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LEAF EPIDERMAL ANATOMY OF SELECTED *ALLIUM* SPECIES, FAMILY ALLIACEAE FROM PAKISTAN

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Abstract

Leaf epidermal anatomy of the selected *Allium* species showed variation in size and shape of stomatal cells, stomatal cavity, micro and macro hairs, trichomes, silica bodies and long cells. Leaf epidermal anatomy prooved a significant tool for the resolution of taxonomic confusions of the *Allium* species. *Allium consanguineum* had most diverse leaf epidermal anatomy. This species had longest stomatal cells (6-14 µm) and silca bodies (6-14 µm). Presence of micro hairs is an important distinguishing character for *A. carolianum*, the length of micro hairs varies from 150-200 µm. Only dumb-bell shaped silica bodies were observed in 6 different species viz., *A. dolichostylum, A. borszczewii, A. micranthum, A. consanguinem, A. stocksianum and A. stoliczki*. Trichomes were present in *A. barszczewksi, A. borszczowii, A. micranthum, A. lamondae, A. miserbile, A. longicollum, A. gilli and A. dolichostylum,* Cluster analysis based on anatomical characters revealed that 18 species of the genus *Allium* were divided into 2 main clusters at the phylogenetic distance of 79%. Lower order classification of the genus *Allium* on the basis of anatomical characters is entirely different from morphological classification.

Introduction

The taxonomic position of *Allium* and related genera has long been the matter of controversy. In early classification of angiosperm (Melchior, 1964), *Allium* was placed in Liliaceae. Later on the basis of inflorescence structure it was more often included into Amaryllidaceae. Recently molecular data have favored a division of Liliaceae into large no of small monophyletic families. In the most recent taxonomic treatment of Monocotyledons, *Allium* and its close relative were recognized as distinct family Alliaceae (Robert, 1992).

In the past criteria for taxonomic studies of *Allium* was morphometery (Nasir, 1972) choronolgy (Kwiatkowski, 1999) and palynology (Kioug *et al*., 1998). Leaf epidermal anatomy for the taxonomic purposes was first time studied by Kioug *et al*., (1998). They found that significant difference is occurred in shape and size of epidermal cells. Leaf of the genus *Allium* L., is the uni facial and there is no difference between its abaxial and adaxial sides (Esau, 1965). The epidermis of the *Allium* species consist of stomata, guard cells, subsidiary cells, mature cells, trichomes, short cells and in some species macro/ micro hairs are also present (Esau, 1965).

Anatomical studies have been used successfully to clarify taxonomic status and help in the identification of different species (Gilani *et al.*, 2002). In the past anatomical studies incorporation with morphological studies for the resolution of taxonomic problems of monocots have been used. Webster (1983) studied the grass *Digiteria* anatomy for the taxonomic purposes. The aim of the present study was to find out the solution of existing taxonomic problems of species, which overlap in most of their morphological characters and to elucidate relationship of the critical taxa by utilization of leaf epidermal characters.

Materials and Methods

Leaves from living and dried specimens were used for anatomical studies. Dried leaves were placed in boiling water for a few minutes to soften the leaf until they became unfolded and were ready for epidermal scrapping. Fresh leaves were used directly for anatomical studies. Leaf samples were prepared according to the modified method of Cotton (1974) who followed Clarke's (1960) technique. The fresh or dried leaves were placed in a tube filled with 88% lactic acid kept hot in boiling water bath for about 50-60 minutes. Lactic acid is used to soften the tissues of leaf due to which its peeling off is made possible.

Allium L., species has unifacial leaf, the leaf was placed on tile, and then it was flooded with 88% cold lactic acid. The epidermis was cut across the leaf and scrapped away together with the mesophyll cells until only the epidermal layer of the leaf remained on the tile. A sharp scalpel blade was used for this purpose. The epidermis was placed outside uppermost and mounted in clean 88% lactic acid. The photographs of these mounted materials were taken using a camera (35mm.) mounted on the microscope.

Anatomical observations were made on available representative specimens of the taxa. The specimens of 16 different species of *Allium* L., were studied. Hierarchical clustering was constructed by un weighted pair group method with arithmetic average (UPGMA). The computer software SPSS v 11.0 was used for this purpose.

Results

Key to species

1a: Trichomes present ……...……..…… 2
1b: Trichomes absent ……..……...… 10

2a: Double celled trichome, 300-400µm, Silica bodies are present ……...…… 3
2b: Single cell trichomes, 100-350µm, silica bodies may or may not present …….. 4

3a: Cells are rectangular, walls wavy, semi isodimetrically arranged ……..…….. …….. *A. borszczewii*
3b: Cells are elongated, walls smooth, compactly arranged, ……..... *A. micranthum*

4a: Short cells present, average length 150µm, long cells average length 200µm …… ……. *A. lamondae*
4b: Short cells absent, long cells 100-250µm in length ……..… 5

5a: Trichome position is horizontal, covers the whole length of long cells ……..…. …….. *A. humile*
5b: Trichome position is vertical, covers more than one long cell ……..… 6

6a: Single cell trichome with pointed tip, 300-350µm long ……... 7
6b: Single cell trichome with round tip, 200-250 µm long ……. 8

7a: Mature cells and silica bodies are present,average length of silica bodies is 3 µm ..... ……... *A. dolichostylum*
7b: Mature cells and silica bodies are absent ……. *A. gilli*

8a: Trichomes originated from guard cells,interwall region is dark in colour …… ……... *A. barszczewksii*
8b: Trichome originated from subsidiary cells, inter wall region is transparent ……..... 9

9a: Stomatal cells evenly distributed, silica bodies absent, long cells average length 100µm ……..……. *A. miserabile*
9b: stomatal cells and silica bodies alternatively present, average length of long cells 235µm ……..… *A. stoliczikii*

10a: Silica bodies 2-6µm long ……..…….. 11
10b: Silica bodies absent ……...…….. 12

11a: Longs cells rectangular, ovarlap with each other give the appearance of double wall cells ……...……. *A. jacquemontii*
11b: Long cells elongated, thick walled, layer of repture cells present ….. *A. stocksianum*

12a: Macro hairs absent, long cells double walled ……..... 13
12b: Macro hairs present, average length 350µm, long cells single wall .... *A. longicollum*

13a: Micro hairs absent, long cells rectangular ……..…… 14
13b: Micro hairs present, average length 310 µm elongated long cells … *A. carolinianum*

14a: short cells present, average length 250µm oil droplets are present ……..…… …… *A. consanguineum*
14b: Short cells and oil droplets are absent ……...… *A. griffithianum*

Discussion

Allium is an important genus of economic and medicinal value. In the past taxonomic information of this genus was based largely on morphological markers, which leads to certain taxonomic confusion. Anatomical studies could be an important tool to resolve taxonomic problems of this genus, as anatomical studies showed variation in size and shapes of stomata, stomatal cavity, long/short cells, Silica bodies, Macro/Micro hairs and trichomes. Epidermis of the *Allium* consists of single layer of cells that are tubular vertically but variable in outline may be isodiametric, elongated, wavy or rectangular in shape. All these shape were found in different species of this genus (Table 1).

A. consanguineum had most diverse leaf epidermal anatomy. This species had longest stomatal cells 6-14 µm, whereas smallest stomatal cells were found in *A. dolichostylum and A. lamondae, A. miserabile and A. barszczewii* (Table 1). In these species length of stomatal cells ranged from 3-4 µm. Micro hairs was the character of only one species (*A. carolianum*). The length of micro hairs varies from 150-200 µm. this is an important distinguishing character for *A. carolianum* form the other species of the genus *Allium*. The other characteristic organelle of leaf epidermis was silica bodies. Only dumb-bell shaped silica bodies were observed in 6 different species (*A. dolichostylum, A. borszczewii, A. micranthum, A. consanguinem, A. stocksianum and A. stoliczki*). Length of silica bodies varies in all these 6 species. Longest silica bodies were found in *A. consanguineum* (6-14 µm).

Table 1. Anatomical characters of some selected species of the genus *Allium* from Pakistan.

Species name	Stomata cells length (µm)	Stomatal cavity Length (µm)	Length of long cells (µm)	Length of short cells (µm)	Shapes of cells	Length of macro hairs (µm)	Length of micro hairs (µm)	Type of trichome	Length of trichom (µm)	Length of silica bodies (µm)
A. jacquemontii Kunth	5-7	3-5	230-430	0	Rectangular	0	0	0	0	0
A. griffithianum Boiss.	3.3-6	2.5-5	200-350	0	Rectangular	0	0	0	0	0
A. humile Kunth	3-5	2-4	80-12	0	Rectangular	0	0	0	0	0
A. wallichii Kunth	5-7	3-6	160-300	0	Rectangular	0	0	0	0	0
A. carolinianum DC.	3-6	2-5	240-310	0	Elongated	0	150-200	0	0	0
A. tuberosum Rottle ex Spreng.	3-5	2-5	150-350	0	Rectangular	0	0	0	0	0
A. cepa Linn.	4-6	3-5	100-290	0	Elongated	0	0	0	0	0
A. dolichostylum Vved.	2-4	2-3	130-530	0	Elongated	0	0	Single cell, Pointed end	100-350	3-5
A. barszczewksii Lipsky	3-4	2-3	130-270	0	Elongated	0	0	Single cell, pointed end	100-230	0
A. borszczowii Regel	3-5	2-4	90-140	0	Rectangular	0	0	Double cell, round end	400-600	3-3.5
A. micranthum Wendelbo	3-5	2-3	140-300	0	Elongated	0	0	Double cell, pointed end	200-300	2.5
A. lamondiae Wendelbo	3-4	2-3	130-270	15-37	Rectangular	0	0	Single cell, pointed end	75-100	0
A. miserabile Wendelbo	3-4	2-3	90-110	0	Elongated	0	0	Single cell, pointed end	70-100	0
A. consanguineum Kunth	6-14	6-8	350-700	120-225	Elongated	0	0	0	0	6-14
A. longicollum Wendelbo	4-5	2-3	120-180	0	Elongated	300-450	0	0	0	0
A. gilli Wendelbo	3-4	2-3	130-320	0	Elongated	0	0	Single cell, pointed end	300-500	0
A. stocksianum Boiss.	3-5	2-4	120-310	0	Elongated	0	0	0	0	2-3
A. stoliczki Regel	4-6	3-4	120-350	0	Elongated	0	0	0	0	3-5

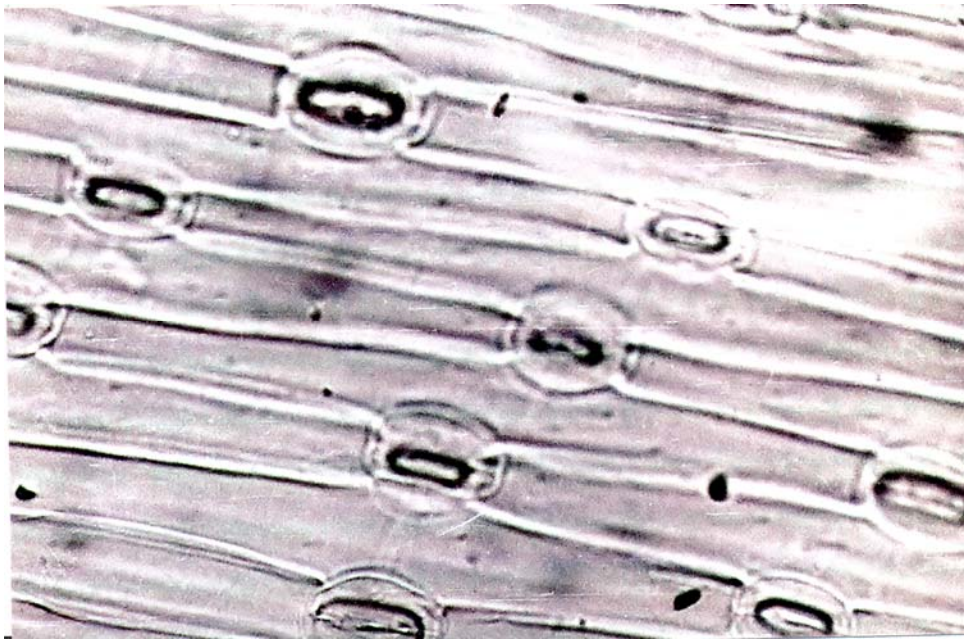

Fig. 1. Leaf epidermal anatomy of *Allium jacquemontii* Kunth.

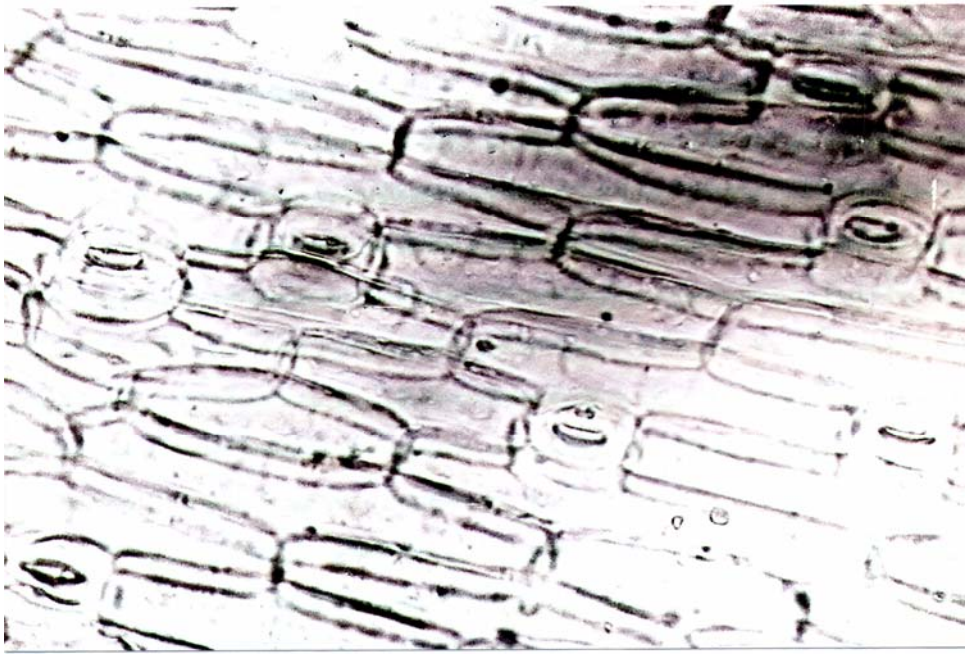

Fig. 2. Leaf epidermal anatomy of *Allium griffithianum* Bioss.

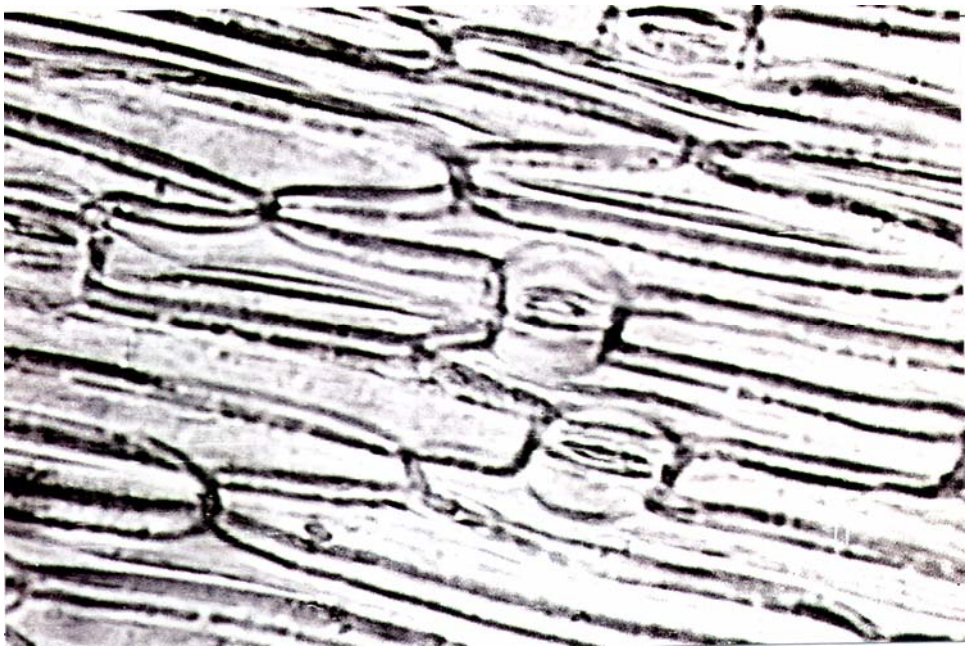

Fig. 3. Leaf epidermal anatomy of *Allium humile* Kunth.

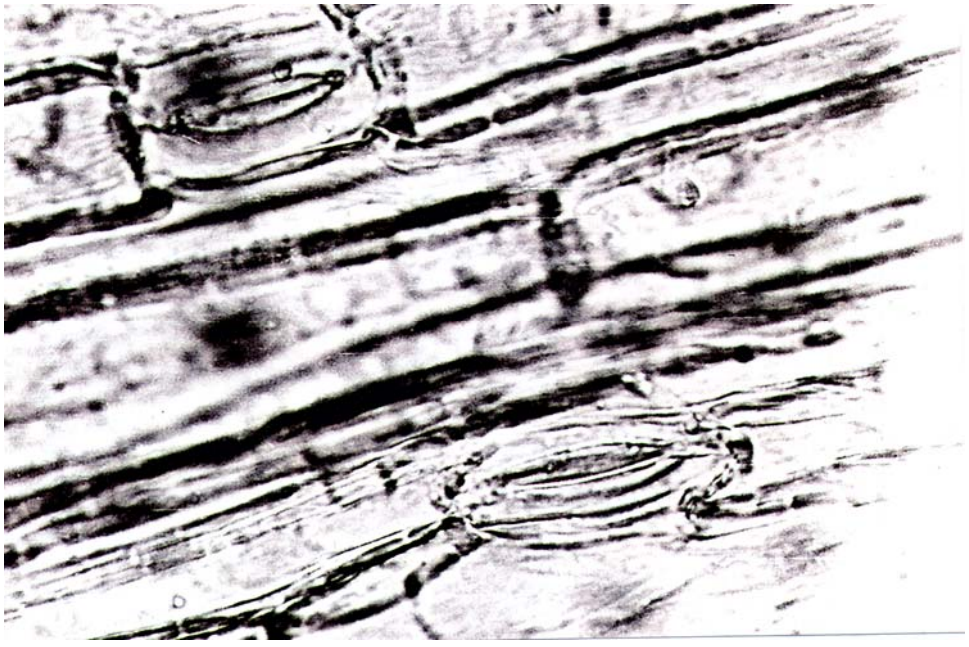

Fig. 4. Leaf epidermal anatomy of *Allium wallichii* Kunth.

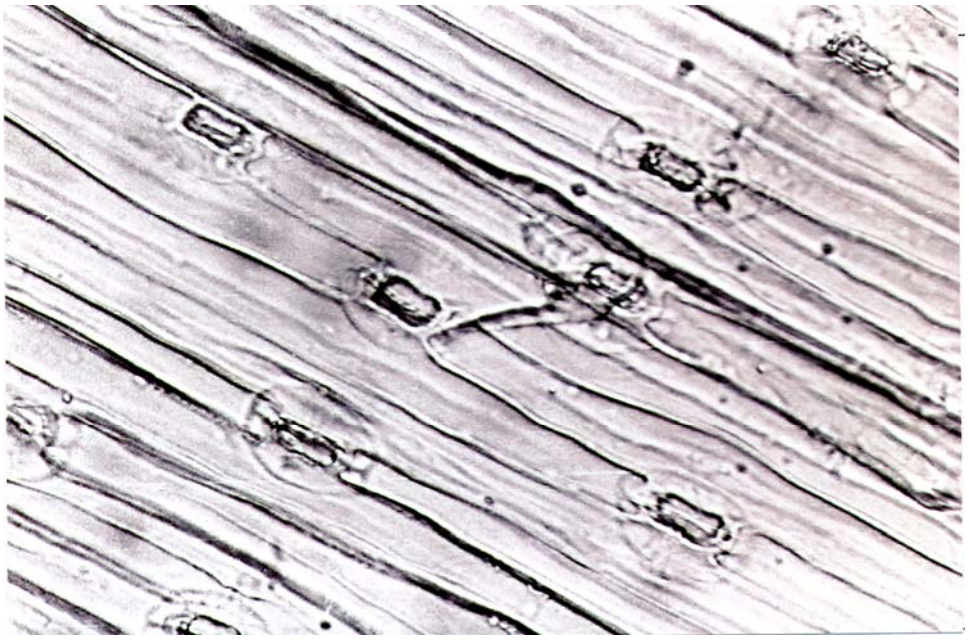

Fig. 5. Leaf epidermal anatomy of *Allium barszczewksii* Lipsky.

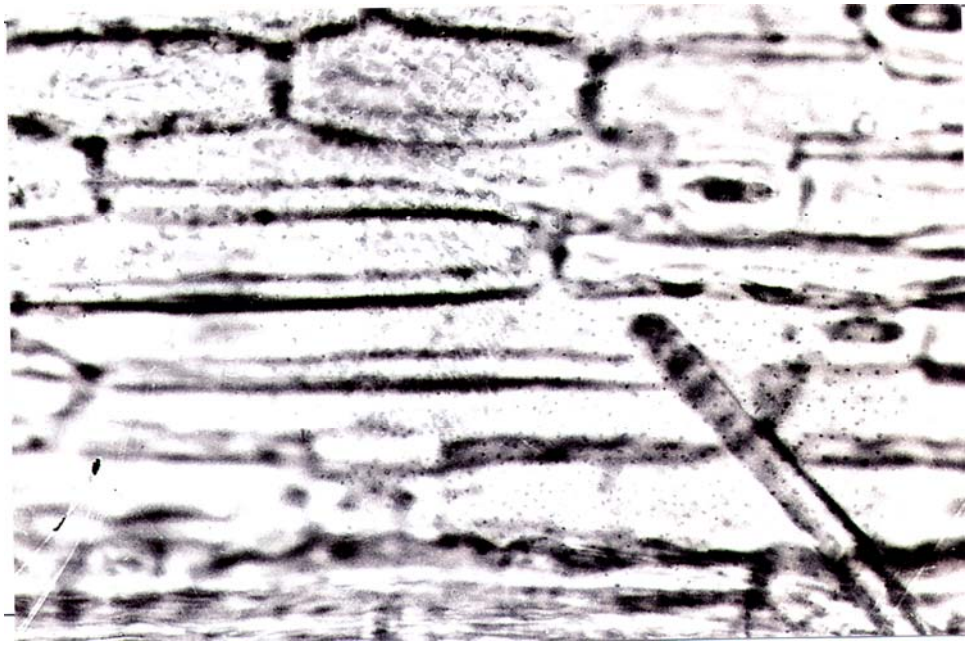

Fig. 6. Leaf epidermal anatomy of *Allium borszczewii* Regel.

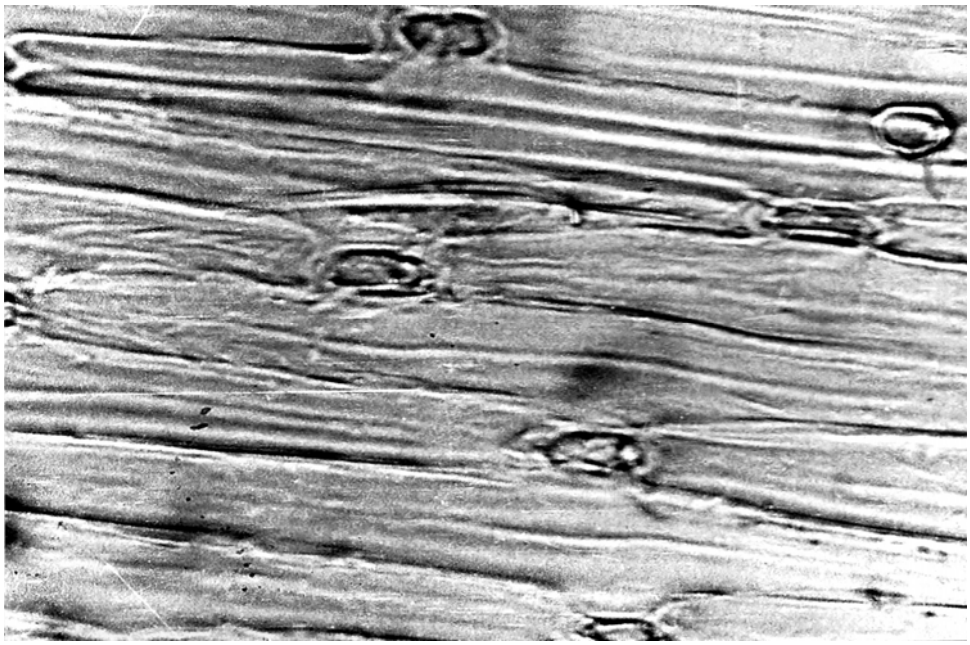

Fig. 7. Leaf epidermal anatomy of *Allium micranthum* Wendelbo.

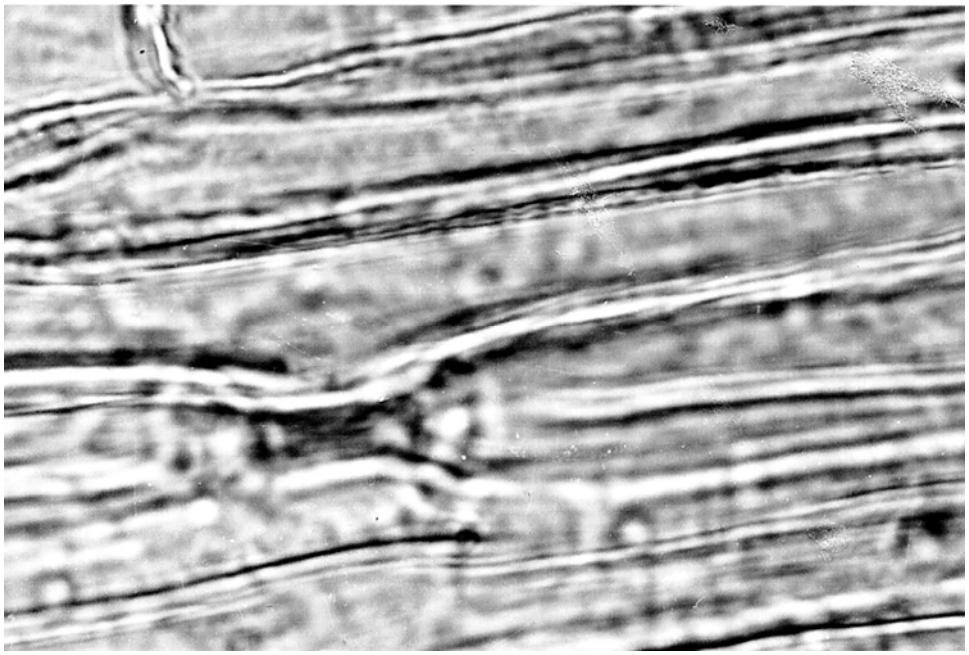

Fig. 8. Leaf epidermal anatomy of *Allium lamondae* Wendelbo.

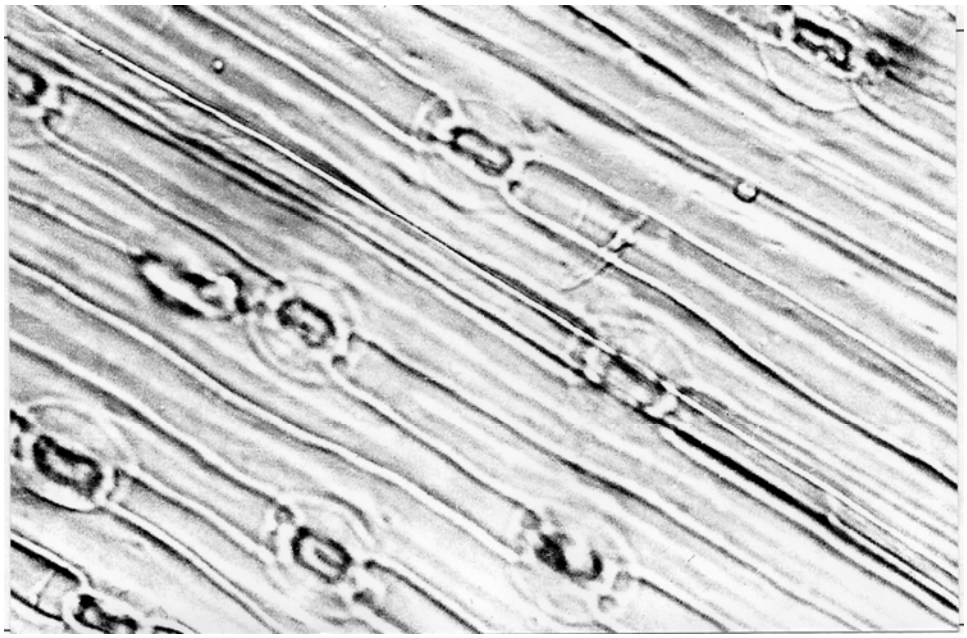

Fig. 9. Leaf epidermal anatomy of *Allium miserabile* Wendelbo.

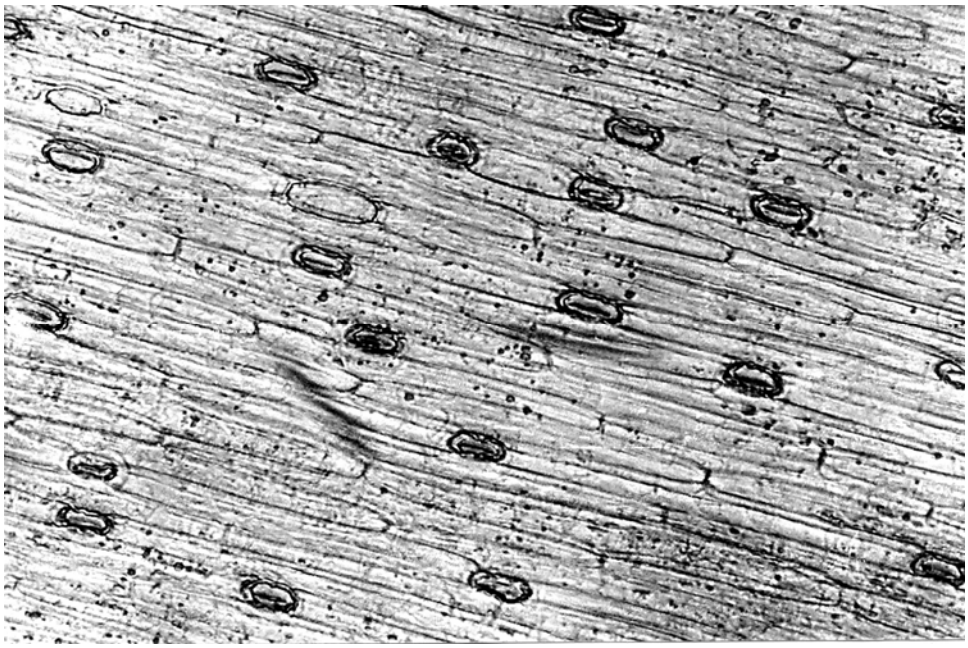

Fig. 10. Leaf epidermal anatomy of *Allium consanguineum* Kunth.

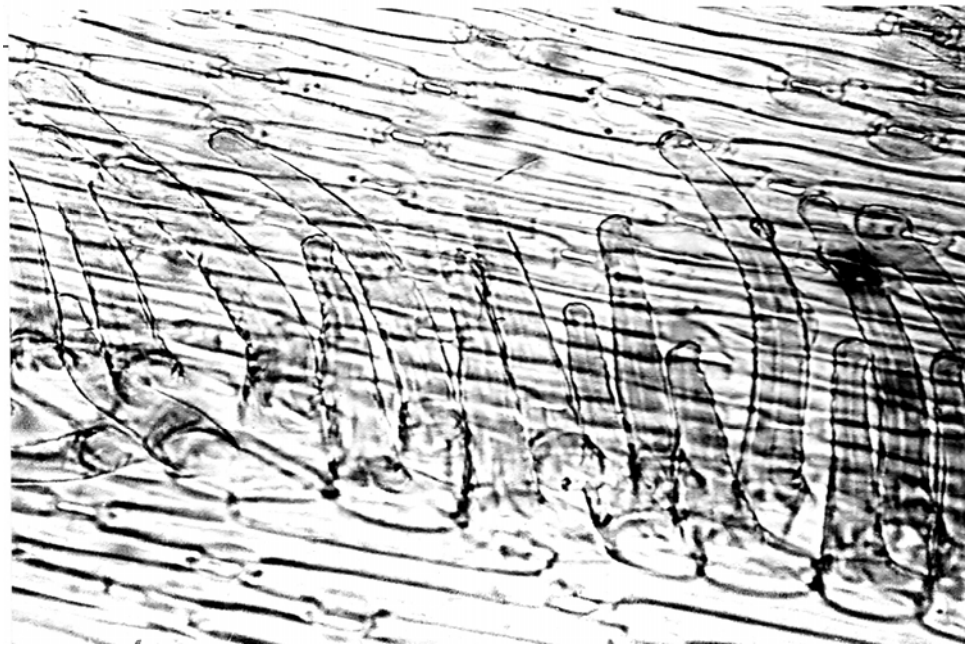

Fig. 11. Leaf epidermal anatomy of *Allium longicollum* Wendelbo.

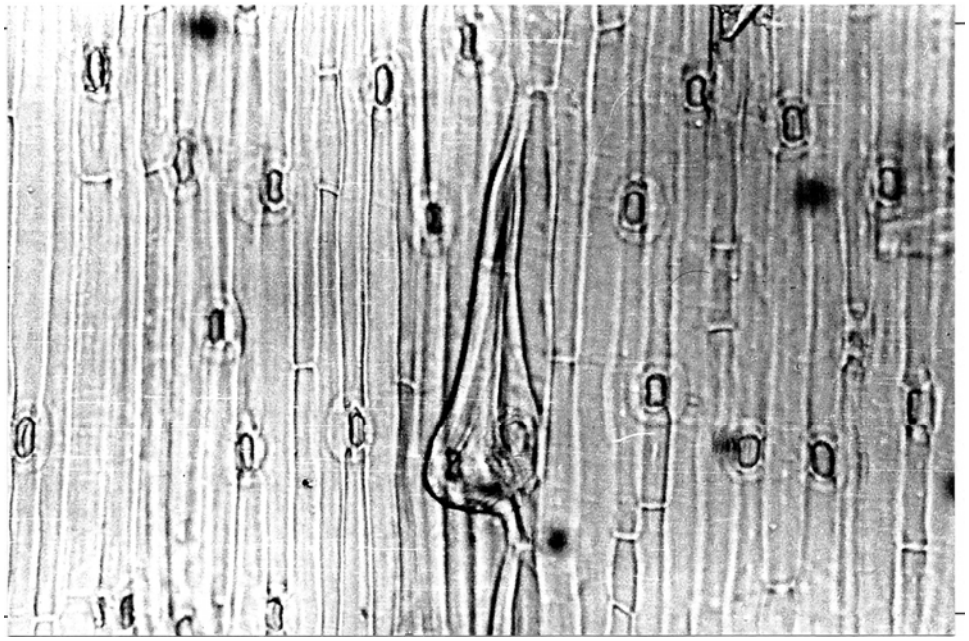

Fig. 12. Leaf epidermal anatomy of *Allium gilli* Wendelbo.

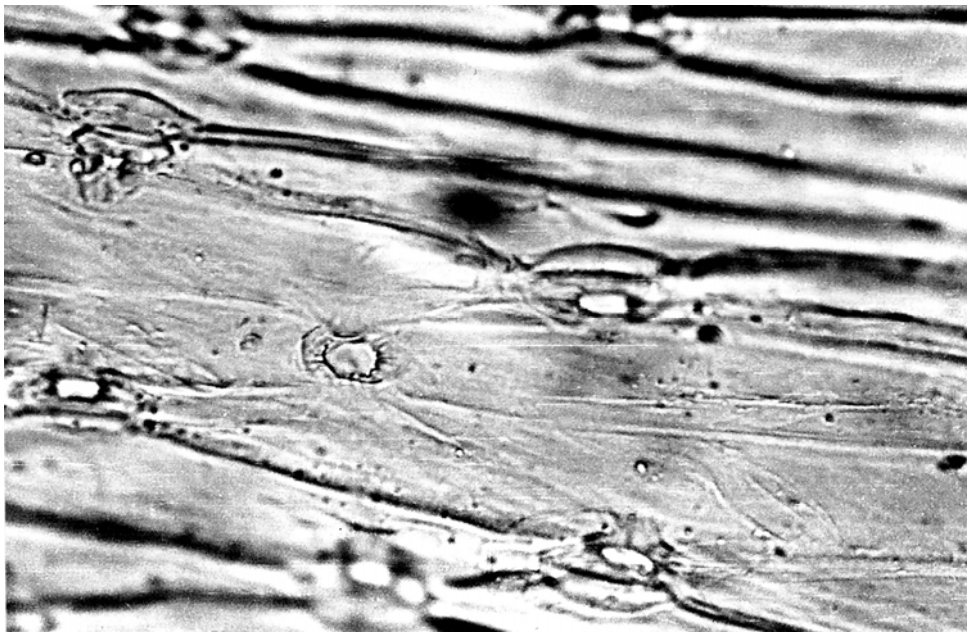

Fig. 13. Leaf epidermal anatomy of *Allium stocksianum* Boiss.

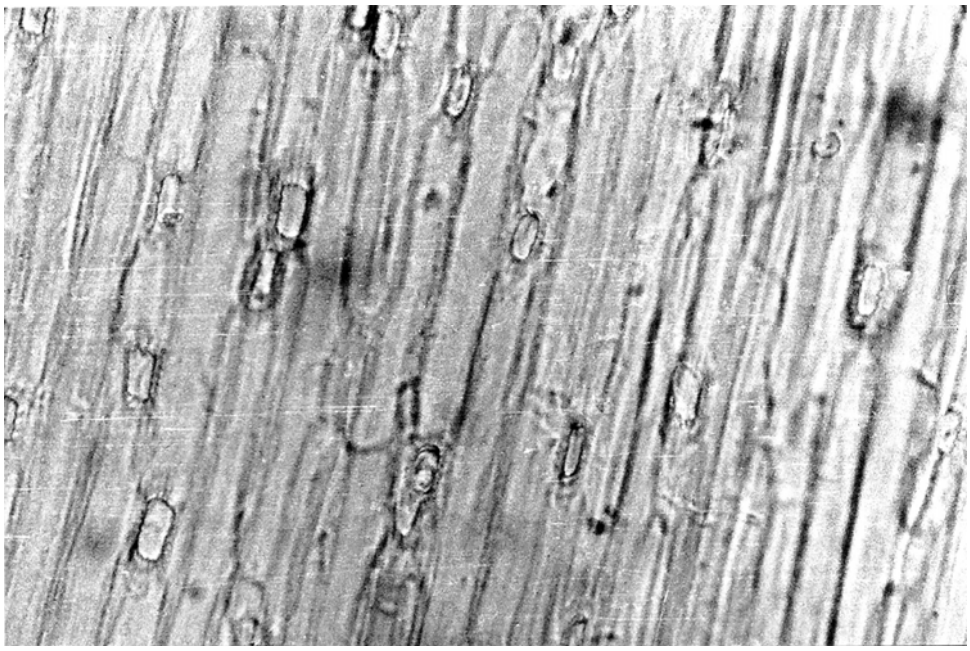

Fig. 14. Leaf epidermal anatomy of *Allium stoliczki* Regel.

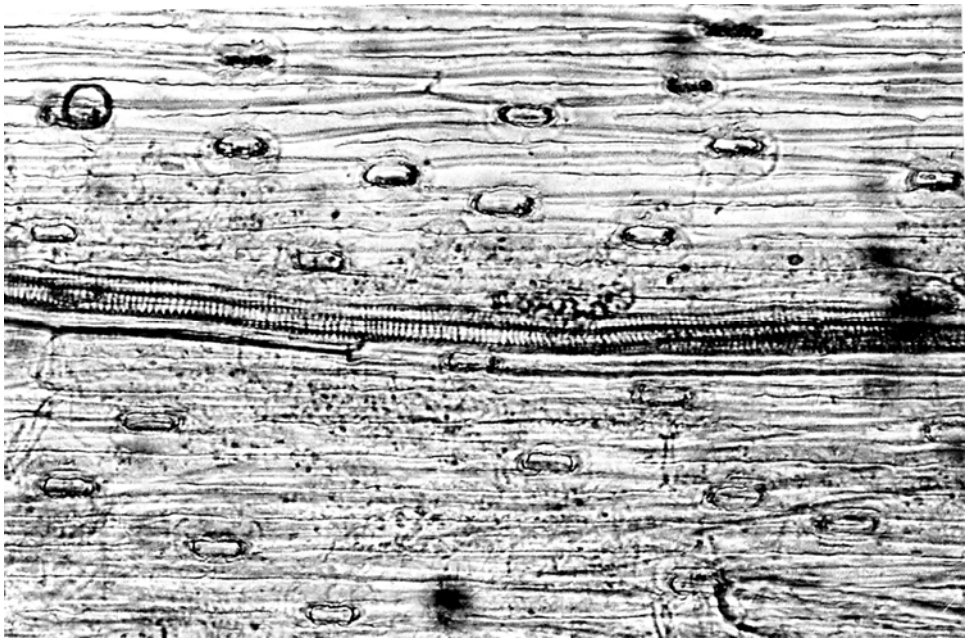

Fig. 15. Leaf epidermal anatomy of *Allium dolichostylum* Vved.

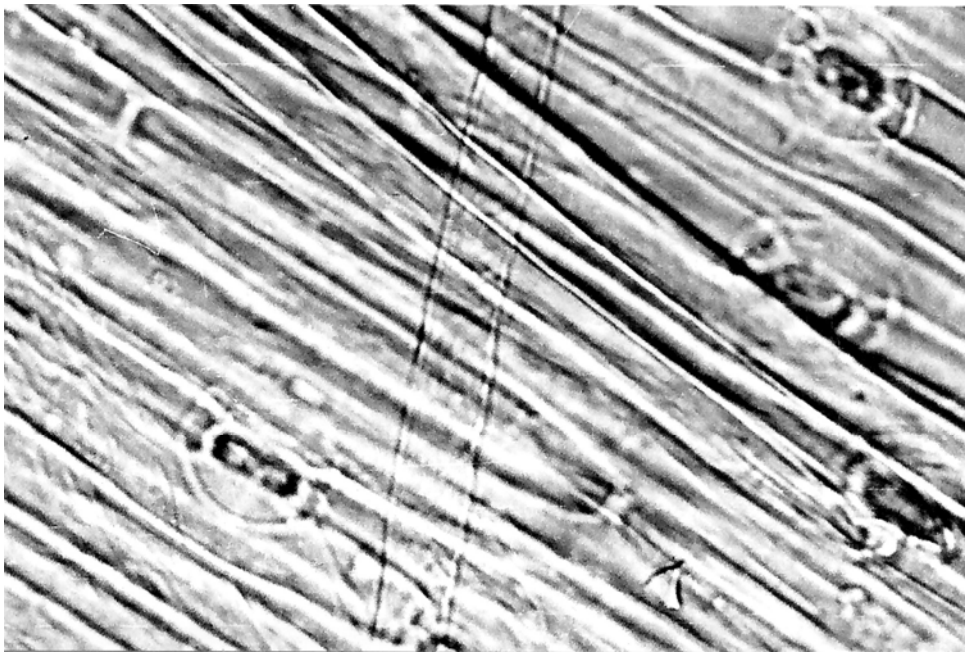

Fig. 16. Leaf epidermal anatomy of *Allium carolinianum* DC.

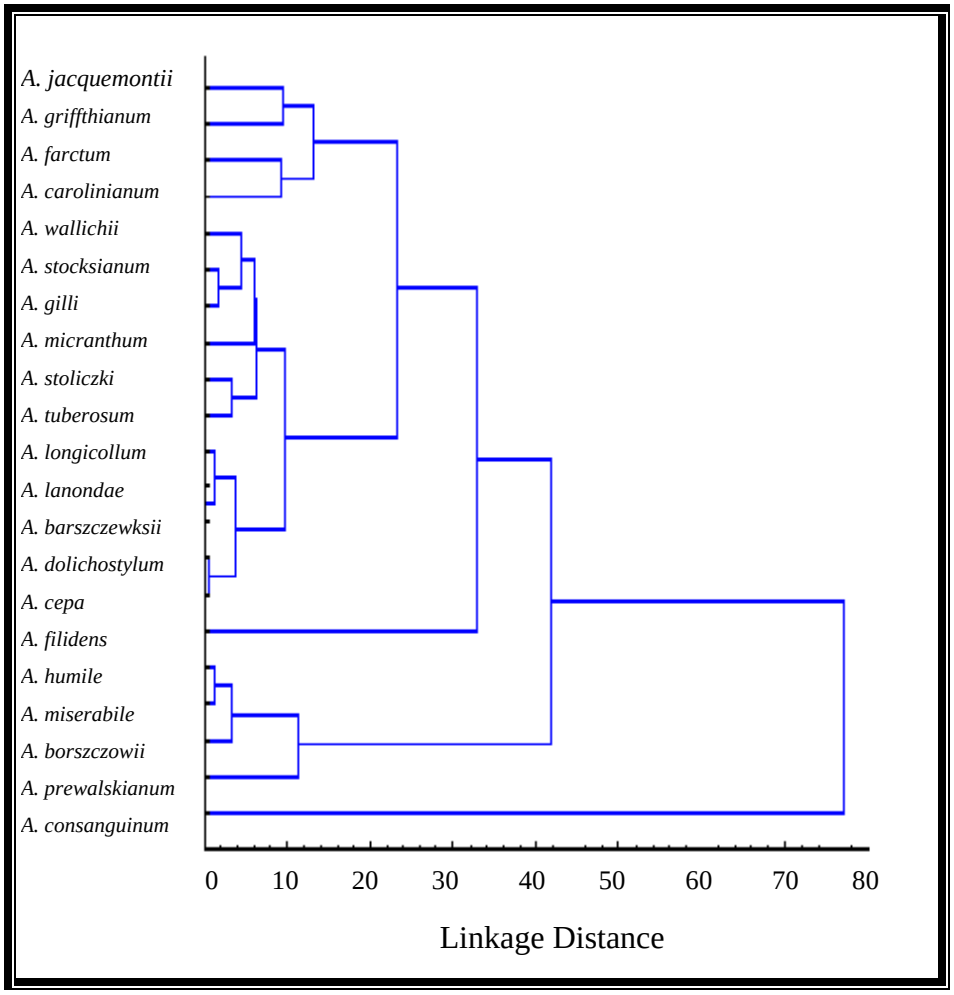

Fig. 17. Cluster analysis of different species of the genus *Allium* based on anatomical characters.

Trichomes formed as outgrowths from an epidermal cell and can be used as one of the important taxonomic marker for *Allium* (Metcalfe, 1960). Trichomes were present in *A. barszczewksi, A. borszczowii, A. micranthum, A. lamondae, A. miserbile, A. longicollum, A. gilli and A. dolichostylum* (Fig. 5). Trichomes not only vary in the size, number of cells but also in the shape. In *A. barszczewksi, A. lamondae , A. miserbile and A. dolichostylum* trichomes were single cell and with pointed tip. However in two species *A. borszczowii* and *A. micranthum* trichomes were double cell. *A. borszczowii* had trichome with round tip whereas in *A. micranthum* it was pointed. In *A. longicollum* Wendelbo trichomes are unicellular and glandular head. In this species many trichomes were present in single row whereas in other species trichomes were scattered on the whole surface. *A. gilli* Wendelbo has single cell trichome with pointed head and round base. Trichomes were present only in few species of the *Allium* and had variation in number and size of cells. This can be utilized as species identifying character (Figs. 6-16).

Cluster analysis based on anatomical characters revealed that 18 species of genus *Allium* were divided into two main clusters at the phylogenetic distance of 79% (Fig. 17). On the anatomical basis *A. griffithianum* Boiss and *A. jacqueomontii* Kunth are closely related species. This is the same result which is obtained from the study of their morphology (Wendelbo, 1971, Yousaf *et al.,* 2004). Lower order classification of *Allium* genus on the basis of anatomical characters is entirely different from morphological classification. *Allium gilli* Wendelbo, *A. miserabile* Wendelbo and *A. micranthum* Wendelbo which are closely related species. These species belongs to subgenus Scoradon (Wendelbo, 1971). On the anatomical basis *Allium micranthum* and *A. gilli* are present in the same group but *A. miserabile* showed resemblance with *A. przewalskianum* Regel and *A. lamondiae* Wendelbo (Fig. 17) while on the morphological basis related *A. griffithianum* Boiss and *A. jacquemontii* Kunth., (Yousaf *et al.,* 2004). All these species belonging to single group i.e. Scordon. *A. consanguineum* Kunth is the most distinct species. Cluster analysis revealed that it separated from all other species at the distance of 79% this species differ on the basis of measurement of stomatal cells, stomatal cavity, short/long cells and silica bodies from the other species of the section Rhiziridemum.

Reference

Clark, J. 1960. Preparation of leaf epidermis for topographic study. *Stain. Techno*., 35: 35-39.

Cotton, R. 1974. *Cytotaxonomy of the genus Vulpia*. Ph.D. Thesis, Univ. Manchester, USA.

Esau, K. 1965. Plant Anatomy (2nd ed.). Wiley & Sons, New York.

Gilani, S.S., M.A. Khan, Z.K. Shinwari and Z. Yousaf. 2002. Leaf epidermal anatomy of selected *Digitaria* species, Tribe Paniceae, family Poaceae of Pakistan. *Pak. J. Bot*., 257-273.

Kioug, E. 1998. Anatomical and Palynological studies of *Allium victoralis* var. *platyphyllium*. *Adv. Exp. Med. Biolo*., 6(7): 11-29.

Kwaitkowski, I. 1999. Chronological analysis of the distribution of *Allium schenoprasum*. *Amer. J. Bot*., 24: 7-11.

Metcalfe, C.R. 1960. Anatomy of the Monocotyledons, *Allium*. Oxford, Clarendon Press.

Nasir, E. 1975. *Flora of Pakistan*, Fascicle 83, Alliaceae. In: (Eds.): E. Nasir and S.I. Ali. Flora of West Pakistan. Karachi.

Robert, C. 1992. *Common families of flowering plants*. Cambridge University Press, Cambridge.

Webster, R.D. 1983. A revision of the genus *Digitaria* Haller (Paniceae, Poaceae) in Australia. *Brunonia*, 6: 131-216.

Wendelbo, P. 1971. Alliaceae. In: Flora Iranica. (Ed.): K.H. Rechinger. Austria.

Yousaf, Z., Z.K. Shinwari, R.A. Qureshi, M.A. Khan and S.S. Gilani. 2004. Can complexity of the genus *Allium* L., be resolved through some numerical techniques? *Pak. J. Bot*., 36(3): 487-501.

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