

Foliar Micromorphology of Subtribe Ischaemineae, Tribe Andropogoneae, Family Poaceae

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Abstract

Leaf epidermal features are very important in grass systematics for characterization of broad groups, within subfamilies and tribes. Numerous species belonging to sub-tribe Ischaemineae, tribe Andropogoneae, family Poaceae are endemic to the Indian subcontinent, but their micromorphological reports were very scarce. Therefore, foliar micromorphological characters of subtribe Ischaemineae have been studied in detail for 16 taxa of subtribe Ischaemineae from Gujarat, India. All the epidermal peels were prepared after following routine scraping method, representative areas were photographed and presented here. Structural diversity as well as metric values for both the epidermises has been recorded. In general, intercostals zones and costal zones are uniform in nature in terms of sinuous papillate long cells, cross-dumbbell-nodular shaped silica cells and triangular-low domed shaped subsidiary cells on stomata. From all the studied micromorphological characters, papillae and microhairs are found to be most useful character to segregate species and genera in subtribe Ischaemineae. Additionally, intercostal silica bodies, hooks, prickles and bulliform cells are also found to be helpful for the same. An artificial key based on observed variable micromorphological features has been also prepared.

Keywords: identification key, macrohairs, microhairs, papillae, silica bodies

Introduction

Family Poaceae are the most diverse in their morphological features and are used for demarcating different genera and species, since Linnaeus (1753). In the recent years, non reproductive organs are also used for identification and segregation; among them, leaf is the most widely used in plant taxonomy (Stebbins and Khush, 1961). Microscopic features such as, epidermal cells, stomata, cuticle, surface contours and ornamentation (hairs, papillae, trichomes) are in use to segregating different taxa (Aydulov, 1931; Prat, 1932). Metcalfe (1960) in his work pointed out that the epidermal characters are quite useful in systematics. General anatomical and micromorphological features for monocots have been described by Metcalfe and Chalk (1964). Later on, Palmer and Tucker (1981, 1983), Palmer *et al.* (1985), Palmer and Gerbeth-Jones (1986, 1988) have described for East African grasses in different publications for specific tribes. In their reports they have given the characteristic pattern, SEM photographs along with the magnification only. Soon after, Clayton and Renvoize (1986) used micromorphological features to solve the taxonomic problems in poaceae. In addition to that, Watson and Dallwitz (1992) have utilized data of abaxial leaf surfaces only at generic level. They have given the structural diversity as well as measurements of few epidermal characters including cells, nature of the walls, stomata, microhairs, silica bodies and papillae for abaxial leaf

surfaces only. But, their voluminous work did not pay any attention to some important epidermal ornamentation such as, prickles, hooks, and macrohairs as these characters are helpful for identification of certain genera and species.

It is well established that foliar anatomy and epidermal features are very important in grass systematics for characterization of broad groups, within subfamilies and tribes (Palmer and Tucker, 1981, 1983; Palmer *et al.*, 1985; Palmer and Gerbeth-Jones, 1986, 1988; Renvoize, 1982 a, b; 1983, 1985, 1986 a, b). Hilu (1984) observed species specific differences in leaf epidermises and suggested that micromorphological variations exist within the genus also. Most of the characters are further described and explained by numerous researchers; such as stomata, trichomes (Metcalfe, 1960), microhairs (Tateoka *et al.*, 1959; Amarasinghe and Watson, 1990) and silica cells (Krishnan *et al.*, 2001; Prychid *et al.*, 2003). Most of above mentioned work include the representative taxa from specific geographical regions. Perusal of literature are suggested that reference to numerous Indian members were not been available.

Subtribe Ischaemineae found to be endemic to Indian subcontinent and represented by seven genera, *viz.*, *Ischaemum*, *Thelepogon*, *Apluda*, *Triplopogon*, *Pogonachne*, *Sehima* and *Andropterum* (Bor, 1960; Karthikeyan *et al.*, 1989). In the earlier work only 3 species of *Ischaemum* and one species of *Sehima* and *Apluda* has been mentioned from the subtribe Ischaemineae (Ahmed, 2009; Ullah *et*

al., 2011; Traiperm *et al.*, 2011). Therefore, in the present communication 16 taxa of 4 genera are studied from their leaf micromorphological point of view and presented here.

Materials and methods

Total 16 different species of 4 genera from the subtribe Ischaemineae, tribe Andropogoneae, family Poaceae were collected from different habitats of Gujarat state, India. Plant identification was done based on available literature (Hooker, 1897; Cooke, 1908; Blatter and Mc Cann, 1935; Shah, 1978). Voucher specimens of all the collected taxa were deposited in BARO herbarium and details are provided in Appendix 1 (Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India).

Leaf epidermal preparation

Leaves from the middle of the culms (2-4) were used throughout the preparation. The peels were made by scraping pieces of fresh or softened dried leaves (Glycerol: Water mixture) with the help of safety razor blade or by Nail-polish method, stained it with the saffranin and

mounted in glycerol (Johnston and Watson, 1976; Ellis, 1979; Hilu and Randall, 1984). Adaxial and abaxial leaf surfaces of investigated taxa were studied at $\times 400$ magnification and individual cells were identified and measured by micrometer. 20-25 peels were made from each species of several dozen of leaves. All the peels were examined and representative areas were photographed using Leica research microscope (Fig. 1 to 4). Final counts of different cells (average of 50 observations) summarized in results. An artificial key has been prepared and presented based on micromorphological characters.

Results and discussion

The epidermis of grasses is made up of cells of two distinct types. The larger cells are commonly referred to as long cells because they elongate horizontally and are parallel with the long axis of the leaf (Metcalfe, 1960). These cells usually constituted slightly less than half of the total epidermal cells present. Long cells are frequently referred to as fundamental elements, undifferentiated cells, or ordinary epidermal cells (Ellis, 1979). In grasses, short cells are products of asymmetric division of intercalary meristem cells (Kaufman *et al.*, 1970), which gives rise to all cells

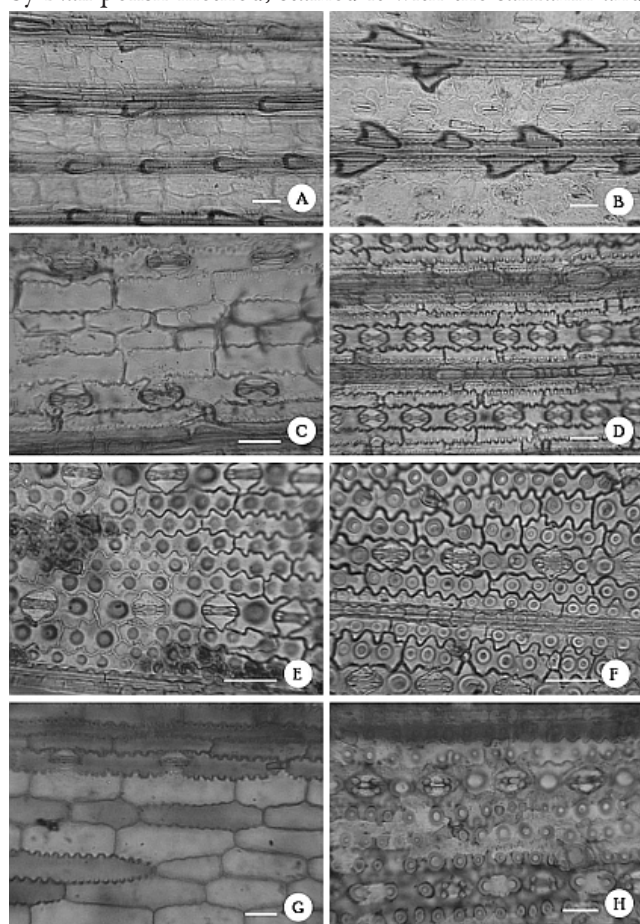


Fig. 1. Photomicrographs of leaf-blades: A, B: *Apluda mutica*; C, D: *Ischaemum afrum*; E, F: *I. barbatum*; G, H: *I. bombaiense*; A, C, E, G: Adaxial epidermises; B, D, F, H: Abaxial epidermises

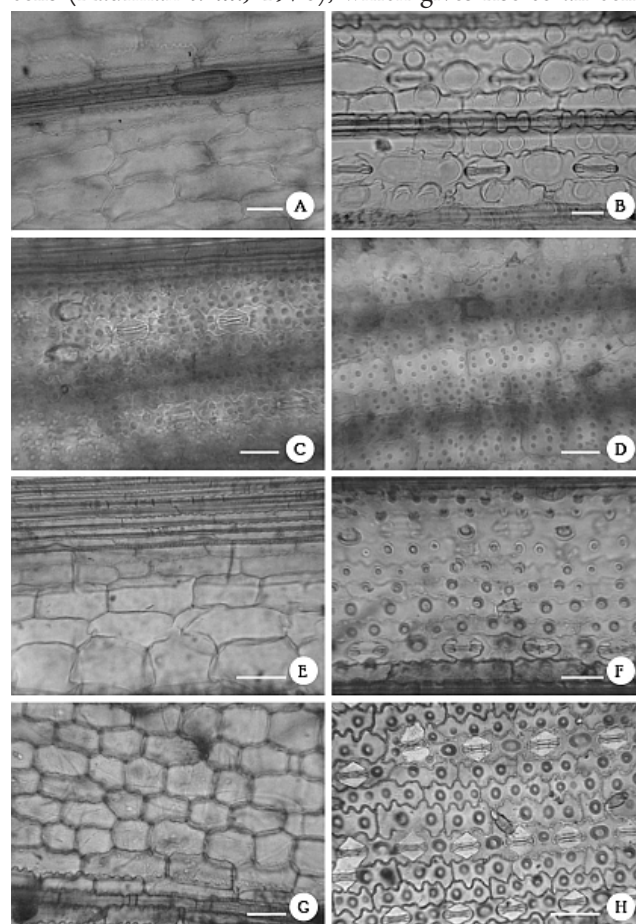


Fig. 2. Photomicrographs of leaf-blades: A, B: *Ischaemum diplopogon*; C, D: *I. indicum*; E, F: *I. molle*; G, H: *I. muticum*; A, C, E, G: Adaxial epidermises; B, D, F, H: Abaxial epidermises

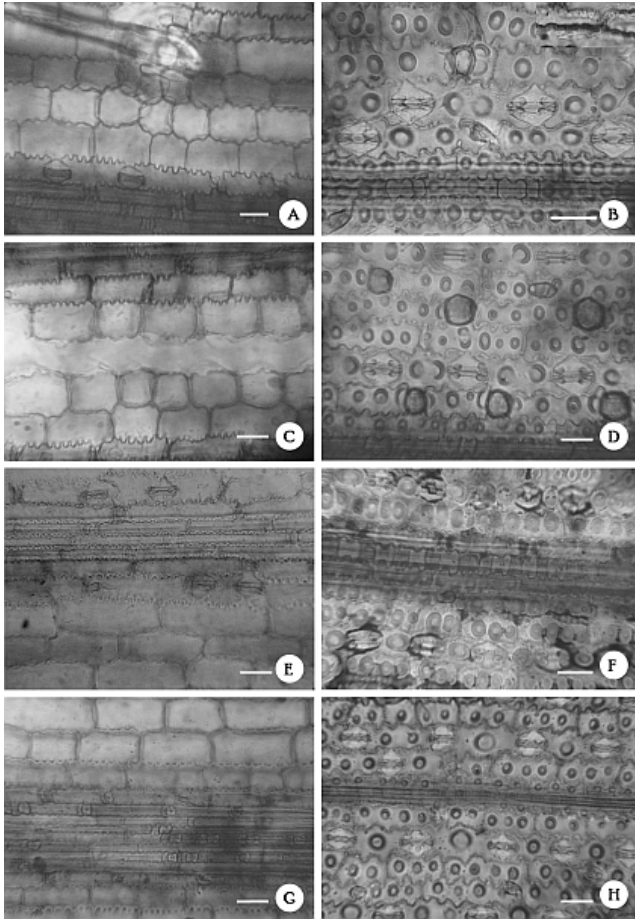


Fig. 3. Photomicrographs of leaf-blades: A, B: *Ischaemum rugosum*; C, D: *I. santapauai*; E, F: *I. sayajiraoi*; G, H: *I. semisegittatum*; A, C, E, G: Adaxial epidermises; B, D, F, H: Abaxial epidermises

except long cells. It produces cork-silica cell pairs, prickles, macrohairs, microhairs and stomata (McWhorter *et al.*, 1993). Metcalfe (1960) reported that guard cells of stomata are dumbbell shaped, however there are some variations in shape of subsidiary cells. The dumbbell shaped stomata of grasses are generally believed to represent a more evolutionary advanced than the kidney shaped stomata (Palevitz, 1981), of which the tall dome shaped subsidiary cells is a primitive character over low dome and triangular one (Shouliang *et al.*, 1996). As the tribe Andropogoneae considers being an advanced one, almost all the members depict triangular-low domed subsidiary cells.

Micromorphologically all the studied taxa of subtribe Ischaemineae, i.e., *Apluda mutica*, *Triplopogon ramosissimus*, 12 species of *Ischaemum* and both the species of *Sehima* depict overall similarity in major characteristic features and showed a common structural pattern for the family Poaceae. Till today, 86 genera of the tribe Andropogoneae has been reported for their leaf anatomy and micromorphological features of which only 5 taxa of subtribe Ischaemineae were mentioned (Metcalfe, 1960; Renvoize, 1982a; Hilu, 1984; Davila and Clarke, 1990; McWhorter *et al.*, 1993; Abid *et al.*, 2007; Folorunso and Oeytunji, 2007; Desai *et al.*, 2009;

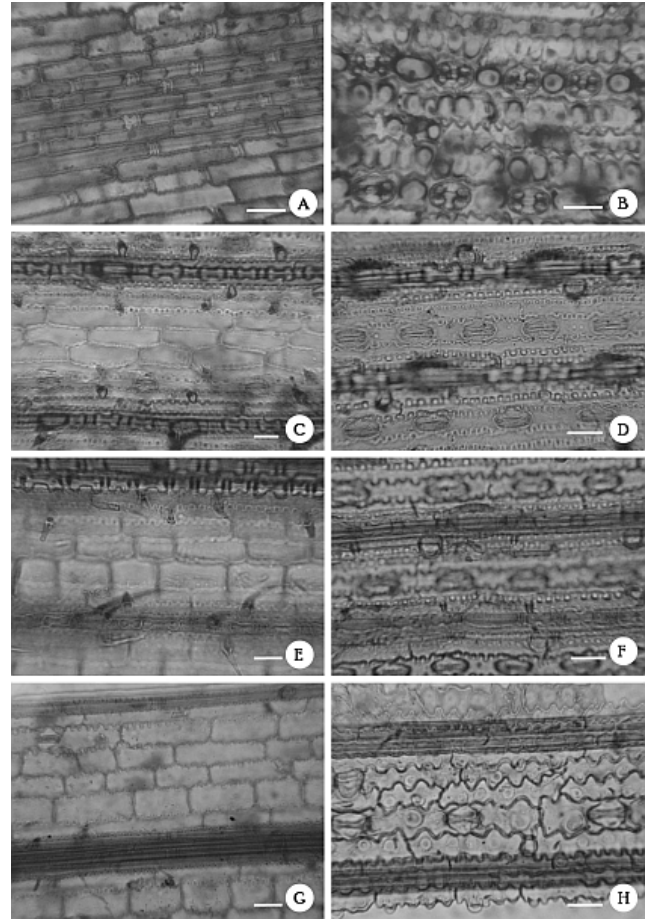


Fig. 4. Photomicrographs of leaf-blades: A, B: *Ischaemum travancorense*; C, D: *Sehima nervosum*; E, F: *S. sulcatum*; G, H: *Triplopogon ramosissimus*; A, C, E, G: Adaxial epidermises; B, D, F, H: Abaxial epidermises

Ullah *et al.*, 2011; Triaperm *et al.*, 2011). During present investigations, intercostal zone and costal zone are uniform in nature in terms of rectangular sinuous papillate long cells, triangular-low domed stomata and cross-dumbbell-nodular shaped silica cells. This silica perform various functions like mechanical stability, pathogen, insect, herbivore and drought resistance, facilitation of light and alleviation of nutrient deficiency (Epstein and Bloom, 2005; Motomura *et al.*, 2006). Shape of silica bodies is a valuable character because a great variety of shape occurs (Clifford and Watson, 1977). In the present study, different kinds of silica bodies are found in different taxa, such as dumbbell, cross, saddle, rounded, tall and narrow and some intermediate types.

It has been already proven that various micromorphological characters are used to segregate the genera in the tribe or subtribe. In the present study *A. mutica* is unique owing to presence of arundo type of microhairs and tall domed stomata along with non papillate long cells, which are in accordance with Traiperm *et al.* (2011). Ullah *et al.* (2011) reported cross-dumbbell shaped silica bodies and panicoid microhairs in *Apluda*, but in present findings only long nodular silica cells are recorded on the costal region along with arundo type microhairs (Fig. 1 A-B; Tab.

Tab. 1. Comparison of studied qualitative and quantitative micromorphological characters in the tribe Ischaemineae (L: lower/abaxial epidermis, U: upper/adaxial epidermis, Ch: Chloris type, P: Panicoid type)

Plant name	<i>Apluda mutica</i>	<i>Ischaemum afrum</i>	<i>Ischaemum barbatum</i>	<i>Ischaemum bombaiense</i>	<i>Ischaemum diplopogon</i>	<i>Ischaemum indicum</i>	<i>Ischaemum molle</i>	<i>Ischaemum muticum</i>	<i>Ischaemum rugosum</i>	<i>Ischaemum santapau</i>	<i>Ischaemum sayajiraoi</i>	<i>Ischaemum semisegittatum</i>	<i>Ischaemum travancorense</i>	<i>Sebima nervosum</i>	<i>Sebima sulcatum</i>	<i>Triplopogon ramosissimus</i>
Long cell: Rectangular, Sinuous																
Size (μ)	65-96×28-36	76-100×14-20	65-120×12-20	80-200×24-30	96-150×20-28	50-100×20-28	86-116×26-32	60-90×15-30	86-100×22-30	80-130×38-42	75-116×20-30	40-83×16-24	86-132×38-46	76-100×14-20	76-100×14-20	64-122×22-26
Short cell / Silica cell																
Intercostal	Absent	Absent	Crescent	Absent	Crescent	Absent	Absent	Absent	L: Absent U: Cubical	Cubical	L: Absent U: Cubical	Absent	L: Cubical U: Absent	Absent	Absent	Cubical
Costal : Type and Size(μ)	Nodular 20-55×6-8	Dumbbell 20-33×10-12	Cross-dumbbell -nodular 10-17×6-7	Dumbbell- cross 10-20×10-13	Dumbbell- cross 34-42×12-16	Cross- dumbbell 13-17×6-7	Dumbbell 16-17×6-10	Cross- dumbbell 6-17×10	Cross- dumbbell 10-27×13-17	Cross- dumbbell 16-26×13-16	Cross-dumbbell 10-17×17-20	Cross- -Dumbbell 6-17×6-7	Cross- dumbbell 16-26×16-17	Dumbbell 20-33×10-12	Dumbbell 20-33×10-12	Cross-dumbbell- nodular 8-28×10-14
Si-Cr pairs	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Costal Prickle (μ)	30-45×22-28	26-40×20-26	Absent	Absent	L: Absent U: 72-86×14-20	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	26-60×20-26	26-60×20-26	Absent
Hooks	Absent	Present	Absent	Absent	Absent	Present	Absent	Absent	L: Present U: Absent	L: Present U: Absent	Absent	Absent	Absent	Present	Present	Present
Macrohair	Absent	Absent	Absent	Absent	Absent	L: Tubercled hair	Tubercled hair	L: Tubercled hair	U: Tubercled hair	L: Tubercled hair	U: Tubercled hair	Absent	Absent	Absent	Absent	Tubercled hairs
Bicellular Microhair																
-Type	Arundo	Panicoid	Chloris	L: Chloris U: Panicoid	L: Chloris U: Zizania	Chloris	Zizania	L: Zizania U: Chloris	L: Chloris and Panicoid U: Panicoid	L: Chloris U: Panicoid	L: Zizania U: Panicoid	L: Chloris U: Zizania	L: Chloris U: panicoid	Panicoid	Panicoid	Panicoid
-Size (μ)	34-42×5-7	42-47×6-8	12-17×6-8	L: 29-32×10-13 U: 43-50×6-8	L: 18-20×6-7 U: 34-38×6-7	20-23×6-7	30-34×6-7	L: 18-20×6-7 U: 34-37×6-7	Ch: 20-24×7-8 P: 35-39×7-8	L: 18-27×10-12 U: 33-63×6-7	L: 23-26×10-12 U: 33-36×10-12	L: 22-26×10-12 U: 26-30×6-7	L: 28-32×10-12 U: 42-50×10-12	42-47×6-8	42-47×6-8	54-60×10-12
Papillae/cell	Absent	Absent	L: 3-5, 1 row U: Absent	L: 4-8, 1-row U: Absent	L: 2-5, 1-row U: Absent	12-22, 2-3 row	L: 4-7, 1-row	L: 3-5, 1-row	L: 4-10, 1-row	L: 3-5, 1-row	L: 5-10, 1 row	L: 4-8, 1 row	L: 3-5, 1-row	Absent	Absent	L: 3-6, U: Absent
Stomatal complex																
-Subsidiary cell type	Triangular- tall dome	Triangular	Triangular	Triangular- low dome	Low dome	Triangular	Triangular- Low dome	Triangular	Triangular	Triangular	Triangular - Low dome	Triangular	Triangular- Tall dome	Triangular	L: Triangular	Triangular
-Size(μ)	30-35×22-28	30-35×18-27	20-24×20-24	36-40×16-30	36-40×18-24	23-27×23-27	23-33×16-23	17-20×26-28	30-36×26-33	26-40×26-28	26-33×16-20	20-24×16-20	23-33×33-36	30-35×18-27	30-35×18-27	30-34×24-30
-No. of Papillae overarchng the stoma	Absent	Absent	Absent	L: 4	L: 4	Absent	L: 4	Absent	L: 4	L: 4	L: 4	Absent	L: 4	Absent	Absent	Absent

1). Likewise, presence of bulliform cells on both the epidermises is the delimiting character of genus *Sehima* from rest of the studied taxa. It is well accepted fact that bulliform cells plays a significant role in rolling and spreading of the leaves due to prevailing stressed or favourable conditions (Grigore *et al.*, 2010). Rest other taxa from *Apluda*, *Ischaemum* and *Triplopogon* shows presence of bulliform cells in adaxial epidermises only. Abaxial surface of all the species of *Ischaemum* depict presence of 2-8 papillae, but *I. indicum* depicts numerous small sized papillae on both epidermises (Fig. 2 C, D; Tab. 1) and *I. afrum* is exception due to its absence (Fig. 1 C, D; Tab. 1). Four papillae are present on the stomatal apparatus in all the *Ischaemum* spp. except *I. semisegittatum*, *I. indicum* and *I. muticum*. It is also not recorded from *A. mutica* and *Sehima* spp. Costal zone is also peculiar in *Apluda*, *I. afrum*, *I. diplopogon* and *Sehima* as prickles are observed (Fig. 1 A-D; Fig. 2 A, B; Fig. 4 C-F; Tab. 1). Long tubercled macrohairs are recorded from the *I. indicum*, *I. muticum*, *I. molle*, *I. rugosum* and *I. santapau* and hooks from the *I. indicum*, *I. rugosum*, *I. afrum*, *I. santapau* and *I. conjugatum*. But, in the work of Triperm *et al.* (2011) hooks and macrohairs were absent in *I. muticum* and *I. rugosum*.

Although type of microhairs is found to be the diagnostic characters and it is constant within the species or even in genera and tribe, *Ischaemum* shows great diversity within/between the species (Amarasinghe and Watson, 1990). Presence of only panicoid type of microhairs is characteristic features of genus *Sehima* and *Triplopogon* and *Ischaemum afrum*. Panicoid type of microhairs is present in the upper epidermis of all the species, except *I. semisegittatum*, *I. molle*, *I. muticum*, *I. diplopogon* and *I. barbatum*. Along with that, in *I. barbatum*, *I. bombaiense*, *I. diplopogon*, *I. indicum*, *I. muticum*, *I. rugosum*, *I. santapau*, *I. semisegittatum* and *I. travancorensis* chloris type bicellular microhairs are noticed only on the lower epidermises. At the same time zizania type microhairs are observed in *I. diplopogon*, *I. molle*, *I. muticum*, *I. sayajiraoi* and *I. semisagittatum* (Fig. 2 A, B, E-H; Fig. 3 E-H; Tab. 1). Presence of different kind of microhairs in *I. rugosum* was reported by Metcalfe (1960) and Ullah *et al.* (2011), but not mentioned in the works of Renvoize (1982a) and Triperm *et al.* (2011).

Conclusions

Present paper demonstrated considerable differences in quantitative and qualitative micromorphological traits among 16 taxa of subtribe Ischaeminae from Gujarat, India. From all the studied micromorphological characters, papillae and microhairs are found to be most useful to delineate the species and genera in subtribe Ischaeminae. In addition to that, intercostal silica bodies, hooks, prickles and bulliform cells are also found to be helpful for the same. In general, micromorphological features alone are insufficient for the species segregation; it has been proven

that they have considerable systematic values to provide additional support for the species characterization.

Identification Key based on foliar micromorphological features for taxa studied:

1. Papillae absent on both the leaf epidermises
2. Microhairs are of arundo type.....*Apluda mutica*
2. Microhairs other than arundo type
3. Bulliform cells present in both the epidermises
4. Leaves amphistomatic.....*Sehima nervosum*
4. Leaves hypostomatic.....*Sehima sulcatum*
3. Bulliform cells present in adaxial epidermis only... *Ischaemum afrum*
1. Papillae present either on one or both the leaf epidermises
5. Papillae on both epidermises, small and numerous....*Ischaemum indicum*
5. Papillae on lower epidermis only, 2-8/cell in one row
6. Tubercled macrohairs present
7. Microhairs of zizania type only on both epidermises
7. Microhairs of different types on different epidermises
8. Microhairs of panicoid type present
9. Hooks present on epidermises
10. Panicoid type of microhairs present on both the epidermises.....
Triplopogon ramosissimus
10. Panicoid type of microhairs present along with chloris type
11. Microhairs chloris + panicoid type on lower epidermis.....
Ischaemum rugosum
11. Microhairs chloris type on lower epidermis....*Ischaemum santapau*
9. Hooks absent on epidermises.....*Ischaemum sayajiraoi*
8. Microhairs of panicoid type absent...*Ischaemum muticum*
6. Tubercled macrohairs absent
12. Prickles on upper epidermis present..... *Ischaemum diplopogon*
12. Prickles on upper epidermis absent
13. Microhairs of chloris type only on both epidermises.....*Ischaemum barbatum*
13. Microhairs of different types on different epidermises
14. Microhairs of chloris and zizania types...*Ischaemum semisegittatum*
14. Microhairs of chloris and panicoid types
15. Intercostal silica cells present on lower epidermis...*Ischaemum travancorensis*
15. Intercostal silica cells absent on lower epidermis....*Ischaemum bombaiense*

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Appendix 1: List of Studied taxa and the voucher specimens deposited in BARO herbarium

Genus no.	Species no.	Taxon name	Voucher Specimen Number
1	1	<i>Apluda mutica</i> L.	RJD/ 89, 154, 220, 345
2	2	<i>Ischaemum afrum</i> (J.F.Gmel.) Dandy	RJD/ 3, 432, 456
	3	<i>Ischaemum barbatum</i> Retz.	RJD/ 95, 520, 702
	4	<i>Ischaemum bombaiense</i> Bor	RJD/ 652, 742, 748, 769
	5	<i>Ischaemum diplopogon</i> Hook.f.	RJD/ 854, 855, 870, 884
	6	<i>Ischaemum indicum</i> (Houtt.) Merr.	RJD/ 80, 118, 275
	7	<i>Ischaemum molle</i> Hook.f.	RJD/ 95, 163, 458
	8	<i>Ischaemum muticum</i> L.	RJD/ 127, 578, 665
	9	<i>Ischaemum rugosum</i> Salisb.	RJD/ 162, 216, 733
	10	<i>Ischaemum santapauai</i> Bor	RJD/ 517, 558, 646
	11	<i>Ischaemum sayajiraoi</i> Raole & R. J. Desai	RJD/ 32 (Holotype), 33, 166, 518, 523
	12	<i>Ischaemum semisegittatum</i> Roxb.	RJD/ 183, 193, 382, 739
3	13	<i>Ischaemum travancorense</i> Stapf ex C.E.C.Fisch.	RJD/ 34, 47
	14	<i>Sehima nervosum</i> (Rottb.) Stapf	RJD/ 235, 312, 454
	15	<i>Sehima sulcatum</i> (Hack.) A. Camus	RJD/ 860, 867, 883
4	16	<i>Triplopogon ramosissimus</i> (Hack.) Bor	RJD/ 472, 515, 451, 861