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Original Contribution

COMPARATIVE MORPHOLOGY, PALYNOLOGY AND ANATOMY OF TWO ASTERACEAE SPECIES

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ABSTRACT

In this study, *Tragopogon* L. *Lactuceae* (Asteraceae) collected from Pakistan has been investigated for its morphology, leaf epidermal anatomical feature and pollen grain characteristics. It is a perennial herb. In this project 2 species i.e. *Tragopogon dubius* & *Tragopogon gracilis* are described. It was found that the basal leaves were sessile, whilst the basal, middle and upper leaf blade was linear-lanceolate. Furthermore, the phyllaries were in one row in all. The middle and inner phyllaries were absent. The leaves were bifacial and stomata cells were anomotetracytic. The pollen type of the species was trizonocolporate and tetrazonocolporate. All morphological, palynological and anatomical studies are illustrated for the first time.

Key words: Morphology, palynology, anatomy, *Tragopogon* L., scanning electron micrograph, Pakistan

INTRODUCTION

The *Tragopogon* L. taxa are naturalized in Pakistan probably introduced as contaminants of crop or pasture seed, although some have been propagated for their ornamental appeal. To date no comprehensive morphological, palynological and anatomical study has been done on this genus, the species of which are often difficult to distinguish from each other and whose infra-generic classification is only preliminary. The present paper is an introduction and first attempt at morphological, palynological and anatomical studies on *Tragopogon* L. from Pakistan. *Tragopogon* (Asteraceae) comprises approximately 150 species native to Eurasia (1). Although some 20 species are listed in *Flora Europaea*, Richardson (2) limited the presence in the Iberian Peninsula to only four. Blanca & Diaz de la Guardia (3), on the other hand, listed eight Iberian species, seven previously described (*T. porrifolius* L., *T. angustifolius* Bellardi ex Willd., *T. crocifolius* L., *T. castellanus* Levier, *T. pratensis* L., *T. lamottei* Rouy and *T. dubius* Scop.) and one new species (*T. pseudocastellanus* Blanca & C. Diaz). *Tragopogon hybridus* L. has been excluded from the genus, as it is considered to belong to the monotypic genus *Geropogon* L. (4, 5, 6).

Most species are diploid (2n = 12), but some polyploidy species, *Tragopogon dubius, T. porrifolius* and *T. pratensis*, were introduced from Europe into the Palouse region of eastern Washington and adjacent Idaho, USA, in the early 1900s (7). The introduction of these diploid species into the Palouse brought them into close contact, something that rarely occurs in the Old World, where they are largely allopatric. Using morphology and cytology, Ownbey (7) demonstrated that *Tragopogon* mirus and *Tragopogon miscellus* were allotetraploids (2n = 24) whose diploid (2n = 12) parents were *T. dubius* and *T.* porrifolius, and *Tragopogon dubius*, *T. pratensis*, respectively.

Three diploid species of *Tragopogon* were introduced to the Palouse region from their native ranges in Europe near the beginning of the twentieth century (8). Ownbey (7) demonstrated through

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morphological and cytological studies that *T. mirus* was a tetraploid whose progenitors were diploids *T. dubius* and *T. porrifolius,* and that *T. miscellus* was also tetraploid, with diploid progenitors *T. dubius* and *T. pratensis.* All five species occur in waste places, empty lots, and disturbed sites in towns on the Palouse; only *T. dubius* tends to occur outside of towns, along highways, in old fields, and occasionally at old logging sites on the eastern edge of the Palouse. In some places, such as along trails, it occurs in native vegetation. The allotetraploids have not been reported in the diploidś native ranges in the Old World, and the diploids did not co-occur on the Palouse prior to about 1928 (7). *Tragopogon dubius*, is a biennial composite, its 1st yr is a low rosette; during its 2nd yr, if its root crown diameter is sufficiently large (9), it sends up a long stem, which eventually produces 1-30 flowering stalks. This widespread plant is a favoured dietary item for pocket gophers. *Tragopogon dubius*, like most plants in natural settings, is subjected to myriad depredations (10).

Tragopogon porrifolius L. subsp. *porrifolius* is an annual or biennial herb of 30-125 cm high with lilac to reddish-purple ligules, which is native to the Eastern and Central Mediterranean region and Asia Minor. The taxon was formerly widely cultivated as a vegetable in most parts of Europe and therefore occurs as an introduced plant in the entire Mediterranean region and as a casual further North (11, 2). Usage as a vegetable decreased in Southern and Central Europe in recent decades. However, white salsify is still quite popular in the United Kingdom (12). The species *Tragopogon porrifolius* is subdivided into three subspecies: *T. p*. subsp. *porrifolius*, which is the only subspecies used as a vegetable (2). Achenes of all the *Tragopogon* species from the Iberian Peninsula were examined by means of scanning-electron microscopy and stereomicroscopy. The achenes of the eight species are described, illustrated and compared. The results are contrasted with the systematics of this genus. The isolated position of *Tragopogon lamottei* with regard to the other seven species has been noted (13).

The only tetraploid Iberian species is *Tragopogon castellanus* (2n = 24) (14), although *Tragopogon pseudocastellanus* also appears to be tetraploid based on the size of its pollen (3), given that polyploids have larger pollen grains than diploid species (15). Pollen development in *Tragopogon porrifolius* has been studied in the scanning electron microscope to establish the origin of taxonomically significant features. As in other members of the tribe recently investigated the echinolophate condition is shown to result from differential deposition of the tetrad callose wall and, subsequently, of primexine. *Tragopogon* differs from other *Lactuceae* in the absence of tectum and columellae in the lacunae; this also appears to be the result of differential primexine deposition. The development of onci and their extrusion at the apertures have been described (16). The outer wall of pollen grains and spores of fern allies are composed of sporopollenin, an organic substance highly resistant to decay (17). Morphological, palynological and anatomical evidence is provided here for its recognition as a distinct species.

MATERIALS AND METHODS

Tragopogon is most common in the North West Frontier Province and Punjab of Pakistan. Collections were made from nine sites, given in the map (**Figure 1**). Most of the material used for this study was collected directly from wild populations, and the voucher specimens for each of the samples studied have been placed in herbarium of Pakistan Islamabad (ISL), Faculty of Biological Sciences, and Department of Plant Sciences, Quaid-i-Azam University Islamabad. The specimens were also studied from herbarium of Pakistan Museum of Natural History, Islamabad (PMNH) and National herbarium, Islamabad (RAW). The parts of the plants were measured with hard ruler or for finer details, under a dissecting microscope. Magnifiers of 5X, 10X, 20X were also used for observation of various parts. For morphological studies 10 specimens per species were used for assessment of morphological characters. Mature achenes from the peripheral florets of the capitulum were measured at low magnification under a stereomicroscope. The terminology of fruit-coat surface sculpturing follows that already described (18, 19).

Pollen was removed from herbarium (ISL) sheets. General preparation consisted of acetolyzing mature pollen grains as described by Erdtman (20), then the removal of undigested plant debris (21, 22) and, finally, separating the samples for SEM. Whole pollen grains and fragments of pollen walls obtained by cryomicrotomy Skvarla et al. (23) were stained and dried using the repeat method of osmium and thiocarbohydrazide (i.e., OTOTO) as has been described in another study (24). Pollen was then mounted on double-stick tape and pulse sputter coated

for 3 min with a gold / palladium target in a Hummer VI Sputter Coating System (21). Secondary electron imaging and photography were done with a JEOL 880 scanning electron microscope equipped with a lanthanum hexaboride gun operating at 15-keV accelerating voltage.

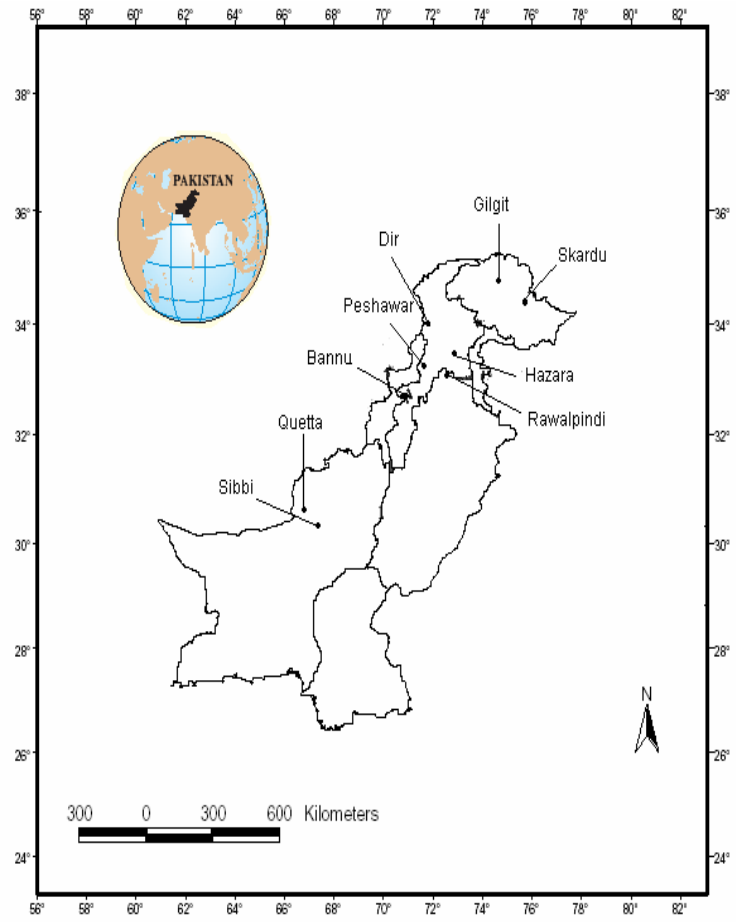

Figure 1*. Location of Tragopogon sampling sites from Pakistan*

Leaf samples were taken from the sixth/seventh nodes of the main stem at the full flowering stage between the hours of 9:00 and 10:00 am. Dried leaves were placed in boiling water for few minutes to soften the leaf until they became unfolded and were ready for epidermal scrapping. Fresh leaves were used directly for anatomical studies. The fresh or dried leaves were placed in a test tube filled with 88% lactic acid kept hot in boiling water bath for about 50 or 60 minutes. Lactic acid softens the tissue of the leaf to make peeling off possible. To prepare the abaxial surface, the leaf was placed keeping its adaxial surface upward and then was flooded with 88% cold lactic acid. The adaxial epidermis was cut across the leaf using a sharp scalpel blade and scraped away together with the mesophyll cells until only the abaxial epidermis of the leaf remained on the tile. The epidermis was placed outside uppermost and mounted in clean 88% lactic acid. Same procedure was followed to prepare the adaxial epidermis. For anatomical studies, sections from fixed samples were taken by hand using a razor and their photographs were taken with a camera marked Nikon FDX-35 for examining them under a microscope. The length and width of the stomata were measured with an ocular micrometer using surface sections from the upper and lower parts of the leaf epidermis. The number of stomata was evaluated by preparing slides from the clear nail polish impression on both lower and upper epidermis of the leaves. Stomata were counted and measured using a 10X, 20X and 40X magnifications.

All original slides and photographic negatives are kept at the Centre for Microscopy and Microanalysis, Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan. A statistical analysis was conducted in order to ascertain whether there is support for the taxa that have been discriminated based on their vegetative and pollen characters (**Tables 2** and **4**).

RESULTS

Tragopogon L. Sp. Pl. 2: 789. 1753; Gen. Pl. ed. 5, 346. 1754.

Taxonomic Treatment

Annual to perennial, herbs; Stem erect, usually branched proximally, glabrous or tomentulose to floccose, often glabrescent. Leaves basal and cauline, sessile, blades linear to lance-linear or lanceolate to oblong, grass like, bases clasping, margins entire. Capitula terminal, solitary, homogamous, large with many florets. Peduncle often inflated distally, ebracteate. Involucre campanulate to cylindric. Phyllaries usually in one row, linear lanceolate to triangular lanceolate, ± equal, narrowly pellucid, apices acute. Receptccle naked, convex, smooth, glabrous, epaleate. Florets bisexual. Corolla ligule yellow or purple, apex 5-toothed. Anthers appendages basally sagittate. Style arms slender and long. Achenes cylindric, distinct ribs, tuberculate or not, apex attenuate or abruptly acuminate into a beak. Pappus many, densely plumose and interwined with bristles, often apically scabrid and barbellate.

About 150 species introduced in Eurasia, N. Africa; Australia, 2 species in Pakistan.

Key to the Species

1. Plant is 60-100 cm tall, involucre campanulate, phyllaries lanceolate to linear-lanceolate ……..2
1a. Plant is 20-35 cm tall, involucre cylindric, phyllaries lanceolate……...2
2. Achene with 10-15 ribs, beak as long as achene …… *Tragopogon dubius*
2a. Achene with 10 ribs, beakless……*T. gracilis*

1. *Tragopogon dubius* Scop. Flora Carniolica, ed. 2. 2:95. 1772

Biennial to perennial herbs, 60-100 cm tall herb. Stem often simple or branching from the base. Leaves basal and cauline. Basal leaves sessile, linear lanceolate, glabrous, 12-15 X 1-1.5 cm, broad sheathing base, apex acuminate, margin entire. Middle and upper stem leaves similar to basal leaves, 8-10 X 0.6-1.1 cm. Capitula solitary, terminal on strong inflated peduncle. Involucre campanulate, 4-6 X 0.8-1 cm. Phyllaries in one row, 8-13, lanceolate to linear-lanceolate, 3.8-6.2 X 0.4-0.8 cm, margin smooth, apex acuminate. Florets yellow, shorter than involucral bracts. Achenes slender, fusiform, 20 X 1.3 mm, slightly curved, long beaked, beak as long as achene, 10-15 ribbed. Pappus yellowish brown to white, 17-28 mm long, plumose below and scabrous above, persistent.

Flowering and Fruiting: June-September

2. *Tragopogon gracilis* D. Don. In Mem. Wern. Soc. 3:414. 1820

Herbs, perennial, 20-35 cm tall. Stem slender, branched from base. Leaves basal and cauline. Basal leaves sessile, linear lanceolate, 10-15 X 1-2.5 cm, expanded into sheath base. Middle and upper stem leaves similar to basal leaves, 6-8 X 1-2 cm. Capitula terminal on strong not inflated peduncle. Involucre cylindric, 3-5.2 X 1-1.5 cm. Phyllaries in one row, 5-7, lanceolate, 2.8-4.5 X 0.6-0.9 cm, margin smooth, apex acuminate. Florets not of same colour, abaxially purplish red, adaxially yellow. Achenes fusiform, yellow, 1.4 X 0.5 cm, with 10 muricate ribs, apically truncate, beakless. Pappus pale yellowish brown, 1.5-2 cm, persistent.

Flowering and Fruiting: June-August

DISCUSSION

Taxonomic studies of the genus *Tragopogon* L. were carried out for the first time from Pakistan. Literature on the taxonomic studies of the genus *Tragopogon* L. and even on the tribe *Lactuceae* of family Asteraceae from Pakistan has not been published yet. So the studies were started on the materials available in the herbaria of Pakistan. Observations on the species were recorded in the field in their habitat in living form.

The main morphological characters which are useful in distinguishing and to describe the species are habit, height of plant, basal leaves, middle and upper stem leaves, capitula, inflorescence, phyllaries, achene size, shape, number of ribs and surface between ribs, achene beak and pappus. Plant morphology in all its aspects is hugely important and the first step in plant identification. In the research it was found that the basal leaves were sessile. It was also determined that basal, middle and upper leaf blade was linear-lanceolate. Furthermore, it was found that phyllaries were in one row in all. The middle and inner phyllaries were absent. Achenes were fusiform and number of ribs in achene is important taxonomic character which varies in different species, 10-15 ribs in *T. dubius* and 10 ribs in *T. gracilis*. In *T. dubius* the achene beak is long to the size of achene while in *T. gracilis* beak was absent. Pappus was persistent in all.

Figure 2. *Tragopogon dubius*

Figure 3. *Tragopogon gracilis*

Table 1: *Differences between related species of Tragopogon L.*

Character	*Tragopogon dubius*	*T. gracilis*
Habit	Biennial to Perennial	Perennial
Height of plant	60-100 cm	20-35 cm
Basal leaves	Sessile	Sessile
Basal leaf blade	Linear-lanceolate	Linear-lanceolate
Middle stem leaves	Linear-lanceolate	Linear-lanceolate
Upper stem leaves	Linear-lanceolate	Linear-lanceolate
Phyllaries	1 row	1 row
Outer phyllaries	Lanceolate to linear	Lanceolate
Middle phyllaries	Absent	Absent
Inner phyllaries	Absent	Absent
Florets	Yellow	Adaxially yellow and abaxially purplish red
Achene	Fusiform	Fusiform
Size of Achene (L X W)	20 X 1.3 mm	1.4 X 0.5 cm
No. of ribs on each face	10-15 ribs	10 ribs
Achene beak	Beak as long as achene	Beak less
Pappus	Persistent	Persistent
Size of Pappus	17-28 mm long	1.5-2 mm long

Systematic and evolutionary themes based on Asteraceae pollen morphology were definitively set forth by Wodehouse in publications from Wodehouse (25, 26); although from an historical perspective, it is noteworthy that Steetz (27) is credited as the first to employ pollen as a taxonomic character in the Asteraceae (28, 29, 30). Wodehouse recognized four pollen morphological forms in the family: simple echinate, sub-echinolophate, echinolophate, and psilolophate together with many intermediates. Wodehouse (25) showed one morphological form to be present in the genus *Tragopogon* L. of tribe *Lactuceae*. Blackmore's (31) defining study of lophate pollen recognized, minimally, 23 different pollen types in the Asteraceae, and lophate pattering was most variable. The ultraviolet microscopy of Stix (32) provided the foundation for interpreting structural types throughout the family. Robinson significantly extended the pollen morphological / taxonomic database (29, 30, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43).

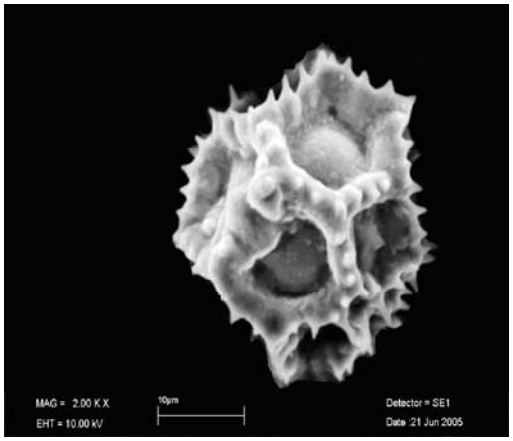

Figure 4. *Scanning Electron Micrograph of Tragopogon dubius, Polar view*

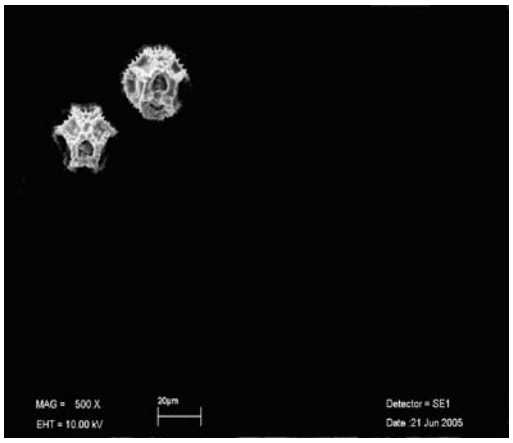

Figure 5. *Scanning Electron Micrograph of Tragopogon dubius, Polar and Equatorial View*

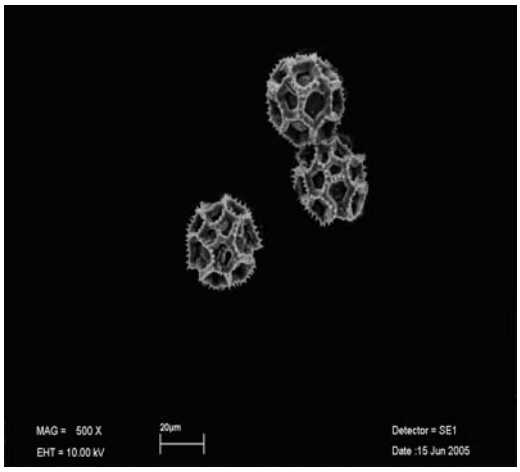

Figure 6. *Scanning Electron Micrograph of Tragopogon gracilis, Polar and Equatorial view*

The influence of pollen morphology in Asteraceae systematics is understood by his comments (29) *"...pollen is one of a series of characters such as stylar bases and anther appendages, observable with the compound microscope, that prove useful in delimiting natural groups. The point has been reached where I believe every taxonomic treatment in the Lactuceae should include mention of pollen type"*.

Figure 7. *Tragopogon dubius upper epidermis at 20X; stomatal pore (P), guard cell (G), epidermal cell (E)*

Later he reinforced these feelings by stating *"The pollen is one of the most useful characters available, and it is inexplicable that taxa would be described at this time without detailed description of the pollen"*. Similarly, Bolick and Keeley (44) concluded, *"Within the tribe, pollen is a good character for delimiting sections, subsections and series"*. Based on the sculpturing all pollens were echinate in *Tragopogon*. The pollen class was trizonocolporate in *Tragopogon dubius*, tri or tetrazonocolporate in *Tragopogon gracilis*. There is variation of pollen size in *Tragopogon*. Dimension of the polar axis ranged from 26 µm (*T. gracilis*) to 50.5 µm (*T. dubius*), and equatorial axis varied between 35 to 43.5 µm (*T. dubius*). The ratio of polar to equatorial axis (P/E) varies between 0.8 (*T. gracilis*) and 1.2 (*T. dubius*). The exine thickness ranged from 5 µm (*T. gracilis*) to 10 µm (*T. dubius*). The spines are present in both species. The character of pollen spine shows an impressive variation which is of significance at the specific and generic level and has also been helpful to understand the process of spine evolution within the tribe *Lactuceae*. It ranged from 2.5 µm (*T. gracilis*) to 5 µm (*T. dubius*). Number of spine rows between colpi considerably differed in the *Tragopogon*. It varies from 4 (*T. gracilis*) to 9 (*T. dubius*). Wodehouse (26) reported that pollen grains of Compositae were unique and true to form and he outlined the principles of morphological evolution of

spine form of the family in which he suggested the reduction series from long to minute spines. There seems to be a potential indicating the evolutionary processes of pollen spines in the *Lactuceae*. The peculiar spine character perhaps represents a climax in the apertural evolution. The occurrence of spines and its absence indicate a trend of evolution of spine reduction in the tribe *Lactuceae*. The reduction and the absence of spines is an evolved character in *Lactuceae* and the genus with spinate pollen as in *Tragopogon*, indicate primitive feature as compared to the genera with spineless pollen which are considered as advanced features within the tribe. The data may be used in establishing relationship at the generic and specific level of the tribe *Lactuceae* within the family Asteraceae.

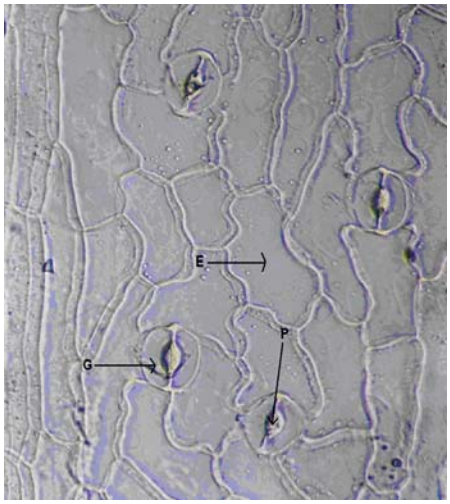

Figure 8. *Tragopogon dubius lower epidermis at 20X; stomatal pore (P), guard cell (G), epidermal cell (E)*

Both species had anomotetracytic stomata (**Table 3**). All the species had stomata on both surfaces. Metcalfe and Chalk (45) reported that due to the diversity of habits, Asteraceae species show various anatomical structures and some present ecological specialization. The micro hairs were found to be absent on both the abaxial and adaxial surfaces of leaf epidermis. Trichome was absent in *Tragopogon.* The crystals were found to be present in all except absent in adaxial surface of *T. gracilis*. The % of open stomata varies from 60 % (*T. dubius*) to 80 % (*T. gracilis*) and % of close stomata ranges from 20 % to 42.85 % (*T. gracilis*) (**Table 4**). The size of stomatal complex varies from 45 µm to 190 µm (*T. gracilis*). The size of epidermal cell ranges from 43 µm to 140 µm (*T. dubius*). Number of stomata and epidermal cells varies 5-8 /mm^2 and 29-42 / mm^2. The % of each type in abaxial and adaxial surface is 100%. The length of guard cells varies from 30 µm (*T. gracilis*) to 42 µm (*T. dubius*) whiles the width of guard cells varies from 8 µm (*T. gracilis*) to 13 µm in (*T. dubius*). The length of stomatal pore ranges from 18 µm to 23 µm (*T. dubius*) while the width of stomatal pore varies from 1 µm to 11 µm.

In view of this, it could be concluded that pollen morphology and anatomy shows evolutionary sequences comparable to those in other organs, than it may need to be given as much weight as any other morphological character.

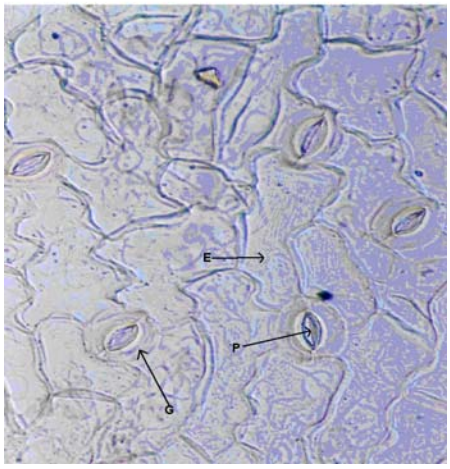

Figure 9. *Tragopogon gracilis upper epidermis at 20X; stomatal pore (P), guard cell (G), epidermal cell (E)*

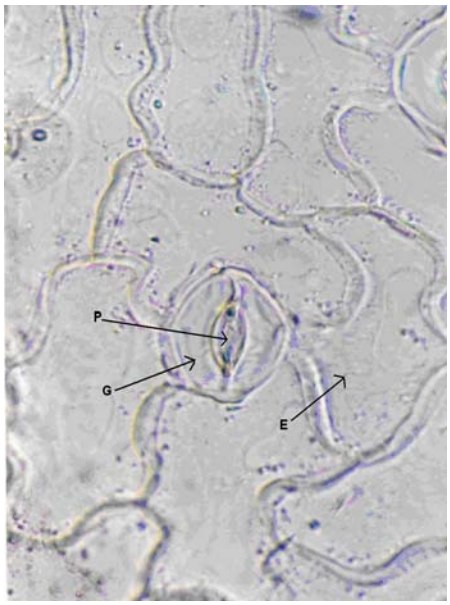

Figure 10. *Tragopogon gracilis lower epidermis at 40X; stomatal pore (P), guard cell (G), epidermal cell (E)*

Table 2: *List of species examined, measurements of equatorial diameter, polar diameter, P/E ratio, exine thickness, spine length, spine rows, shape and sculpturing features in Tragopogon L. of tribe Lactuceae*

Taxon	*Equatorial Diameter (µm)*	*Polar Diameter (µm)*	*P/E*	*Exine Thickness (µm)*	*Spine Length (µm)*	*Number of Spine rows b/w colpi*	*Shape in polar view*	*Shape in Equatorial view*	*Aperture type*	*Pollen Class*	*Sculpturing*
Tragopogon dubius Scop.	35 – 43.5 39.7±1.3	42 – 50.5 47.1±1.5	1.2	6 – 10 8±0.6	3.5 –5 4.2±0.2	8 – 9 8.6±0.2	Semiangular	Spheroidal	Nonlacunate	Trizonocolporate	Echinate
T. gracilis D. Don.	42 – 43 42.5±0.2	26 – 44 32.5±3.5	0.8	5 – 7 6.3±0.3	2.5 –4 3.3±0.2	4 – 8 6±0.7	Rectangular to Semilobate	Spheroidal	Lacunate to non lacunate	Tri or tetra zonocolporate	Echinate

Table 3: *Percentage of different types of stomata, and epidermal cells in the upper (U.E.) and lower (L.E.) epidermis of Tragopogon L.*

Name	*Type of Cell Wall*		*Type of Stomata*		*% of Each Type*		*No. of Stomata /mm²*		*No. of Epidermal cell/mm²*	
	U.E.	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*
Tragopogon dubius	Undulate	Undulate	Anomotetracytic	Anomotetracytic	100	100	6	7	29	34
T. gracilis	Angular, Tubular	Angular, Tubular	Anomotetracytic	Anomotetracytic	100	100	5	8	33	42

U.E. = Upper Epidermis *L.E. = Lower Epidermis*

Table 4*: Dimension and state of epidermal cell, stomatal complex and % of open & close stomata.*

Name	*Size of Epidermal Cell (µm)*		*Size of Stomatal Complex (µm)*		*% of Open Stomata*		*% of Close Stomata*		*Crystals*		*Length of Trichome (µm)*		*Base of Trichome (µm)*	
	U.E.	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*
Tragopogon dubius	43-60 51.8±2.6	51-140 95.4±14.3	92-156 124±11.1	111-182 149±11.7	66.66	60	33.33	40	+	+	Absent	Absent	Absent	Absent
T. gracilis	61-130 95.4±11.1	40-56 48.8±2.4	104-170 139.2±11	45-190 123±23	80	57.14	20	42.85	Absent	+	Absent	Absent	Absent	Absent

U.E. = Upper Epidermis *L.E. = Lower Epidermis* *± Standard Error*

Table 5: *Dimension and state of stomatal complex*

Name	*Length of Guard Cell (µm)*		*Width of guard Cell (µm)*		*Length of Stomatal Pore (µm)*		*Width of Stomatal Pore (µm)*		*Size of Microhairs (µm)*	
	U.E.	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*	*U.E.*	*L.E.*
Tragopogon dubius	39	42	13	12	18	23	1	9	Absent	Absent
T. gracilis	40	30	12	8	20	20	11	3	Absent	Absent

U.E. = Upper Epidermis *L.E. = Lower Epidermis*

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