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The use of taxonomic studies to the identification of wetlands weeds

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Abstract: Background: Palyno-anatomy of wetland species belonging to 10 families was studied in the present research work through light microscopy (LM) and scanning electron microscopy (SEM), to find valuable taxonomic characters.

Objective: This study aims to provide baseline information of the micro-morphological characters of 24 wetland species which will be helpful for further identification of wetlands weeds flora.

Methods: 24 weeds were collected from different wetlands of Azad Kashmir. Taxonomic tools like light microscopic and scanning electron microscopic techniques are used for the proper identification of wetland weeds.

Results: The results show diversity among the qualitative and quantitative characters of epidermal cells, stomata, trichomes, and stomatal pore on both leaf surfaces. In accordance with these variations, a taxonomic key was prepared by using these characters for the identification and differentiation of wetland plant species. In pollen evaluations, variations were observed among exine sculpturing, the number of pores, exine thickness, and diameter of pollens.

Conclusions: Based on our findings it will be helpful for the taxonomist to identify other wetland species by using these micro-morphological characters. This study also indicates that at different taxonomic levels, LM and SEM of pollen and epidermal morphology is explanatory and significant to identify plants up to the species level.

Keywords: Wetland weeds, Taxonomy, Stomata, Trichomes, Pollens, Exine ornamentation

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1. Introduction

The term anatomy is used to study the internal structure of plants. The study of anatomical characters has much importance in different types of systematic research work. The foliar epidermal studies are essential for delimitation, classification, and for arranging the evolutionary and phylogenetic difficulties which include interrelationship among the plant species (Munir et al., 2011; Pereira et al., 2017; Ahmad et al., 2017). The foliar epidermal anatomy of plants is valuable in studying systematics and phylogenetic relationships of the taxa and it plays a significant role in the field of cytology (Bunawan et al., 2011; Khan et al., 2017; Lersten, Curtis, 1992). The epidermis contains numerous types of functionally specialized cells and plays an important role in controlling water loss, defense against intense sunlight and wind, regulate gaseous exchange, photosynthesis, and attract the pollinators, respiration, transpiration, flexibility, and mechanical strength. According to (Wetzel et al., 2017) to study systematics and characterization within subfamilies and tribes, foliar epidermal features are very important.

The epidermis is the most external cellular layer whose characters are under gene control and is influenced to some extend by environmental factor (El-Ghamery et al., 2017). Bio systematically, foliar epidermal characters such as size and shape of stomatal complex, their placement, structural specialties of epidermal cell walls, and typical forms of trichomes help in the identification of many plant families (Chaudhari et al., 2014; Özdemir et al., 2016). The previous literature shows that leaf epidermal features play a key role in systematic botany similar to the use of modern methods such as the search for a pattern of evolution, and biochemical composition (Uka et al., 2014; Waly, 2013). Stomatal characteristics include the arrangement and distribution of stomata, shape of stomata, subsidiary cells, stomatal edge and guard cells, and stomatal size (Wang et al., 2015).

Wetland is a land that is either temporary or permanently covered with water and the basic factor that differentiates wetland from other landforms is the vegetation of wetland plants (Keddy 2010). Wetland vegetation is an important part of the world's flora (Sardar, 2008). Previous studies show that much work has been done on family Pontederiaceae (Arber, 2010). Some work is also done regarding palynology on family Haloragaceae (Kubitzki, 2007), family Mimosaceae, and Nymphaceae (Bhunia, Mondal, 2012). Pollen studies of the Lemnaceae family were studied by (Hesse, 2006;

Alwadie, 2008), family Potamogetonaceae (El-Amier, 2015), Typhaceae family (Arenas, Scarpa, 2003), Podostemaceae (de Sá-Haiad et al., 2010), and Eriocaulaceae family (Borges et al., 2009).

Wetland plants are remarkable plants due to their moist habitat in which they live. Wetland plants include species of different habitats, as emerged or submerged, true aquatics which are free-floating. Plants that are growing between water and land are considered aquatic plants (Özdemir et al., 2016; Santos et al., 2004). The present research work is done in several rivers, lakes, reservoirs, tanks, waterfalls, ponds, ditches, and puddles. Water bodies provide a constant habitat; therefore, the anatomical structures of wetland plants are less different than those of xerophytes and mesophytes. Pakistan is a fairly large country gifted with a variety of climates, ecological zones, and topographical regions. Particularly the region of Azad Kashmir, which is bestowed by plant diversity and natural water resources provides a great habitat for wetland flora. In terms of wetland, Azad Kashmir flora is less explored and the location of the research area is near the agricultural field because this area is lush green. In creating ecological balance in a crop system weeds play a sufficient role by supporting different life forms. *Amaranthus viridis*, *Cynodon dactylon, Phragmites australis* and *Phyla nodiflora* was reported as important weeds of cotton crop (Memoni et al., 2014). Memoni et al., 2013 illustrated the *Cynodon* sp., *Phragmites* sp., *Polypogon* sp., *and Ranunculus* spp. as weed flora of wheat crop. Similarly, *Achyranthes aspera* is a common weed in sugarcane (*Saccharum officinarum* L.) (Jogil et al., 2019) and on chickpea fields, *Plantago lanceolata* was documented as a major weed (Hassan et al., 2010). The correct identification of species is determinant for developing any weed management program according to its different ecological aspects. The taxonomic studies are preliminary research towards the use of identification and classification of wetlands weeds of the study area, which could be in the same way useful for weed scientists as well as for botanists. Thus, the present study aims to provide a baseline of the palyno-anatomical characters of wetland weed species through taxonomic approaches for the proper identification.

2. Material and methods

2.1 Plant material

A total of 24 weeds belonging to 10 families was collected from different wetlands of Azad Kashmir. Azad Kashmir is situated with GPS coordinates of 33° 30’ 46.76” North and 73° 45” 20.07” East with an area of 13,297 km^2 (Rafique et al., 2013). Plants samples were collected from different wetlands of Azad Kashmir. These specimens were identified and confirmed with the help of Flora of Pakistan and available literature (Ali, 1980; Nasir, Ali, 1970). A voucher number was given to each specimen collected. Collected specimens were pressed in a presser, dried in newspapers, identified, and mounted on herbarium sheets. The identified voucher specimens were deposited in the herbarium of Pakistan Quaid-i-Azam University Islamabad. The list of plants their altitude and distribution are presented in Table 1.

Table 1 - Plant sampling, herbarium specimens, and their locality.

Sr.No	Plant species	Collector	Locality	Distract/Province	Altitude (meter)	Voucher Number
1.	*Achyranthus aspera*L. (Amaranthaceae)	Maryam Akram Butt, Essam Akram Butt	Mangla lake	Mirpur/Azad Kashmir	458	MA-71
2.	*Alisma plantago-aquatica* L. (Alismataceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-60
3.	*Alternathera sessilis* (L.) DC. (Amaranthaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-61
4.	*Amaranthus spinosus* L. (Amaranthaceae)	Maryam Akram Butt, Essam Akram Butt	Mangla lake	Mirpur/Azad Kashmir	458	MA-66
5.	*Amaranthus viridis* L. (Amaranthaceae)	Maryam Akram Butt, Essam Akram Butt	Mangla lake	Mirpur/Azad Kashmir	458	MA-87
6.	*Arundo donax* L. (Poaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-68
7.	*Bacopa monnieri* (L.) Wettst. (Plantaginaceae)	Maryam Akram Butt, Essam Akram Butt	Mangla lake	Mirpur/Azad Kashmir	458	MA-59
8.	*Brachiaria reptans* (L.) C.A.Gardner & C.E.Hubb. (Poaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-30
9.	*Cynodon dactylon* (L.) Pers. (Poaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-29
10.	*Dichanthium annulatum* (Forssk.) Stapf (Poaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-12

11.	*Echinochloa colona* (L.) Link (Poaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-63
12.	*Persicaria maculosa* S.F. Gay, Nat.(Polygonaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-29
13.	*Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-49
14.	*Phragmites karka* (Retz.) Trin. Ex Steud. (Poaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-76
15.	*Phyla nodiflora* (L.) Greene (Verbenaceae)	Maryam Akram Butt, Essam Akram Butt	Mangla lake	Mirpur/Azad Kashmir	458	MA-79
16.	*Plantago lanceolata* L. (Plantaginaceae)	Maryam Akram Butt, Tariq Butt, Usman Tariq, Essam Akram Butt	Banjosa lake	Poonch/Azad Kashmir	1,981	MA-55
17.	*Plantago major* L. (Plantaginaceae)	Maryam Akram Butt, Tariq Butt, Usman Tariq, Essam Akram Butt	Banjosa lake	Poonch/Azad Kashmir	1,981	MA-99
18.	*Polypogon monspeliensis* (L.) Desf. (Poaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-80
19.	*Ranunculus repens* L. (Ranunculaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-78
20.	*Ranunculus scleratus* L. (Ranunculaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-45
21.	*Salvia plebeia* R.Br. (Lamiaceae)	Maryam Akram Butt, Essam Akram Butt	Mangla lake	Mirpur/Azad Kashmir	458	MA-89
22.	*Typha angustifolia* L. (Typhaceae)	Maryam Akram Butt, Tariq Butt	Sehnsa wetland	Kotli/Azad Kashmir	679	MA-12
23.	*Verbascum thapsus* L. (Scrophulariaceae)	Maryam Akram Butt	Mangla lake	Mirpur/Azad Kashmir	458	MA-77
24.	*Veronica anagallis-aquatica* L. (Plantaginaceae)	Maryam Akram Butt	Mangla lake	Mirpur/Azad Kashmir	458	MA-88

2.2 Palynological studies

In the present study 13 species of wetland flora have been studied regarding their pollen morphology (Table 4). For pollen analysis, the method proposed by Borsch (1998) has been followed. In which anthers from the flowers were separated and put on a glass slide, and then added 1–2 drops of Acetic acid. They were crushed with the help of a glass rod, mounted with glycerin jelly for staining on a glass slide. Several pollen micro-morphological characters were measured with the help of a Leica Dialup 20 microscope. For Scanning Electron Micrographs, the pollen grains in a droplet of water were suspended and were transferred directly to double side tape affixed to a stub with a fine pipette and gold-coated sputter to 150 A. The pollen specimens inserted on slides were observed and photographed with a Jeol JSM-T200 SEM at 15 kV.

2.3 Leaf epidermal anatomy

For leaf anatomy, we followed the modified techniques of (Nazir et al., 2013). Leaves were collected from mature plants for anatomical study. Leaf samples were taken in the test tube and dip in lactic acid 70% and 30% nitric acid, boiled for 2 minutes. The leaves were poured into Petri dishes; epidermis was isolated through lax camel brush and mounted on glass slides. Both upper and lower epidermis surfaces were separated. Separated epidermis transparent samples were washed twice with water and after that with lactic acid for 5 to 6 seconds. Afterward, the samples were covered with coverslips. The corner of coverslips on glass slides was coated with transparent nail polish, for making permanent slides. Six samples of both adaxial and abaxial surfaces were made for each species. The samples were studied under the light microscope (Model: XSP-45 LCD) for different epidermis parameters. Under the microscope, we observed quantitative and qualitative characters. Quantitative characters include epidermal cell length (L) and width (W), stomata length (L) and width (W), stomatal pore length (L) and width (W), number of epidermal cells, number of stomata, width, and length of the cell wall. The qualitative characters included types of stomata, shape of epidermal cell, shape of anticlinal wall, shape of subsidiary cells, and shape of trichomes.

2.4 Statistical analysis

The qualitative and quantitative features are summarized in (Tables 2 and 3), respectively. Quantitative features are represented by minimum – maximum = mean ± standard error (for example: 27.7 – 105 = (85.2 ± 10.8). Five readings of each character were noted for each adaxial and abaxial surface. The quantitative data were noted and processed using SPSS 16.0 software to determining minimum maximum, mean and standard error; these data are very helpful in the identification of species and the nature of different epidermis characters. These indices give us information about the length and width of stomata, epidermal cells, subsidiary cells, stomatal pore, trichomes, and percentages of the stomatal complex.

Table 2 – Qualitative characters, foliar epidermal anatomy of wetland flora of Azad Kashmir.

Plant species	Epidermal cells shape		Stomata		Type of stomata		Trichome/ Glands		Trichome type	
	Ad*	Ab**	Ad*	Ab**	Ad*	Ab**	Ad*	Ab**	Ad*	Ab**
Alternathera sessilis (L.) DC.	Irregular	Irregular	P	P	Anomocytic	Anomocytic	A	P	__	Multicellular capitate
Achyranthus aspera L.	Elongated	Irregular	P	P	anomocytic	Paracytic	P	P	Attenuate and bulbous base	Attenuate and bulbous base
Amaranthus spinosus L.	Irregular	Irregular	P	P	Anomocytic	Anomocytic	A	A	__	__
Amaranthus viridis L.	Irregular	Irregular	P	P	Anisocytic Anomocyic	Anisocytic Anomocyic	A	A	__	__
Alisma plantago-aquatica L.	Polygonal	Polygonal	P	P	Anomocyic	Anomocyic	A	A	__	__
Plantago lanceolata L.	Isodiametric	Isodiametric	P	P	Anomocytic	Anomocytic	P	P	__	__
Plantago major L.	Polygonal	Polygonal	P	P	Anisocytic	Anisocytic	p	P	Barrel-shaped	Barrel-shaped
Bacopa monnieri (L.) Wettst.	Irregular	Polygonal	P	P	Diacytic	Anisocytic	P	P	Peltate / Glandular sessile trichomes	Peltate
Veronica anagallis-aquatica L.	Irregular	Irregular	P	P	Anomocytic	Anomocytic	P	P	__	Unicellular non-glandular
Ranunculus scleratus L.	Wavy	Wavy	P	P	Paracytic	Paracytic	A	A	__	__
Ranunculus repens L.	Irregular	Irregular	P	P	Paracytic	Paracytic	P	P	Unicellular Non-glandular	Unicellular Non-glandular
Phyla nodiflora (L.) Greene	Isodiametric	Isodiametric	P	P	Anisocytic	Anisocytic	A	A	__	__
Verbascum thapsus L.	Irregular	Irregular	P	P	Anomocytic	Anisocytic	P	P	Stellate	Stellate
Salvia plebeia R.Br.	Irregular	Irregular	P	P	Anomocytic	A	P	P	Simple Multicellular Non-glandular	Simple Multicellular Non-glandular
Typha angustifolia L.	Rounded	Rounded	P	P	Paracytic	Paracytic	A	A	__	__
Arundo donax L.	Irregular	Irregular	P	P	Paracytic	Paracytic	P	P	Prickles	Prickles
Polypogon monspeliensis (L.) Desf.	Elongated	Elongated	P	P	Paracytic	Paracytic	A	A	__	__
Phragmites australis (Cav.) Trin. ex Steud.	Irregular	Irregular	P	P	Paracytic	Paracytic	A	A	__	__
Phragmites karka (Retz.) Trin. Ex Steud.	Irregular	Irregular	P	P	Paracytic	Paracytic	A	A	__	__
Cynodon dactylon (L.) Pers.	Irregular	Irregular	P	P	Paracytic	Paracytic	P	P	Prickles	Prickles
Brachiaria reptans (L.) C.A.Gardner & C.E.Hubb.	Irregular	Irregular	P	P	Paracytic	Paracytic	A	A	__	__
Echinochloa colona (L.) Link	Irregular	Irregular	P	P	Paracytic	Paracytic	P	P	Prickles	Prickles
Dichanthium annulatum (Forssk.) Stapf	Irregular	Irregular	P	P	Paracytic	Paracytic	P	P	Prickles	Prickles
Persicaria maculosa S.F. Gay, Nat.	Tetragonal	Tetragonal	P	P	Anomocytic	Anomocytic	P	P	Unicellular Glandular	Unicellular Glandular

Ad*: Adaxial; Ab**: Abaxial; P: Present; A: Absent.

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Table 3 – Quantitative characterization of foliar epidermal anatomy of wetland Flora of Azad Kashmir.

Plant species	LxW	Epidermal cell Min-Max=Mean+S.E (µm)		Stomata Min-Max=Mean+S.E (µm)		Trichome Min-Max=Mean+S.E (µm)		Stomata Pore Min-Max=Mean+S.E (µm)		Stomatal Index	
		Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
Alternathera sessilis	L	—	—	12.5-20=16.5+1.2	17.5-25=21.5+1.5	—	—	12.5-17.5=15+0.79	12.5-17.5=15.5+0.93	11	9
	W	25-50=30+4.7	25-30=27.5+1.1	10-15=12.5+1.11	12.5-17.5=14.5+0.93	350-450=400+35.3	50-70=60+4.47	2.5-5=3+0.5	2.5-5=3+0.5		
Achyranthus aspera	L	35-45=40+5	87.5-102.5=95+7.5	22.5-27.5=25+2.5	20-25=22.5+2.5	45-55=50+5	72.5-85=78.75+6.25	12.5-17.5=15+2.5	10-15=12.5+2.5	9.7	34.3
	W	20-25=22.5+2.5	15-22.5=18.75+3.75	15-20=17.5+2.5	19-23=21+2	12.5-20=16.25+3.75	12.5-20=16.25+3.75	2.5-7.5=5+2.5	5-7=6+1	—	—
Amaranthus spinosus	L	50-75=62.5+12.5	47.5-87.5=67.5+20	12.5-25=18.75+6.25	12.5-20=16.25+3.75	70-92.5=81.25+11.25	62.5-87.5=75+12.5	10-17.5=13.75+3.75	10-17=13.5+3.5	7.4	3.5
	W	22.5-55=38.75+16.25	30-60=45+15	10-20=15+5	10-17.5=13.75+3.75	15-20=17.5+2.5	12.5-22.5=17.5+5	5-12.5=8.75+3.75	5-7.5=6.25+1.25	—	—
Amaranthus viridis	L	50-75=62.5+12.5	77.5-97.5=87.5+10	17.5-22=19.75+2.25	20-25=22.5+2.5	—	—	12.5-15=13.75+1.25	7.5-10=8.75+1.25	21.6	23
	W	50-62.5=56.25+6.25	30-37.5=33.75+3.75	12.5-17.5=15+2.5	12.5-17.5=15+2.5	—	—	5-10=7.5+2.5	5-7.5=6.25+1.25	—	—
Alisma plantago-aquatica	L	52.5-116.25=85.8+11.71	35-50.25=43.3+3.02	37.25-51.25=43.45+2.24	30-49.75=41.2+3.76	—	—	25-33.75=29.7+1.57	16-37=29.8+3.67	6.81	29.55
	W	18.75-37.5=30+3.37	32.5-52.5=41+3.24	17.75-32.25=23.2	21.25-30=24.2+1.57	—	—	4.75-10=7.2+0.86	5-9.5=7.46+0.73	—	—
Plantago lanceolata	L	14-31=22.5+8.5	40-56=48+8	17-26=21.5+4.5	27-33=33+3	—	—	12-16.5=14.25+2.25	12-16=14+2	26.5	34.5
	W	27-50=38.5+11.5	24-33=28.5+4.5	7-9.5=8.25+1.25	11-13=12+1	—	—	4.5-8=6.25+1.75	7-10.5=8.75+1.75	—	—
Plantago major	L	17-30=23.5+6.5	36-70=53+17	22-29.5=25.75+3.75	20-31=25.5+5.5	—	—	14.9-18.5=16.7+1.8	14.5-19.5=17+2.5	32.5	22.5
	W	11-14.5=12.7+1.75	23-34=28.5+5.5	14.5-18=16.25+1.75	16-19=17.5+1.5	—	—	4.70-7=5.85+1.15	4.5-7=5.75+1.25	—	—
Bacopa monnieri	L	29-31=30+1	30-61=45.5+15.5	25-31=28+3	19-29=24+5	—	—	16.5-29=22.75+6.25	16.9-24.5=20.7+3.8	16.6	15.6
	W	26-36.5=31.25+5.25	24-48=36+12	14-19=16.5+2.5	14.5-19.5=17+2.5	—	—	7-9.5=8.25+1.25	7.5-9.5=8.5+1	—	—
Veronica anagallis-aquatica	L	48-60=54+6	39-58=48.5+9.5	24-36=30+6	17-29=23+6	—	—	17-26=21.5+4.5	25.5-29.9=27.7+2.2	23.4	28.2
	W	24-37=30.5+6.5	26-47=36.5+10.5	12-19=15.5+3.5	16.5-19.5=18+1.5	—	—	2.6-7=4.8+2.2	2.4-7.9=5.15+2.75	—	—
Ranunculus scleratus	L	15-20=17+0.93	17.5-25=21+1.27	15-20=18+0.93	11-12.5=11.75+1.06	—	—	10-12=11+0.61	10-15=12.5+0.79	86.5	28.9
	W	5-7.5=6+0.61	7.5-10=9+0.61	15-17.5=16+0.61	6-7.5=6.75+1.06	—	—	2.5-5=3+0.5	2.5-5=3.5+0.61		
Ranunculus repens	L	45-77.5=61.5+5.39	36.25-75=51+7.23	45-62.5=54.27+2.84	37.5-56.25=49.75+3.31	212.5-412.5=312.5+41.61	275-425=350+26.69	30.25-49.75=41.85+3.46	25-45=36.2+3.92	39.81	33.66
	W	35-47.5=40.5+2.15	32.5-47.5=39+2.69	33-42.5=38.6+1.6	30-40=33.75+1.76	22.5-42.5=34.5+3.48	11.25-25=19.4+2.79	7.5-13.75=10.05+1.16	8-12=9.8+0.73		
Phyla nodiflora	L	29-53=41+12	36-80=58+22	24.5-31=27.75+4.59	25-29=27+2	—	—	14-21=17.5+4.94	14.5-19=16.75+2.25	19.1	19.5
	W	31-43=37+6	34-51=42.5+8.5	21-29=25+5.65	17-24.75+3.75	—	—	7-12=9.5+3.5	2.5-8=5.35+2.75	—	—
Verbascum thapsus	L	37-80=58.5+21.5	72-125=98.5+26.5	12-21=16.5+6.36	19-29=24+5	120-220=170+50	60-145=107+42.5	9-21=15+8.48	18-31=24.5+6.5	4.6	8.5
	W	23-47=35+12	17-36.5=26.75+9.75	10.5-14=12.25+2.47	9.5-14.5=12+2.5	7-16=11.5+4.5	11-14==12.5+1.5	2-4.5=3.25+1.76	2.3-4.5=3.4+1.1	—	—

Salvia plebeia	L	48-96=72+24	36-90=63+27	19-29=24+5	17-29.5=23.5+6.25	40-70=55+15	36-78=57+21	17-23=20+3	14.5-19.5=17+2.5	25.3	14
	W	23-37=30+7	21.5-36.5=29=7.5	14.5-25.5=20+5.5	15-19=17+2	8-15.5=11.75+3.75	36-78=57+21	7-12=9.5+2.5	2.5-4.8=3.65+1.15	__	__
Typha angustifolia	L	29-51=40+11	31-43=37+6	14.5-19=16.75+2.25	14.5-19.5=17+2.5	__	__	14-18=16+2	12-18=15+3	16.9	19.5
	W	17-28=22.5+5.5	14.5-29=21.75+7.25	12-17=14.5+2.5	12-14.5=13.25+1.25	__	__	2.6-4.9=3.75+1.15	5-5.5=5.25+0.25	__	__
Arundo donax	L	75-115=95+20	72-102=87+15	24-33=28.5±4.5	24.5-29.5=27+2.5	__	__	14.5-19=16.75+2.25	24.7-29.5=27.1+2.4	24.5	29.7
	W	11.5-21=16.25=4.75	12-18=15+3	12-21=16.5+4.5	10.5-14.5=12.5+2	__	__	2.5-3=2.75+0.25	2.3-3=2.65+0.34	__	__
Polypogon monspeliensis	L	60-78=69+9	53-82=67.5+14.5	17-24=20.5+4.94	19.5-24.5=22+2.5	__	__	17-23=20+4.24	19.5-23.8=21.65+2.15	16.9	30
	W	16.5-29.5=23+6.5	14-36=25+11	15-21=18+4.24	16-24.5=20.25+4.25	__	__	15-19=17+2.82	15-19=17+2	__	__
Phragmites australis	L	36-53=44.5+8.5	45-54=49+4.5	24-31=27.5+27.5+4.94	24-29=26.5+2.5	__	__	14.5-17=15.75+1.76	12-17=14.5+2.5	22	38.3
	W	14-21=17.5+3.5	14-21=17.5+3.5	14-21=17.5+4.94	15.5-19=17.25+1.75	__	__	2.4-5.5=3.95+2.19	2.5-4.8=3.65+1.15	__	__
Phragmites karka	L	36-48=42.25+6.25	31-47=39+8	19-24.5=21.75+2.75	17-24.5=20.75+3.75	__	__	12-19=15.5+3.5	14-19=16.5+2.5	24.5	23.5
	W	12-18=15+3	11.5–16.9=14.2+2.7	12-23.5=17.75+5.75	12-16=14+2	__	__	5-6.5=5.75+0.75	5-7.9=6.45+1.45	__	__
Cynodon dactylon	L	42-60.5-51.25+9.25	37-53=45+8	19-29=24+5	20-29=24.5+4.5	__	__	19-28.5=23.75+4.75	16.9-24.5=20.7+3.8	18.7	22.3
	W	14-21=17.5+3.5	12-17=14.5+2.5	12-18=15+3	13-18=15.5+2.5	__	__	2.5-4.5=3.5+1	4.7-5.3=5	__	__
Brachiaria reptans	L	43-59.5=51.25+8.25	42-63=52.5+10.5	29-34.5=31.75+2.75	31-34=32.5+1.5	__	__	12-17=14.5+2.5	12-17=14.5+2.5	16.6	27.2
	W	24-38=31+7	22-36.5=29.25+7.25	14.5-21=17.75+3.25	15.5-19.7=17.6+2.1	__	__	2.5-5.5=4+1.5	2.5-4.6=3.55+1.05	__	__
Echinochloa colona	L	70-85=77.5+7.5	42-63=52.5+10.5	21-32=26+1	25.5-32=28.75+3.25	__	__	14-16.5=15.25+1.25	14.5-16.8=15.65+1.15	27.3	18
	W	24-31=27.5+3.5	22-36.5=29.25+7.25	15-17=16+1	15.4-17=16.2+0.8	__	__	2.5-4.9=3.7+1.2	2.5-4.8=3.65+1.15	__	__
Dichanthium annulatum	L	23.9-68=45.95+22.05	63-88=75.5+12.5	17-29=23+6	17-29=23+6	__	__	16.5-29=22.75+6.25	17-29.5=23.25+6.25	16.6	31.3
	W	29-36=32.5+3.5	19-31=25+6	14.6-19=16.8+2.2	12-17=14.5+2.5	__	__	4.5-7=5,75+1.25	5-7.8=6.4+1.4	__	__
Persicaria maculosa	L	38.75-50=45.25+2.17	27.5-87.5=56+10.62	22.25-27.5=25.35+0.86	22.25-27.25=24.3+0.91	100-237.5=168.5+24.28	__	15-18.75=16.75+0.71	18.75-24.7=21.4+1.02	21.18	96.15
	W	30-42.5=36.25+2.3	15-40=26.45+4.72	16.25-23.75=20.25+1.44	16.25-21.25=18.4+0.91	30.25-60.25=44.9+4.92	__	1.25-5.25=3.2+0.76	3-7.75+4.9	__	__

L: length; W: width; Ad: Adaxial; Ab: Abaxial; Min: minimum; Max: maximum; S.E: standard error; µm: micrometer.

Plant name	Polar diameter (µm)	Equatorial diameter (µm)	P/E ratio (Polar axis/ equatorial axis)	Exine thickness (µm)	No. of Pores	Exine Ornamentation
Achyranthus asperaL. (Amaranthaceae)	7(8.2±0.3)9.1	6(6.9±0.8)10.5	1.05	0.8	36	Psilate to scabrate
Alisma plantago-aquatica L. (Alismataceae)	17.2(18.9±1.3)23.5	18.9(22.1±1.1)24	2.20	0.9	-	Psilate
Alternathera sessilis (L.) DC. (Amaranthaceae)	14 (18.25 ±1.2) 22.5	15 (18.6 ± 1.0) 22.5	1.00	0.8	15	Microechinate
Amaranthus spinosus L. (Amaranthaceae)	8(9.7±0.3)11.1	8.3(9.4±0.2)9.9	0.90	0.7	24	Psilate
Amaranthus viridis L. (Amaranthaceae)	5.6(6.6±0.2)7.8	6.6(7.7±0.9)9.2	0.91	0.8	16	Microechinate
Bacopa monnieri (L.) Wettst. (Plantaginaceae)	20 (22.5±1.0)25.0	22.5 (26.25± 1.3) 30	0.85	1.635	-	Psilate
Cynodon dactylon (L.) Pers. (Poaceae)	21.4(24.5±0.1)26.5	13.1(15.4±0.4) 22.5	1.51	1.21	-	Psilate
Plantago lanceolata L. (Plantaginaceae)	21.5(23.6±0.1)24.0	22.9(25.1±0.12)26	0.97	2.5	-	Psilate
Plantago major L.	18.9(22.1±0.17)27.4	14.7(19±0.2)23.5	1.04	1.9	8	Granulate
Ranunculus repens L. (Ranunculaceae)	25.5 (30.29±0.6) 32.75	25 (32.5±0.4) 31	0.90	2.2	-	Scabrate
Ranunculus scleratus L. (Ranunculaceae)	22.6 (19.4±0.6)16.0	16.5(14.2±0.7)10.1	1.40	1.00	-	Psilate to scabrate
Typha latifolia L.	22.98(24.9± 1.5)28.9	26(31.2 ± 1.4)33.2	0.70	2.35	-	Rugulate to scabrate
Veronica anagallis-aquatica L. (Plantaginaceae)	17.9 (22.2±0.5)25.4	15.9(19.7±0.2)23.5	1.13	2.2	-	Psilate

Table 4 – Qualitative and quantitative features of pollen of some wetland species.

2.4.1 *Stomatal index*

For the stomatal index, we counted the number of stomata and epidermal cells per unit area from 5 different oculars for adaxial and abaxial surfaces.

$$S.I = S/S+E\ (100)$$

(In this equation S.I = stomatal index, S = number of stomata per unit area, E = number of epidermal cell per unit area)

3. Results and discussion

In the present study, examination of foliar epidermis and pollens under light and scanning electron microscope variations were found in both qualitative and quantitative features. A taxonomic key was prepared by using these qualitative characters for the identification and differentiation of wetland plant species.

3.1 Pollen micromorphology

In this study, pollen observed are mostly psilate in sculpturing while some species have microechinate and scabrate exine sculpturing. In some species, the number of pores also varies as *Achyranthes aspera* L has 36 pores while *Amaranthus spinosus* L has 24 numbers pores in its pollen, *Amaranthus viridis* L has 16 pores *Alternanthera sessilis* (L.) R.Br. ex DC has 15 pores and *Plantago major* L has 8 pores. In the species of family Amaranthaceae, spheroidal or sub-spheroidal types of pollens were observed, in species of family Ranunculaceae prolate and sub spheroidal types were observed, in family Plantaginaceae spheroidal types of pollens were found (Figure 5). The maximum polar to equatorial ratio was observed in *Alisma plantago-aquatica* L. which is 2.2 followed by *Cynodon dactylon* (L.) Pers. 1.51, *V. anagallis-aquatica* 1.13. The thickest exine is observed in *Plantago lanceolata* L. 2.5 *Typha latifolia* L. 2.35; *Ranunculus repens* L. 2.2 and *V. anagallis-aquatica* 2.2. The highest diameter in the polar view is found in *R. repens* is 30.29 µm, *T. latifolia* is 24.9 µm and *C. dactylon* is 24.5 µm.

Variations are found in pollen micro-morphological characters including the shape of pollens, size of pollens, exine sculpturing, etc. Pollen morphology has been studied previously by (Perveen, Qaiser, 2012; Hussain et al., 2018) in which they reported that exine sculpturing of the Amaranthaceae family was scabrate to psilate, while in our study *A. sessilis* have micro-echinate exine ornamentation. Similarly, exine of *T. latifolia* has Rugulate to scabrate sculpturing confirmed the findings of (Bahadur et al., 2018). The pollen micro-morphology also provides delimitation of the species within the genus of the family (Dönmez et al., 2008).

Exine of the pollen was the basic character for differentiation between the species of different families. The exine is often micro-reticulate scabrate and psilate in this study which is a taxonomically significant feature examined under SEM. Similarly, Butt et al. (2018), Kosenko (1999), Sufyan et al. (2018), Ullah et al. (2018), investigated pollen of various taxa, using LM and SEM techniques and reported that the pollen morphological characteristics were of important characters for the taxonomic identifications of species and genera in different plant families.

3.2 Foliar epidermal anatomy

3.2.1 *Epidermal cells*

Great variations in epidermal cells have been observed in their number size and shape. Epidermal cells are found to be irregular in most of the species of family Amaranthaceae, Plantaginaceae, Ranunculaceae, Scrophulariaceae, Lamiaceae, Poaceae, and other species having polygonal, elongated, Isodiametric, tetragonal and wavy type of cells. Isodiametric epidermal cells are found in *P. lanceolata* and *Phyla nodiflora* (L.) (Figure 4). While tetragonal cells are present in *Persicaria maculosa* Gray (Figure 1) and elongated cells are found in *Polypogon monspeliensis* (L.) Desf and *A. aspera*. Polygonal cells are the character of *A. plantago-aquatica* and *P. major* (Figure 2). Different length of epidermal cells was found in the same species, the variation in cell size, large cells were observed in *Arundo donax* L. having irregular walls ranges from 75-115(95 ± 20) µm while minimum size was 22.5 µm studied in *P. lanceolata* on the upper surface. The maximum length on the lower surface was examined in *A. aspera* 95 µm while the minimum length 21µm on the lower surface. Based on width large cells having irregular walls and size ranges from 56.25 µm in *A. viridis* to minimum size 6 µm *Ranunculus sceleratus* L. in the adaxial surface. On the abaxial surface, the maximum sizes of the cell were 42.5µm *P. nodiflora* to a minimum of 9 µm in *R. scleratus*.

3.2.2 *Stomatal morphology*

Variations among stomata were also observed in upper and lower surfaces of the leaf epidermis in all 24 species of wetland. Three types of stomata were studied, i.e. Anomocytic, Anisocytic, and Paracytic. Paracytic stomata were observed in 12 species; anomocytic type of stomata was found in 11 species remaining 2 species had anisocytic stomata. Large stomatal sizes were observed in *R. repens* and minimum size was observed in *A. sessilis* on the upper surface, while on lower surface size ranges from 49.75 µm *R. repens* to minimum (11.75) µm in *R. scleratus* as mentioned in Table 3. Based on width, lower and upper surfaces showed variation. The largest stomata on the upper surface are found in *R. repens* 38.6 µm and smaller are found in *P. lanceolata* 8.25 µm. On the lower surface, *R. repens* 33.75µm has a maximum stomata size while the minimum size is observed in *R. scleratus* 6.75 µm (Figure 5). The upper epidermal surface was observed to have a large size of stomatal pore than lower surfaces in a continuous manner. *R. repens* 41.85 µm has the largest size of stomatal pore on the upper epidermis while *R. scleratus* L (11 µm) has a minimum mean size of the stomatal pore. Similarly, on lower epidermis *R. repens* 36.2 µm has the largest size of

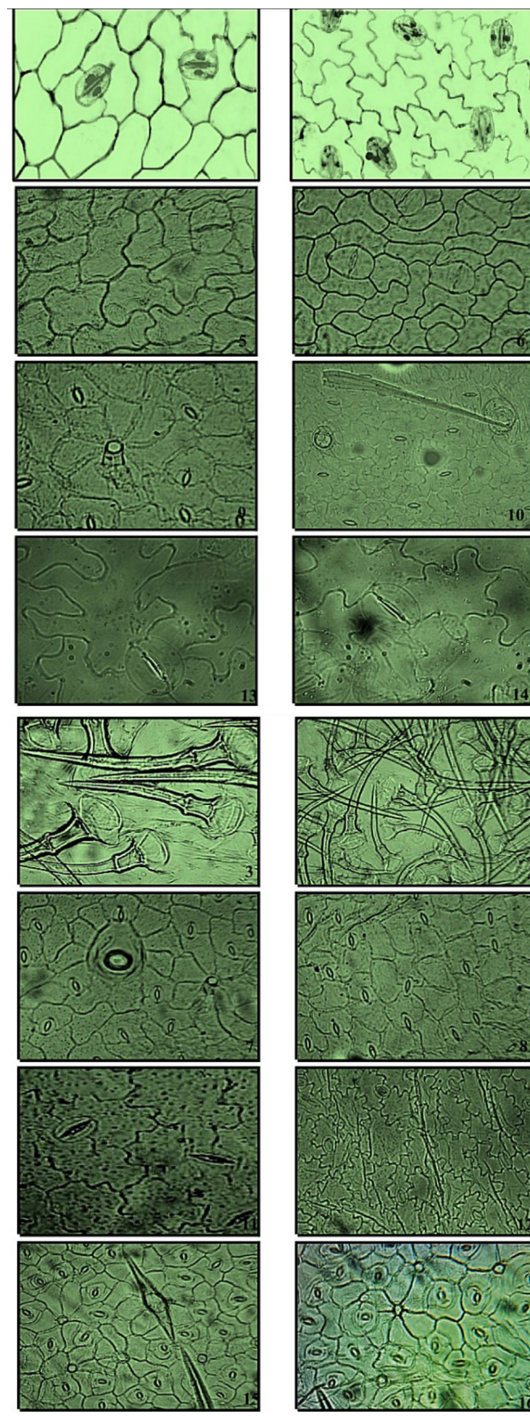

Figure 1 - Light microphotographs (LM 10X) of *Alternanthera sessilis* (1-2), *Achyranthus aspera* (3-4), *Alisma plantago-aquatica* (5-6), *Plantago lanceolata* (7-9), *Bacopa monnieri* (10), *Veronica anagallis-aquatica* (11-12), *Ranunculus sceleratus* (13-14), *Phyla nodiflora* (15-16).

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pore while *A. viridis* 8.75 µm has the smallest pore size. The stomatal index of the species varied from the highest 86.5% *R. sceleratus* to the minimum 4.6% *Verbascum thapsus* L. on the upper epidermis, while at the lower epidermis maximum stomatal index is 96.15% *P. maculosa* and minimum 3.5% *Amaranthus spinosus* L. (Table 3) (Figure 3).

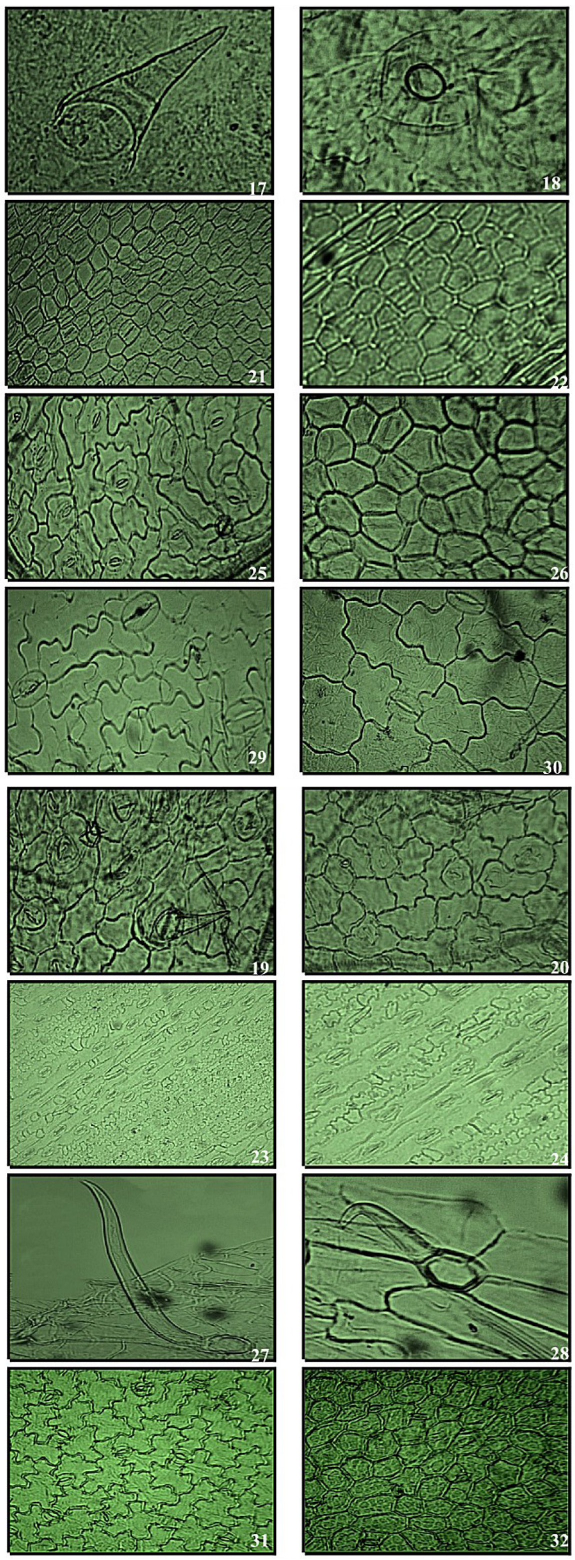

Figure 2 - Light microphotographs (LM 10X) of *Salvia plebeia* (17-20), *Typha angustifolia* (21-22), *Arundo donax* (23-24), *Persicaria maculosa* (25-28), *Ranunculus muricatus* (29-30), *Amaranthus spinosus* (31-32).

3.2.3 *Trichomes morphology*

In 9 species, trichomes were present on both leaf surfaces. Diverse trichomes and foliar appendages i-e prickles are mostly found on the upper surface but also observe on lower surfaces. Foliar appendages were observed in *A. donax, C. dactylon, Echinochloa colona* (L.) Link, *Dichanthium annulatum* (Forssk.) Stapf. Barrel-shaped trichomes were found in *P. major* while stellate trichomes were also observed in *V. thapsus* and peltate type was present in *Bacopa monnieri* (L.) Wettst. Based on width largest trichome was observed in *A. sessilis* (400 µm), (60 µm) on adaxial and abaxial surfaces. Lengthwise the largest trichome size was observed in *R. repens* (312.5 µm), (350 µm) on both surfaces respectively. The foliar epidermal anatomy of *A. aspera* in our study showed that the adaxial surface of the cells is elongated while the abaxial side observed irregularly. Stomata are anomocytic on adaxial surface, and paracytic type of stomata was studied on abaxial surface, while in the study of (Ogundipe, Kadiri, 2012) the shapes of cells on both upper and lower surfaces were irregular. Stomata types showed that were observed anomocytic and paracytic two types on both surfaces. In our study we observed trichomes attenuate and bulbous base while previously study showed that trichomes were absent. In *A. spinosus* epidermal cells are irregular and stomata anomocytic on both adaxial and abaxial surfaces as observed by (Ogundipe, Kadiri, 2012). The foliar epidermal cells of *A. viridis* showed in the previous study that the shape of cells is irregular same in our finding, the stomata types in the study of (Ogundipe, Kadiri, 2012) was anomocytic and paracytic type in our results the stomata is Anisocytic and Anomocyic.

Foliar epidermal anatomy study of *A. donax*, *P. monspeliensis*, *Phragmites karka*, *C. dactylon*, and *D. annulatum* show similar stomata type paracytic types with the recent study of (Khan et al., 2017) working on family Poaceae. *E. colona* have tetragonal epidermal cells in our finding while (Ferreira et al., 2002; Jattisha, Sabu, 2015) studied showed that the species had wavy cells. The shape of epidermal cells in *P. major* in our study showed polygonal shape, while in the study of (Ahmad

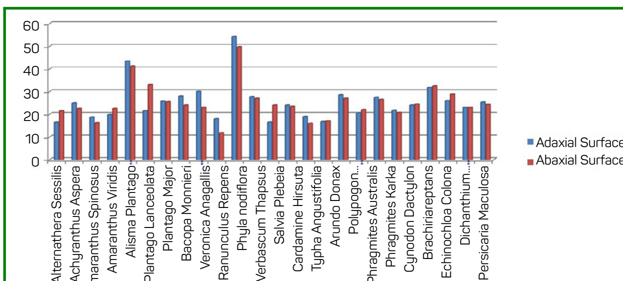

Figure 3 - Graphical representation of stomatal cells length of both surfaces.

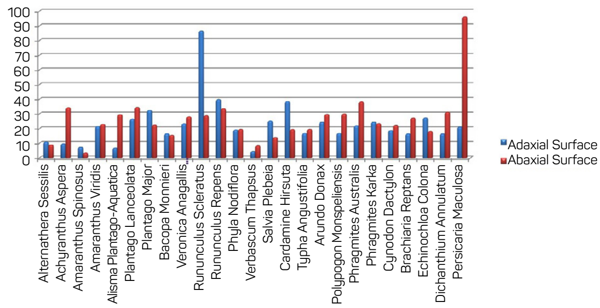

Figure 4 - Graphical representation of stomatal index of both surfaces.

et al., 2010) observed tetragonal to polygonal. In the study of (Lan et al., 2008) leaf epidermal anatomy of *R. scleratus* was irregular to polygonal while in our study the shape of the cells is wavy. In the present study *Salvia plebeia* R.Br. having anomocytic while in the study of (Dimitrova, Yurukova, 2005) the relative species had diacytic type of stomata. *T. angustifolia* in our research we examined round shape of epidermal cells previously (Ni et al., 2014) had also round epidermal cells. *P. maculosa* have anomocytic type of stomata on both surfaces while unicellular trichomes were also observed while in previous study the species had paracytic type of stomata and the trichomes were absent (Yasmin et al., 2010).

Twenty-four plant species belonging to 11 families were investigated in this work; palyno-anatomical features of these species were studied both qualitative and quantitatively. In the case of anatomical studies, clear differences were observed on the epidermal adaxial and abaxial surfaces. While in pollen morphological investigation a significant difference was observed in pollen shape, size, and diameter of the polar and equatorial axis, exine thickness, and sculpturing. The present research work was compared with the previous study reveals and confirms that the leaf epidermal light microscopy is of very important value for the identification of wetland flora. These characters have great information and possess important information for the correct identification of plants. In this research work, we adopted different taxonomic keys based on qualitative characters like trichomes types, epidermal cells shape, stomata types, prickles, etc. These features play a vital role in the correct identification of plants and delimitation of complex species.

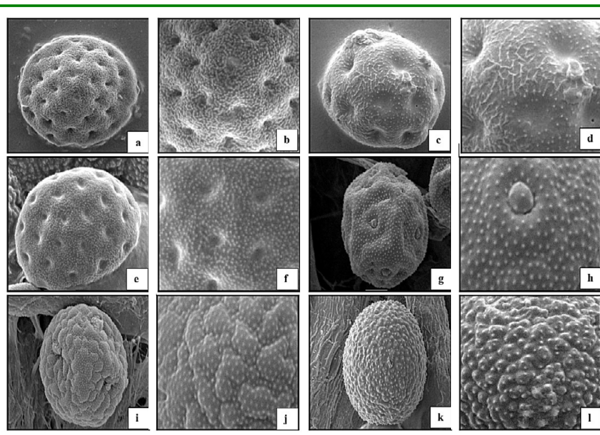

Figure 5 - SEM micrographs of *Achyranthus aspera* (a-b), *Alternanthera sessilis* (c-d), *Amaranthus spinosus* (e-f), *Plantago lanceolata* (g-h), *Plantago major* (i-j), *Ranunculus repens.*

3.3 Economic importance and limitation management of wetland weeds

Wetland plants play important role in maintaining the nutrient balance in the water. Turbidity and excessive erosion are prevented by the diversity of wetland plants (Marwat et al., 2011). Similarly, Khan et al. (2013) documented the medicinally important 73 weed species used for the management of different disorders. These weed species are helpful for the manufacturing of different plant yields and also useful for the indigenous sellers of crude drugs of plants. The pattern of distribution, abundance, and richness to the diversity of weed species (e.g *P. lanceolate, A. viridis, V. Thapsus, D. annulatum, C. dactylon*) depend upon the environmental variables such as pH, $CaCO_3$, soil texture, organic matter, electrical conductivity, phosphorous and nitrogen concentration. Rafay et al. (2014) reported that in agroecosystem weeds play a significant role by giving the environmental heterogeneity and floral diversity is increase for the cropping system. Williams and Kremen (2007) described that different types of invertebrates are supported by non-crop plants because many insects for survival depend upon specific weed. On the other hand, weeds play a serious role in crop production and threat to the cultivated lands because of the use of nutrients from the soil. Weeds badly affect the yield and growth of crop plants because of nutrient demand. Weed vegetation is eliminated by the use of different control measures for example chemical approaches, cultural procedures, and biological mechanisms. In cultural operations, weeds are thrown away from the rice field (Khan et al., 2013). Identification of different weed species and farming practices plan with revised management is suggested (Iqbal et al., 2017). *A. aspera* is a common weed for cane crop and Jogil et al. (2019) reported that greater yield for sugarcane crop with highest height and density was produced if the crop treated with Buctril M @ 3.75 kg ha^{-1}. Similarity the judicious management is recommended for the harvesting of potential chickpea production (Hassan et al., 2010).

3.4 Taxonomic keys based on leaf micro-morphological features

1 + Epidermal cells irregular on both surfaces, stomata anomocytic .... *Alternanthera sessilis*
- Epidermal cell elongated on adaxial surface, irregular on abaxial, stomata paracytic on abaxial surface 2

2 + Trichomes present on both surfaces with bulbous base .... *Achyranthus aspera*
- Trichomes are absent on both surfaces........................3

3 + Epidermal cells on both surfaces irregular, stomata anomocytic on both surface .... *Amaranthus spinosus*
- Epidermal cells are irregular on surface 4
4 + stomata anisocytic and anomocytic...*Amaranthus viridis*
- stomata anomocytic 5
5 + epidermal cells are polygonal on both surfaces............. .... *Alisma plantago-aquatica*
- epidermal cells Isodiametric 6
6 + stomata anomocytic ....*Plantago lanceolata*
- epidermal cells polygonal.. 7
7 + stomata anisocytic .... *Plantago major*
- Stomata anomocytic on both surfaces..........................8
8 + epidermal cells on abaxial surface is polygonal.............. .... *Bacopa monnieri*
- Epidermal cells on both surfaces are irregular..............9
9 + trichomes are present on abaxial surface unicellular..... .... *Veronica anagallis-aquatica*
- Trichomes are absent 10
10 + Epidermal cells are wavy...............*Ranunculus scleratus*
- Epidermal cells are irregular 11
11 + Trichomes unicellular non-glandular.......................... ....*Ranunculus repens*
- Trichomes are absent...12
12 + Epidermal cells are isodiametric .... *Phyla nodiflora*
- Epidermal cells are irregular..................................... 13
13 + Trichomes are stellate.....................*Verbascum Thapsus*
- Trichomes are multicellular non glandular.............. 14
14 + Stomata are anomocytic on adaxial surface and absent on abaxial surface...................................*Salvia plebeian*
- Stomata paracytic... 15
15 + Epidermal cells are rounded .... *Typha angustifolia*
- Epidermal cells Irregular... 16
16 + Trichomes are in prickles........................*Arundo donax*
- Trichomes are absent..17
17 + Epidermal cells elongated........*Polypogon monspeliensis*
- Epidermal cells are irregular.....................................18
18 + stomata are paracytic....................*Phragmites australis*
- Stomata are anomocytic 19
19 + Epidermal cells are tetragonal.......*Persicaria malculosa*
- Epidermal cells are irregular.....................................20
20 + Trichomes are absent..........................*Phragmites karka*
- Trichomes are prickles 21
21 + Epidermal cells irregular................... *Cynodon dactylon*
- Epidermal cells on adaxial surface irregular and wavy on abaxial surface...22
22 + Trichomes are absent on both surfaces ....*Brachiaria reptans*
- Trichomes are prickles on both surfaces 23
23 + Epidermal cells Irregular ....*Echinochloa colona*
- Epidermal cells wavy.............. *Dichanthium annulatum*

4. Conclusions

24 plant species belonging to 11 families were investigated in this work; palyno-anatomical features of these species were studied both qualitative and quantitatively. In the case of anatomical studies, clear differences were observed on the epidermal adaxial and abaxial surfaces. While in pollen morphological investigation a significant difference was observed in pollen shape, size, and diameter of the polar and equatorial axis, exine thickness, and sculpturing. The present research work was compared with the previous study reveals and confirms that the leaf epidermal light microscopy is of very important value for the identification of wetland weeds. These characters have great information and possess important information for the correct identification of plants. In this research work, we adopted different taxonomic keys based on qualitative characters like trichomes types, epidermal cells shape, stomata types, prickles, etc. These features play a vital role in the correct identification of plants and delimitation of complex species.

Author's contributions

MAB: primary author of this manuscript the data belongs to her. MZ: proofread the whole manuscript and helps in the collection of specimen. MA: helps in the write-up section. SK: helps in quantitative data analysis: SB, and FU: helps in experimental work. SK: helps in plant identification.

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