.79
Cymbopogon nardus	Abaxial epidermis Length & width	17.69 ± 0.33	14.37 ± 0.30
	Adaxial epidermis length & width	23.3 ± 0.72	22.92 ± 0.68

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Table 4. One-way ANOVA (Multiple Comparisons Tukey post hoc test)

Stomata length & width		Sum of Squares	Df	Mean Square	F	Sig.
Lower epidermis Length	Between Groups	177792.239	4	44448.060	932.965	0.01
	Within Groups	11672.217	245	47.642		
	Total	189464.456	249			
Lower epidermis Width	Between Groups	75185.777	4	18796.444	508.731	0.01
	Within Groups	9052.190	245	36.948		
	Total	84237.967	249			
Upper epidermis Length	Between Groups	42581.068	4	10645.267	240.248	0.01
	Within Groups	10855.817	245	44.309		
	Total	53436.885	249			
Upper epidermis Width	Between Groups	98156.680	4	24539.170	621.160	0.01
	Within Groups	9678.825	245	39.505		
	Total	107835.505	249			

Table 5: Locality, Habitat, Elevation, and soil pH of Five *Cymbopogon* species

Taxa	Habitat	Elevation	Soil pH	Locality	Geo-coordinates
Cymbopogon polyneuros	Plains	48 msl	6.2	M.S.U campus, TN	Latitude 8.764303, ongitude 77.651091
Cymbopogon citratus	Grasslands and Forest bufferzone	230 msl	6.13	Sirumalai hills, Dindigul, TN	Latitude 10.217267 Longitude 77.982873
Cymbopogon nardus	Mountain grasslands	580 msl	7.12	Ponmudi, Kerala	Latitude 8.74045 Longitude 77.12377
Cymbopogon iwarancusa	Grasslands and plains	260 msl	7.10	Kallaru, Western Ghats, TN	Latitude 8.803056 Longitude 77.305278
Cymbopogon martini	Rocky slope grasslands	421 msl	7.15	Thullukkarmottai, KMTR, TN	Latitude 8.631767 Longitude 77.306550

Results and Discussion

Micromorphological examination of the leaf epidermis in five *Cymbopogon* species, *C. polyneuros*, *C. citratus*, *C. iwarancusa*, *C. nardus*, and *C. martini*, revealed substantial interspecific variation in both quantitative and qualitative epidermal characters (Tables 1–3). All taxa possessed paracytic stomata with dome-shaped guard cells and dumbbell-shaped subsidiary cells, which are characteristic of Poaceae (Metcalfe, 1960; Folorunso & Oyetunji, 2007; Prasad & Mondal, 2021). However, distinct species-specific differences were noted in stomatal dimensions, density, and surface ornamentation.

Stomata variations

Stomatal length and width varied significantly among species. *C. polyneuros* exhibited the largest stomata (82.43 × 51.44 µm abaxially; 76.12 × 56.39 µm adaxially), whereas *C. iwarancusa* showed the smallest (16.78 × 13.39 µm abaxially; 30.44 × 25.54 µm adaxially). *C. martini* and *C. nardus* displayed intermediate dimensions. ANOVA confirmed significant interspecific differences ($p < 0.01$) in both stomatal length and width on the adaxial and abaxial surfaces (Table 4), indicating that stomatal size serves as a robust taxonomic discriminator within *Cymbopogon* (Saxena et al., 2020).

Stomatal density exhibited an inverse relationship with stomatal size. The highest density occurred in *C. nardus* (470 ± 1.58 mm^{-2}) and *C. martini* (450 ± 1.58 mm^{-2}), while *C. polyneuros* had the lowest (228 ± 2.54 mm^{-2}). Similar size–density trade-offs have been reported in other grasses (Liu et al., 2023; Nunes et al., 2022), representing adaptive strategies that optimize the balance between water conservation and gas-exchange efficiency. Species bearing smaller, denser stomata (*C. nardus*, *C. iwarancusa*) tend to occupy drier, high-elevation habitats with greater vapour-pressure fluctuations, whereas the larger stomata of *C. polyneuros* facilitate higher conductance under moist lowland conditions (Hetherington & Woodward, 2003).

The stomatal index ranged from 28.57 in *C. polyneuros* to 38.46 in *C. martini*, corresponding with their ecological distributions. *C. nardus* and *C. martini*, collected from mountain grasslands and rocky slopes (580 msl and 421 msl; pH 7.12–7.15), possessed smaller but more numerous stomata, indicating xeromorphic adaptations (Table 5). Conversely, *C. polyneuros*, sampled from moist lowlands (48 msl; pH 6.2), exhibited larger and fewer stomata suited to high humidity conditions (Kumar et al., 2021). Such altitudinal trends in stomatal traits are consistent with patterns documented in Poaceae (Wang et al., 2022).

All species were amphistomatous, showing higher stomatal density on the abaxial surface. Amphistomy in grasses is often associated with enhanced photosynthetic performance under high light intensities and open habitats (Mott et al., 2021). However, increased stomatal area also elevates water-loss potential, which is often mitigated by features such as trichomes, papillae, or thickened cuticles. This adaptive combination was particularly evident in *C. citratus* and *C. polyneuros*, both of which exhibited abundant papillae and micro hairs on both leaf surfaces.

Silica Cells

Silica bodies were predominantly bilobate in all taxa, while trilobate short cells occasionally occurred in *C. citratus* and *C. iwarancusa*. Variation in phytolith shape provides valuable taxonomic information for grass systematics (Hošková et al., 2021) and contributes to leaf mechanical strength and herbivore defence (Pokrovsky et al., 2024). The bilobate and trilobate patterns observed here correspond closely with diagnostic features described for *Cymbopogon* and related Andropogoneae genera (Singh et al., 2022).

Epidermal cell variations

Epidermal cell density also varied significantly among the studied species. The highest epidermal cell count per mm^2 was recorded in *C. citratus* (826 ± 3.67), followed by *C. nardus* (768 ± 2.12), suggesting structural reinforcement in species occupying more exposed or arid environments (Chaudhary et al., 2020). Trichomes were present in *C. polyneuros*, *C. citratus*, *C. nardus*, and *C. martini*, but absent in *C. iwarancusa*. These surface appendages, together with papillae and prickle cells, are known to reduce transpiration and provide mechanical protection (Menezes et al., 2022). *C. martini*, which inhabits rocky grasslands, exhibited dense trichomes and papillae, features that likely minimize excessive water loss under intense solar radiation.

The ANOVA results further supported the morphological differentiation among the five species, emphasizing that stomatal attributes can serve as reliable taxonomic markers within *Cymbopogon* (Ahmad et al., 2011; Vivek & Nair, 2021). Nonetheless, as noted by Liu et al. (2023), stomatal traits can display plasticity in response to environmental fluctuations, suggesting that the observed differences may integrate both genetic and ecological influences. Hence, future research should integrate micromorphological data with molecular markers (e.g., *rbcL*, *matK*) and physiological analyses to resolve taxonomic ambiguities and elucidate adaptive mechanisms at the genomic level.

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Key Based on leaf epidermal micromorphological characters:

1.Single band stomata between the veins ……………………………………………..................**2**

Two banded Stomata between the veins……………. ………………………………..**3**

2. Micro hairs present, stomata longer (82 µm)………………………………..***C. polyneuros***

Micro hairs absent, stomata shorter 16.9 µm……………………………***C. iwarancusa***

3. Papillae present, prickles absent, highest stomatal index 38.46…………………***C. martini***

Papillae absent, prickles present, stomatal index 37.96 ……………………***C. nardus***

Bilobate silica bodies, stomatal index is greater than 28.57 …………………………**4**

4. Micro hairs, papillae, and prickles present on both surfaces ……………………***C. citratus***

Overall, the combination of quantitative stomatal data, trichome and papilla patterns, and silica-body morphology offers a powerful diagnostic framework for distinguishing closely related *Cymbopogon* species. These micromorphological variations align closely with habitat and elevation, underscoring the ecological and taxonomic value of leaf epidermal anatomy. The present findings, supported by recent studies (Nunes et al., 2022; Liu et al., 2023; Pokrovsky et al., 2024), highlight the evolutionary adaptability of the genus and reaffirm the utility of epidermal features as complementary tools in grass taxonomy and ecological research.

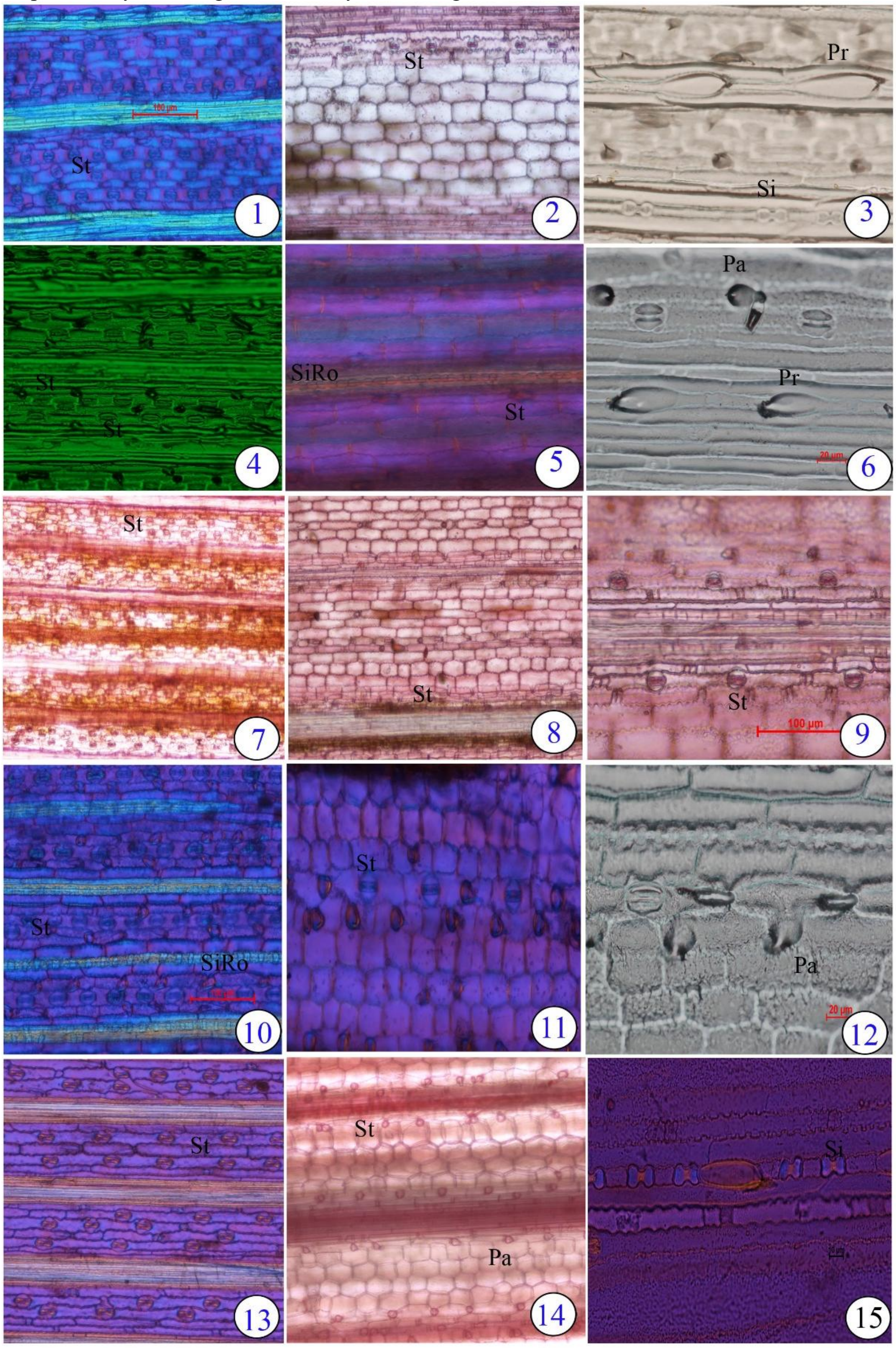

Plate – 1. Different foliar micromorphological characters Stomata, Prickles and Papillae arrangement can be seen on both abaxial and adaxial surfaces at the light and polarized microscopic images, Fig. 1-3 *C. citratus*, Fig. 4-6 *C. martini*, Fig. 7-9 *C. nardus*, Fig. 10-12 *C. polyneuros* and Fig. 13-15 *C. iwarancusa*. St-Stomata, Si-Silica bodies, Pr-Prickle, Pa-Papillae, SiRo – silica row arrangement.

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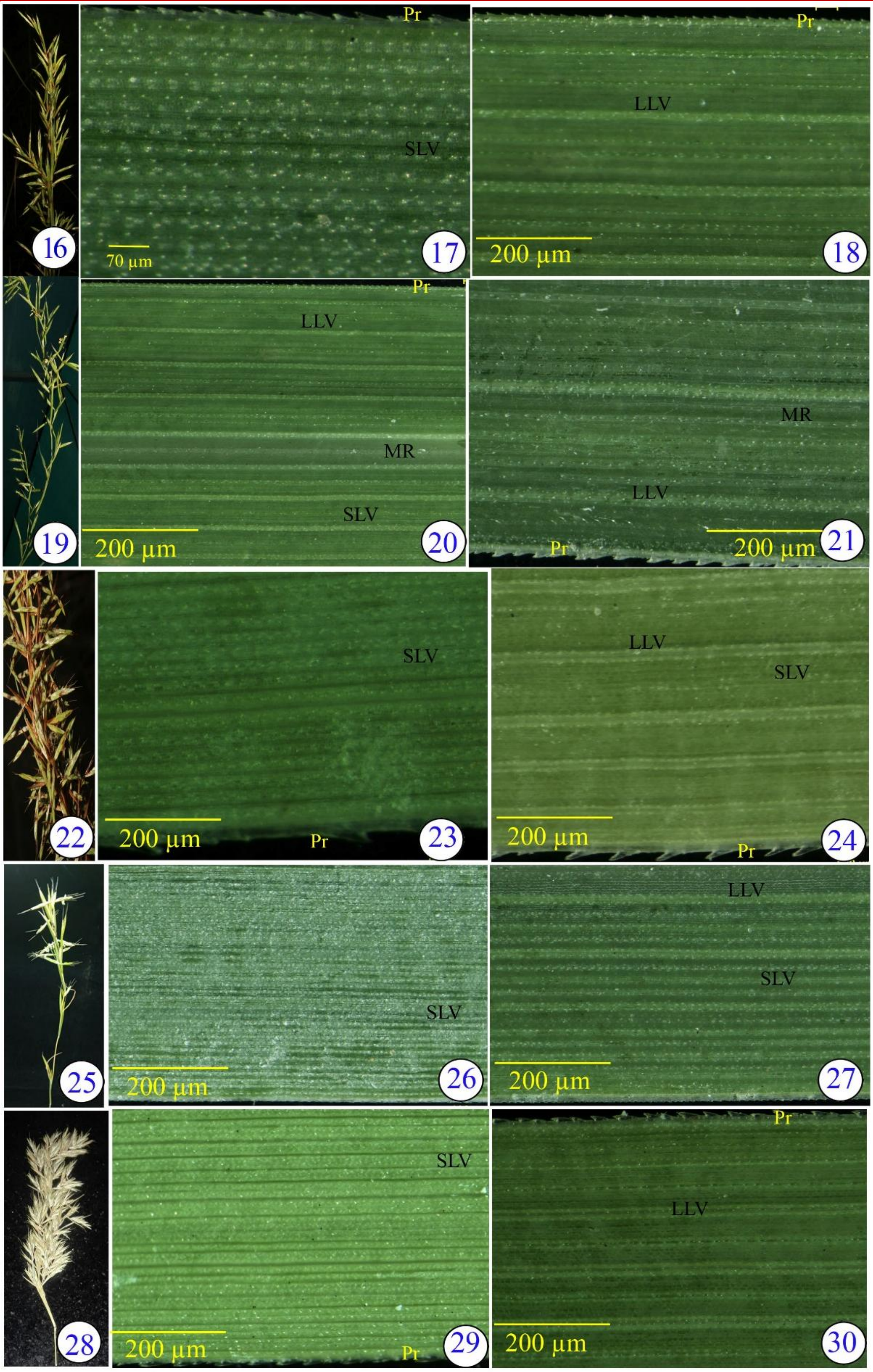

Plate – 2. Inflorescence morphology and leaf abaxial and adaxial surfaces of *Cymbopogon* species at Canon camera and light microscopic images, in *C. citratus* (16-18), *C. martini* (19-21), *C. nardus* (22-24), *C. polyneuros* (25-27) and *C. iwarancusa* (28-30). LLV – Large longitudinal vein, SLV – Small longitudinal vein, Pr – Prickle, MR – Midrib.

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