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EPIDERMAL AND POLLEN MORPHOLOGY OF TWO SPECIES OF *HYGROPHILA* ROXBURGH AND ITS TAXONOMIC RELATION

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ABSTRACT
Hygrophila erecta (Burman *f*.) Hochreutiner and *Hygrophila salicifolia* (Vahl) Nees are quite similar in their external morphology and thus quite difficult to identify the species. The foliar epidermal morphology and pollen morphology were carried out on *Hygrophila erecta* (Burman *f*.) Hochreutiner and *Hygrophila salicifolia* (Vahl) Nees with the aim of determining the patterns of variation in their epidermal characteristics and assessing their value in species identification and classification. Relatively significant higher stomatal index, stomatal frequency, epidermal cell frequency and presence of trichomes in *Hygrophila erecta* (Burman *f*.) Hochreutiner and their differences with *Hygrophila salicifolia* (Vahl) Nees is taxonomically valuable to identify the species during vegetative phase. The different pollen exine sculpture could be also useful and appropriate measure in revealing species diversity .however both taxa showed inter species similarities in diacytic stomata and heterocolpate pollen grains.

KEY WORDS: Epidermal Morphology, Pollen morphology, Hygrophila *erecta* (Burman *f*.) Hochreutiner, *Hygrophila salicifolia* (Vahl) Nees.

INTRODUCTION

The use of epidermal characters such as stomatal types, stomatal frequency and index, trichome types *etc*. in classification seems to be increasing rapidly because not only the epidermal characters correlate with gross morphological features in most cases, they are often known to be much valuable at the levels where classical methods of cytology and genetics cannot be applied (Stace 1965). In dicotyledons, the systematic values of epidermal and cuticular features have been indicated. Many workers showed that leaves possess many morphological attributes of potential taxonomic significance those are often diagnostic at the genus and species levels (Arroyo, 1985, Edeoga, 1991, Mbagwu & Edeoga, 2006). The shape of epidermal cells, types and arrangement of stomata and size and shape of trichomes are important systematic parameters. In recent time, leaf epidermal features have received very considerable attention by taxonomists (Ayensu, 1970). Following this discovery, epidermal features became widely studied from three main perspectives: ontogenetic, phylogenetic and taxonomic (Mbagwu *et al.*, 2007). Of all the non-reproductive organs, the leaf is the most widely used in plant taxonomy (Stace 1965, 1984). Srivastava (1978) described the leaf epidermis as the second most important character after cytology for solving taxonomic problems. On the other hand the field of palynology has a tremendous contribution to the systematic and phylogeny of angiosperms because of the evolutionary trends in pollen wall architecture which provides an important source of phylogenetic information of major importance. Palynology is unique in that one can obtain tremendous amount of information from a little material in a short time (Walker & Doyle, 1975). The constant features and the sculpturing of the exine make pollen grains a highly recognizable object by which parent genera or even species may be recognized (Harris 1955, Moore & Webb, 1978, Ahmad 1978a,b). Application of pollen morphology in plant taxonomy is best evidenced in the flowering plants, especially in the angiosperms. The largest variety of pollen morph types occurs among the angiosperm plants (Nair 1964). The family Acanthaceae is documented for anatomy of foliar epidermis especially by Solereder (1908), Metcalfe and Chalk (1950) and Karlstrom (1979, 1980). Of late, Indian workers studied occurrence, structure, development and taxonomic significance of foliar epidermal features of the family (Pant & Mehra 1963, Paliwal, 1966, Kumar & Paliwal, 1975, 1978, 1982, Inamdar, 1970, Ahmad, 1974a, b, c, 1975a, b, 1976, 1979a, Selvaraj & Subramanian, 1983, Varma & Murty, 1989). *Hygrophila phlomoides* Nees and *Hygrophila salicifolia* (Vahl) Nees are nearly similar in their morphological characters (Ningombam and Singh, 2010) and thus difficult in their identification. Thus the present work is aimed to generate knowledge into the variation pattern of two species of *Hygrophila* Roxburgh based on their epidermal morphological and palynological data analysis. The aim of this study is to determine the patterns of variation in epidermal characteristics, to assess their value in species identification and classification and also using the pollen morphology in establishing the taxonomic relationships between these two species of *Hygrophila* Roxburgh.

MATERIALS & METHODS

Fresh leaves of *Hygrophila phlomoides* Nees and *Hygrophila salicifolia* (Vahl) Nees were collected from their auto natural habitat. They were confirmed and identified by using standard literature. The leaves were washed in tap water. The epidermal characters were studied from fully differentiated mature leaves. The mature leaves were choked and sample were taken separately from apical (A), middle (M) and basal (B) regions for epidermal preparation. Epidermal peeling was carried out from respective parts of the leaf pieces according to Chandra *et al.* (1996). These peelings were properly dehydrated through alcohol grade and stained with 1% safranin, mounted in glycerine with cover slip and observed under microscope. Pollen samples were obtained from different locality of Tripura or from Tripura University Herbarium (TUH). For SEM the pollen grains are preserved in Formalin Acetic Alcohol (FAA) at 4ºC temperature. The pollen grains were prepared for light and scanning microscopy (SEM) by the standard methods described by Erdtman (1952). For light microscopy, the pollen grains are mounted in glycerine–jelly and observations were made under an Olympus Microscope using a 10x eye piece. For SEM studies, pollen grains were first dried and then directly transferred with a fine needle to a metallic stub using double –sided adhesive tape and coated with gold in an IB2 ion coater. The SEM examination was carried out on a S530 Hitachi Scanning Electron Microscope, Japan at Burdwan University (USIC).

RESULTS & DISCUSSION

The quantitative epidermal characters recorded in the present two taxa are shown in Table 1.

TABLE 1: Epidermal morphological characters of two species of *Hygrophila* Roxburgh

Epidermal Character (Mean ±SD)	Leaf Surface	*Hygrophila phlomoides* Nees				*Hygrophila salicifolia* (Vahl) Nees			
		Apex	Middle	Basal	Mean	Apex	Middle	Basal	Mean
Stomatal Frequency	Upper surface	141.59 ± 17.69	79.64 ± 11.53	168.14 ± 10.83	129.79 ± 45.41***	65.49 ± 10.09	102.65 ± 11.25	65.49 ± 10.09	102.65 ± 11.25
	Lower Surface	164.60 ± 13.42	228.31 ± 13.12	102.65 ± 14.80	165.18 ± 62.83***	77.87 ± 13.12	93.80 ± 11.87	77.87 ± 13.12	93.80 ± 11.87
Stomatal Index	Upper surface	31.00 ± 2.69	19.59 ± 2.59	33.79 ± 1.43	28.12 ± 7.52**	17.90 ± 1.61	26.25 ± 2.67	17.90 ± 1.61	26.25 ± 2.67
	Lower Surface	33.42 ± 1.61	30.74 ± 1.87	23.13 ± 2.07	29.10 ± 5.33**	19.80 ± 2.96	25.44 ± 2.16	19.80 ± 2.96	25.44 ± 2.16
Stomatal Size (μm^2) Length× Breadth	Upper surface	95.92 ± 5.73	95.33 ± 3.59	97.533 ± 6.47	96.26 ± 1.13	124.08 ± 38.53	103.84 ± 15.74	124.08 ± 38.53	103.84 ± 15.74**
	Lower Surface	102.08 ± 20.08	95.04 ± 5.28	102.08 ± 20.08	95.04 ± 5.28	88.88 ± 5.73	102.96±1 4.79	103.84±1 2.67	98.56± 8.39 NS
Cell frequency/per mm^2	Upper surface	315.04 ± 27.70	311.50 ± 27.56	329.20 ± 13.12	318.58 ± 9.36***	299.15 ± 24.55	288.49 ± 13.42	299.15 ± 24.55	288.49 ± 13.42
	Lower Surface	327.43± 13.96	515.04 ±32.15	339.82 ± 19.38	394.09 ±104.92***	315.04 ± 22.21	274.33 ± 18.77	315.04 ± 22.21	274.33 ± 18.77
Cell size (μm^2) Length × Breadth	Upper surface	(47.28±3.81) × (24.12± 3.08)	(49.04± 9.21) × (26.48± 2.68)	(51.4± 7.69)× (25.2± 4.98)	(49.24± 2.06) × (25.26± 1.18)	(66.88 ± 17.70) × (29.92 ± 9.01)	(56.32 ± 9.53) × (34.32± 3.68)	(66.88 ± 17.70) × (29.92 ± 9.01)	(56.32 ± 9.53**) × (34.32 ± 3.68**)
	Lower Surface	(44.88± 4.81) × (25.52 ± 3.68**)	(51.04± 9.12) × (27.28 ±3.68)	(48.4± 7.99)× (24.2 ± 5.38)	(48.10± 3.09)× (25.66 ± 1.54)	(58.96 ± 9.12) × (38.72 ± 6.52)	(53.68 ± 10.03) × (35.2 ± 8.23)	(58.96 ± 9.12) × (38.72± 6.52)	(53.68 ± 10.03**) × (35.2 ± 8.23**)
Size Hairs (µm)	Upper surface	144.1 ± 28.37	116.16 ± 10.59	64.24 ± 20.06	108.16± 40.52	-	-	-	-
	Lower Surface	155.76 ± 28.78	179.52 ± 11.38	118.8 ± 9.33	151.36± 30.59	-	-	-	-

The results of this investigation showed some similarities and some differences those are taxonomically important. The numerical data analysis reveals that both the species shows clear difference in terms of their morphological characters. Characteristic presence of trichome on both surfaces of *Hygrophila phlomoides* leaf recorded (Fig.1), whereas no such trichome is found on either surface of *Hygrophila salicifolia* (Fig. 2). Trichomes on abaxial surface are slightly longer (151.36± 30.59) than those of adaxial surface (108.16± 40.52). Metcalfe (1954) pointed out that certain characters of the epidermis such as micro hairs, shape of the subsidiary cells of the stomata, and silica bodies are important systematically. The stomatal and epidermal characters might be used as an additive tool in taxonomy. The present data reveals that stomata of *Hygrophila salicifolia* are larger (U: 103.84 ±15.74; L: 98.56 ±8.39) than *Hygrophila phlomoides* (U: 96.26 ±1.13; L: 95.04 ± 5.28). However in both the species the stomata are diacytic. The common occurrence of diacytic type of stomata in both the species proves that this character can be used as generic character for *Hygrophila*. Stomata are found to occur on both surfaces of lamina of these two taxa. Higher stomatal frequency of *Hygrophila phlomoides* (U: 129.79 ± 45.41; L: 165.18 ± 62.83) as compared to Hygrophila *salicifolia* (U: 102.65±11.25; L: 93.80± 11.87) also found which is highly significant at $P \leq 0.01$ level. The average epidermal cell size of upper epidermal surface in *Hygrophila salicifolia* are 56.32 ± 9.53×34.32 ± 3.68 while in lower surface it is 53.68 ± 10.03×35.2 ± 8.23 µm while those of *Hygrophila phlomoides* were 49.24± 2.06 × 25.26±1.18 for upper epidermal surface while for lo were epidermal surface it is

48.10± 3.09 × 25.66±1.54 µm, respectively and found highly significant at $P \leq 0.01$ level. The Epidermal Cell Frequency /per mm^2 of upper epidermis of *Hygrophila phlomoides* are found to be 318.58 ± 9.36 whereas those in *Hygrophila salicifolia* are 288.49±13.42. Similarly the epidermal cell frequency/mm^2 of lower epidermis of *Hygrophila phlomoides* is 394.09 ±104.92 whereas those in *Hygrophila salicifolia* are 274.33 ± 18.77. In both the cases the epidermal cell frequency /mm^2 found to be highly significant ($P \leq 0.01$).Thus differential epidermal cell size of upper and lower leaf surfaces as recorded within and between species of *Hygrophila* could be very useful in revealing species diversity and their taxonomic significance. The use of leaf epidermal characters to elucidate the problem of recognition and identification of some members of Costaceae, Onagraceae, Fabaceae and Melastomataceae are reported earlier by Mbagwu & Edeoga (2006). The shape of epidermal cells, types and arrangement of stomata and size and shape of trichome are important systematic parameters (Mbagwu *et al*., 2007). The use of epidermal characters such as stomatal frequency, stomatal types and trichome types and index in classification seems to be much valuable at the levels where classical methods of cytology and genetics cannot be applied (Stace 1965).The significance of foliar morphological characters in differentiation of many plant taxa at species level have also been reported (Ahmad 1978b, Dehgan 1980, Baruah & Nath 1997, Baruah *et al*., 1999, Bhowmik *et al*., 2008, 2011). The Table 2 compares detailed measurements of different palynological features of *Hygrophila phlomoides* and *Hygrophila salicifolia*.Pollen grains of both the species are radially symmetrical, isopolar, heterocolpate, sub –oblate to oblate –spheroidal. In *Hygrophila phlomoides* the exine sculpture is reticulate (Fig 3,4) but in *Hygrophila salicifolia* the exine sculpture is coarsely reticulate (Fig 5,6). Pollen morphology is presently a global accepted tool in consideration of plant taxonomy and evolution, and with the SEM providing information on finer architecture, the application of pollen in comparative morphology has become possible in gaining new knowledge at varietal and even ecosystem levels (Nair, 1964). The constant features and the sculpturing of the exine make pollen grains a highly recognizable object by which parent genera or even species may be recognized (Harris 1955, Moore & Webb 1978). The variation as reported in the present study in terms of exine pattern of the studied species suggests the feasibility of applying the data in the identification of the genus of *Hygrophila*.

TABLE 2: Pollen morphological characters of two species of *Hygrophila* Roxburgh

Characters studied	*Hygrophila phlomoides* Nees	*Hygrophila salicifolia* (Vahl) Nees
Aperture type	Heterocolpate (4 colporate , 15-17 colpate)	Heterocolpate (4 colporate , 14-16 colpate)
Exine Sculpture	Reticulate.	Coarsely reticulate.
Exine Thickness (µm)	1.25 ± 0.22	1.28 ± 0.11
Equatorial Diameter (µm)	54.23 ± 1.29	32.04 ± 2.70
Polar Axis (µm)	46.39 ± 1.03	31.21 ± 1.43
Length of Colpi (µm)	35.59 ± 0.73	27.32 ± 0.57
Breadth of Ora (µm)	9.86 ± 1.27	6.41 ± 0.27

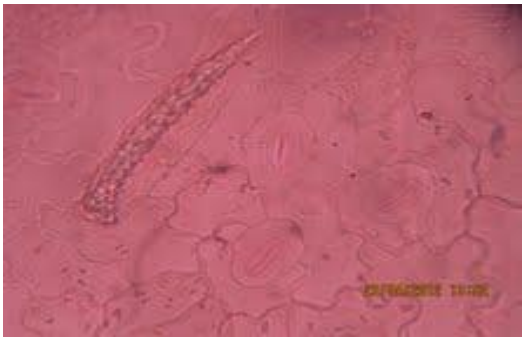

FIGURE 1. Stomata of *Hygrophila phlomoides* Nees showing trichome

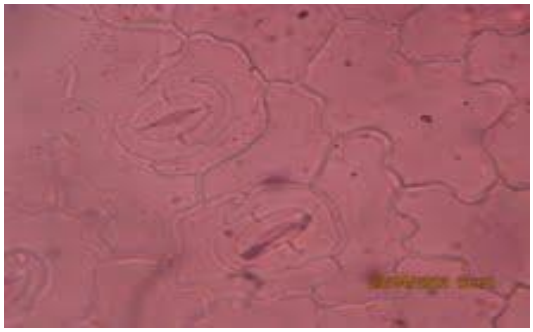

FIGURE 2. Stomata of *Hygrophila salicifolia* (Vahl) Nees)

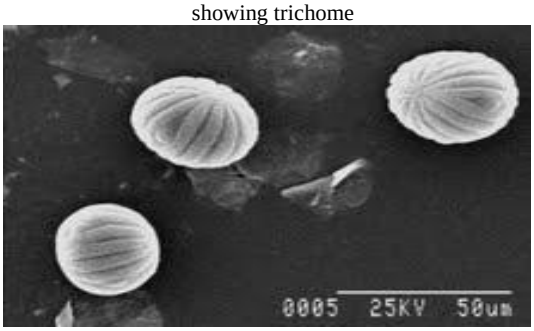

FIGURE 3. Pollen grains of *Hygrophila phlomoides* Nees showing colpi and ora (X800)

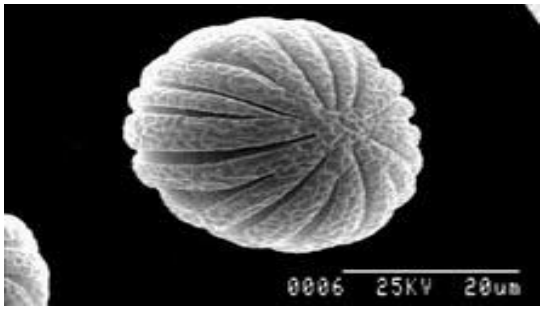

FIGURE 4. Reticule exine of *Hygrophila phlomoides* Nees (X 2000)

FIGURE 5. Pollen grains of *Hygrophila salicifolia* (Vahl) Nees showing colpi and ora (X1000)

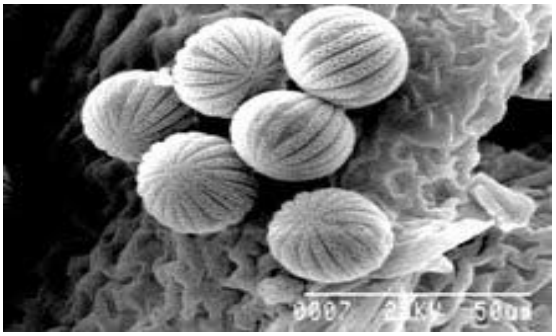

FIGURE 6. Coarsely reticule exine of *Hygrophila salicifolia* (Vahl) Nees (X3000)

The present study reveals that some of the characters, which shows significant differences in between these two species, may be typical of the genus despite the many anatomical variations exists between them. The leaf epidermal characteristics of these taxa have shown the taxonomically importance of this line of evidence, suggesting that similarities in structures showed interspecies relationships while differences in structures showed no relationship among the investigated taxa. Therefore the present study on foliar micromorphometric and palynological characters clearly indicated its significant utility in demarcation and distinction between the two species of *Hygrophila* .The present observation can be attributed due to genotypic variability and diverse ecophysiological adaptive values of these species.

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