A study of the epidermal patterns of the leaf blades in some *Carex* using LM and SEM*

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주사현미경과 광학현미경에 의한 沙草屬 植物의 잎의 表皮型에 관한 分類學的 研究*

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Abstract

The systematic relationship of the *Carex* were elucidated by epidermal patterns studies of the leaf blades. Fourteen species representing twelve section of *Carex* were examined using light microscope (LM) and scanning electron microscope (SEM).

Epidermal patterns of the leaf blades are characteristic and useful in classification of species level; shape and arrangements of subsidiary cell in stoma, epicuticular wax, intercostal cell walls, papillae, prickles and silica body. Characteristically, epidermal data support the present classification and identification of the *Carex* species.

Introduction

Since Metcalfe (1971) published "Anatomy of the Monocotyledons V. Cyperaceae", lots of anatomical works of leaf blades were carried out (Schuyler 1971; Walter 1975; Toivnen and Timonen 1976; Standley 1981; Forbes 1980; Oh 1980, 1985). The author firstly used by the LM and SEM as a technical means of examining the epidermal patterns of the leaf blades. Comparing the results of both LM and SEM observation, the epidermal characters of some taxa of sedges was discussed. In this study the epidermal patterns of leaf blades was designed, using LM and SEM. In present result, the epidermal patterns of the

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leaf blades were found to be useful for the identification and classification of the sedge.

Materials and Methods

The samples for the study were taken from the Wiegand Herbarium sheets in the L. H. Bailey Hortorium at Cornell University, Ithaca, N.Y. U.S.A.

Table 1. Materials and localities tested

Section	Species	Localities	Collector & No.
Dispermae	<i>Carex disperma</i>	Lake Bailey, Keenaw county	F.S. Hermann
Macrocephales	<i>C. macrocephala</i>	New York	J.A. Small
Heleonastes	<i>C. brunnescens</i>	Virginia, Giles county	R.F. Thorne (11325)
Ovales	<i>C. macloviana</i>	Oregon, Mt. Hood	L.F. Henderson
Acutae	<i>C. aquatilis</i>	Montana	Clhitchcock Cvmuhlick (13721)
Stellulatae	<i>C. norvegica</i>	Washington, Sall-marsh Culter	G.G. Kennedy E.F. Williams J.F. Callins M.L. Fernald
Atratae	<i>C. atrata</i>	Colorado, Denver sake, Creek	I.W. Clokey
	<i>C. buxbaumii</i>	Washington, Thomas lake	E.F. Layser
Athrochlaenae	<i>C. pyrenaica</i>	Idaho	J. William Thompson (14020)
Limosa	<i>C. limosa</i>	Canada, Hypnum Swong	W.B. Shofield (1264)
Anomalae	<i>C. maculata</i>	Mexico	Rcruz (115)
Pseudo-cyperus	<i>C. pseudo-cyperus</i>	New York, Seneca	R.T. Clausen (112)
Physocarpae	<i>C. rostrata</i>	Minnesota Vermilion lake	J.C. Arthur (A) L.H. Bailey (B) E. WD. Holway (H)
	<i>C. saxatilis</i>	Alaska, North of Nolan	L.H. Jordal

For both LM and SEM observation

The widest portion of the leaf blades of basal leaves were taken, softened by boiling in Glyciaeri solution (Glycerine 10ml, 10% Aerosol 3ml, Distilled water 90ml) at 60°C (parafin oven) for 24hrs. and then were fixed in F.A.A. (95% Ethyl alcohol 50ml, Glacial acetic acid 5ml, 37-40% Formaldehyde, 19ml, Distilled H₂O 35ml) for 3 days.

The epidermis peeled with a razor blade were observed under a light microscope (Olympus BH type). In measuring the size of stomata, the lateral subsidiary cells were included and an average of 10 measurements was given in micron. To determine the stomata frequency, the number of stomata in a field were counted in 280X.

The wave of the intercostal cell wall was calculated by the Oh's methods(1980): $f(x) = A \sin \frac{2\pi}{B} x$ where A, wave height in μm and B, wave period in cycle/ μm .

For the SEM observation, the fixed leaves were prepared by dehydration in graded acetone sequence, and dried with CO₂ in a Sorval critical point dryer. The specimens were then mounted on aluminum stubs with silver or graphite conducting point and coated with gold/palladium and examined in an scanning electron microscope (Model ARC) at a voltage of 21 KV (500X to 1000X). The electron images were recorded with polaroid type 55.

Results

The comparative studies fourteen species in twelve section of *Carex*, the epidermal patterns of the leaf blade were examined with LM and SEM. Many epidermal characters were found to be important for the species identification and classification of *Carex*: e. g. the shape and arrangements of subsidiary cell in stoma, the wave of intercostal cell walls, forms of silica bodies, epicuticular wax and the presence or absence of papillae and prickles.

LM study; Subsidiary cells in stomata were classified as dome, high-dome, low-dome, triangular and almost round shapes (Metcalf, C. R & M. Gregory 1964). Usually one to several rows of intercostal cells appeared in the intercostal zone (plate I-plate III).

Stomata were sometimes scattered without the relationship of the rows intercostal cells. The smallest stomata were present on the leaf blades of *Carex maculata* (plate II-9) and measure 20-23-24 microns long and 16-18-20 microns wide, whereas the largest of *C. macrocephala* measure 76-80-89 microns long and 48-54-60 microns wide.

As for the shape of subsidiary cells, the high-dome type was observed in *C. atrata* (plate I-5,6,7,); the high-dome to triangular in *C. pseudo-cyperus* and *C. saxatilis* (plate II-3; plate I-3,4); the triangular in *C. aquatilis* (plate II-1,2) and the almost-round shape subsidiary cells in *C. limosa* (plate II-7,8) and the rest of species show either dome or low-dome subsidiary shapes.

The walls of the intercostal cells were variously wavy; deep, sinuous, shallow or sometimes not wavy (Oh 1980). The wall of intercostal cells were deeply wavy in *C. disperma* and *C. macloviana* (plate II-11, 12; plate III-11, 12); sinuous wavy in *C. brunnescens*, *C. buxbaumii*, *C. limosa*, *C. pseudo-cyperus* and *C. rostrata* (plate III-3, 4, 5; plate I-9, 10, 11; plate II-5, 6, 7, 8; plate II-3; plate I-1, 2); shallow wavy in *C. norvegica*, *C. atrata*, *C. pyrenaica* and *C. saxatilis* (plate III-8, 9, 10; plate I-5, 6, 7; plate I-3, 4) and not wavy in *C. macrocephala*, *C. aquatilis*, and *C. maculata* (plate II-1, 2; plate II-9, 10).

The numbers of silica bodies were also consistent in species; one per cell in *C. buxbaumii*, one to two in per cell *C. macrocephala*, *C. macloviana*, *C. aquatilis*, *C. atrata*, *C. pyrenaica*, *C. norvegica*, *C. limosa*, *C. maculata* and *C. pseudo-cyperus* (plate II-4).

Both papillae and prickles were present on both adaxial and abaxial surfaces of *C. buxbaumii* (plate I-8, 9, 10, 11), whereas prickles were lacking in *C. aquatilis*, *C. norvegica*, *C. atrata*, *C. limosa* and *C. maculata* (plate II-1, 2; plate III-8, 9; plate I-5, 6).

The rest of the species showed papillae and prickles on either adaxial surface or abaxial surface only and sometimes none.

SEM study; Subsidiary cells in stomata were generally smooth, swollen, surrounded by papillae and were smooth and sunken and covered with wax-hyphae (Reicosky and Hanover 1976). Species with cuticular wax (Stockey and Taylor 1978) on the abaxial surface only was *C. limosa* (plate II-5, 6), but on both surfaces of the leaf blade included *C. disperma*, *C. macrocephala*, *C. brunnescens*, *C. macloviana*, *C. norvegica*, *C. buxbaumii*, *C. pyrenaica*, *C. maculata* and *C. rostrata* (plate III-1, 2; plate III-3, 4, 5; plate III-11, 12; plate III-7, 8, 9; plate I-9, 10, 11; plate II-9, 10; plate I-1, 2).

The taxon of *C. pyrenaica* and *C. maculata* had well developed cuticular wax (plate II-9, 10). *C. macrocephala*, *C. atrata*, *C. pseudo-cyperus* and *C. rostrata* had smooth subsidiary cells, while *C. disperma*, *C. brunnescens*, *C. macloviana*, *C. aquatilis* and *C. rostrata* had swollen subsidiary cells.

C. norvegica, *C. atrata*, *C. limosa* and *C. maculata* were surrounded by papillae in the stomata. The walls of the intercostal cells were wavy, slightly wavy, helical or rounded projections. The walls of the intercostal cells; slightly wave in *C. atrata*, *C. buxbaumii*, *C. pyrenaica* and *C. rostrata*; helical or tightly helical in *C. macrocephala*, *C. brunnescens*, and *C. saxatilis*. The rest of the species were not confirmed.

Papillae were showed on the both surfaces of *C. aquatilis*, *C. norvegica*, *C. atrata*, *C. buxbaumii*, *C. limosa* and *C. maculata* (plate II-1, 2; plate III-7, 8, 9; plate I-5, 6; plate I-9, 10, 11; plate II-5, 6, 7; plate II-9, 10).

The species of *C. buxbaumii* (plate I-9, 10, 11) possessed prickles and papillae on both surfaces, while *C. pyrenaica* on the adaxial surface only and the species *C. pseudo-cyperus* and *C. rostrata* were present prickles on the adaxial surface only.

Especially, *C. pyrenaica* exhibited well developed wax-hyphae on the epidermal surface. The shape of papillae were irregularly low blunt bodies, blunt/parallel sides, long blunt/sloping sides, parallel sides, irregularly bodies, or bulbous (Walter 1975).

The shape of papillae were blunt bodies/parallel sides in *C. disperma*, *C. aquatilis* (plate II-11, 12; plate II-1, 2); low blunt/irregular bodies in *C. macrocephala*, *C. brunnescens*, *C. macloviana*, *C. buxbaumii*, *C. pyrenaica*, and *C. saxatilis* (plate III-3, 4, 5, 6; plate III-11, 12; plate I-9, 10, 11; plate I-3, 4); long blunt/sloping sides in *C. norvegica* (plate III-7, 8, 9); long blunt/parallel sides in *C. atrata* (plate I-5, 6) and long blunt/irregular bodies in *C. limosa* (plate II-5, 6, 7) and bulbous form in *C. maculata* (plate II-9, 10).

Table 2. Some important characters of *Carex* using LM and SEM

Species	Characters	Size of Intercostal cell (microns) length × width	Size of stomata (microns) length × width	Frequency of stomata	Shape of subsidiary cell LM & SEM	Silica bodies (per-cell)	Wave of cell wall (Unit: cycle/m)	Papillae present(+) /absent(-) LM (ad;ab) SEM (ad;ab)	Prickle present(+) /absent(-) LM (ad;ab) SEM (ad;ab)
<i>C. disperma</i>		81-121-137 × 24-24-24	48-48-48 × 32-35-36	15	dome, swelled	2-3	deep; 4.382	(+;-) (+;-) blunt bodies, parallel sides	(-;-) (-;-)
<i>C. macrocephala</i>		113-129-145 × 36-41-44	76-80-89 × 48-54-60	16	low-dome, smooth, swelled	1-2	not wavy	(-;-) (-;-) low, blunt, irregular bodies	(-;-) (-;-)
<i>C. brunnescens</i>		137-169-209 × 28-30-36	48-48-48 × 36-40-44	13	dome, swelled	2-3	sinuous; 2.955	(+;-) (+;-) low, blunt, irregular bodies	(-;-) (-;-) leaf margin (+)
<i>C. macloviana</i>		145-177-217 × 40-40-40	56-64-68 × 48-55-60	11	dome, swelled	1-2	deep; 3.861	(+;-) (+;-) low, blunt, irregular bodies	(-;-) (-;-)
<i>C. aquatilis</i>		97-145-201 × 28-31-36	40-42-44 × 28-39-44	11	triangular, swelled	1-2	not wavy	(+;+) (+;+) blunt bodies parallel sides	(-;-) (-;-) leaf margin (+) pointed
<i>C. norvegica</i>		64-76-89 × 40-44-56	48-55-56 × 36-41-48	13	dome, surrounded by papillae, sunken	2-3	shallow; 0.816	(+;+) (+;+) long, blunt, sloping sides	(-;-) (-;-)
<i>C. atrata</i>		97-98-105 × 32-32-32	48-56-56 × 48-53-56	15	high-dome surrounded by papillae, sunken, smooth	1-2	shallow; 0.582	(+;+) (+;+) long, blunt bodies parallel sides	(-;-) (-;-)
<i>C. buxbaumii</i>		72-112-153 × 20-27-32	44-48-53 × 36-39-44	28	dome, not confirmed	1	sinuous; 2.499	(+;+) (+;+) low, blunt, irregular bodies	(+;+) (+;+) pointed
<i>C. pyrenaica</i>		113-161-209 × 20-23-28	48-54-56 × 32-40-48	9	low-dome, covered wax-hyphae	1-2	shallow; 0.856	(-;-) (+;-) low, blunt, irregular bodies	(-;-) (+;-)
<i>C. limosa</i>		40-49-64 × 8-14-16	28-30-32 × 28-30-32	32	almost round, surrounded by papillae	2-3	sinuous; 2.571	(+;+) (+;+) long, blunt, irregular bodies	(-;-) (-;-)
<i>C. maculata</i>		24-31-48 × 8-11-12	20-23-24 × 16-18-20	40	low-dome, surrounded by papillae, covered wax-hyphae	2-3	not wavy	(+;+) (+;+) bulbous form	(-;-) (-;-)
<i>C. pseudo-cyperus</i>		80-105-129 × 20-20-20	31-32-32 × 20-25-32	17	high-dome to triangular, smooth	2-3	sinuous; 2.333	(-;-) (-;-) none	(+;-) (+;-) large
<i>C. rostrata</i>		81-99-105 × 10-14-14	28-29-32 × 20-22-24	25	dome, smooth, swelled	1-2	sinuous; 2.554	(-;-) (-;-) none	(+;-) (+;-) large, pointed
<i>C. saxatilis</i>		56-72-89 × 16-19-20	32-32-32 × 26-30-32	11	high-dome to triangular not confirmed	1-2	shallow; 1.982	(+;-) (+;-) low, blunt, irregular bodies	(-;-) (-;-)

It was found that the LM and SEM were very useful to study the epidermal patterns.

Key to the epidermal characters of the leaf blades on genus *Carex*.

- A. Subsidal cells in stoma triangular or high dome to triangular shaped
 - B. Subsidal cells triangular shaped *C. aquatilis*
 - B. Subsidal cells high-dome to triangular shaped, papillae present or absent at adaxial surface
 - C. Papillae present at adaxial surface, low blunt, irregular bodies *C. saxatilis*
 - C. Papillae absent at adaxial surface *C. pseudo-cyperus*
- A. Subsidal cells in stoma high-dome, dome, low-dome, almost-round shaped
 - B. Subsidal cells high-dome or dome shaped
 - C. Subsidal cell high-dome shaped, stoma surrounded by papillae *C. atrata*
 - C. Subsidal cells dome shaped
 - D. Papillae present or absent and prickles present or absent
 - E. Papillae and prickles present at both leaf surfaces *C. buxbaumii*
 - E. Papillae present at both leaf surfaces or adaxial surface
 - F. Papillae present at only adaxial surface
 - G. Papillae blunt bodies, parallel sides *C. disperma*
 - G. Papillae low blunt irregular bodies
 - H. Intercostal cell walls deep wavy *C. macloviana*
 - H. Intercostal cell walls sinuous wavy *C. brunnescens*
 - F. Prickles present at adaxial surface *C. rostrata*
 - D. Only papillae present at both leaf surfaces *C. norvegica*
 - B. Subsidal cells low-dome or almost-round shaped
 - C. Subsidal cell low-dome shaped, papillae present at both leaf surface or one side leaf surface
 - E. Papillae present at both leaf surfaces, stoma surrounded by papillae ... *C. maculata*
 - E. Papillae present at adaxial or abaxial surface
 - F. Papillae present at adaxial surface, covered wax-hyphae *C. pyrenaica*
 - F. Papillae present at abaxial surface *C. macrocephala*
 - C. Subsidal cells almost round shaped, stoma surrounded by papillae *C. limosa*

Discussion

Many systematists studied of the genus *Carex*; gross morphology, spikelet, achene and folage characters and also internal histological patterns of stem and leaves.

Anatomical data were often useful in characterizing species complexes within a genus and in determining evolutionary relationships (Forbes 1980; Standley 1981).

In reference to Cyperaceae, Metcalfe (1964, 1969, 1971) stated that its important and significant anatomical characters were those of the leaf epidermis. Especially, several arthors (Ahuja 1962; Metcalfe and Gregory 1964, 1969, 1971; Koyama 1966. Mehra and Sharma 1965; Govindarajalu 1969; Oh and Lee 1968-1980) studied epidermal patterns of the leaf blade of sedge plants by using light microscope. Recently, Schuyler (1971), Walter (1975), Toivnen and Timonen (1976), Oh (1980, 1985) and Denton (1983) studied the leaf and achene epidermis of certain Cyperaceae with a scanning electron microscope.

In the study by SEM and LM were the epidermal characters for specifying used *Carex* taxonomy. In the present study it seems to be the first time to characterize the epidermal patterns for *Carex* taxonomy by using SEM and LM.

The results by both LM and SEM can be finely compared in every characters of taxonomic phylogeny.

In conclusion, the epidermal patterns of *Carex* seem to be very useful characteristics for classifying species.

It is required to further study that the epidermal characters by combining LM and SEM might be very useful for the study of sedge family taxonomy.

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摘 要

사초속(*Carex*) 식물의 잎의 표피형에 대한 연구로써 12 sect. 14 species에 대하여 광학현미경(LM)과 주사현미경(SEM)을 사용하여 표피형을 조사하였다. 표피형의 구성요소인 기공, 긴세포벽, 유두돌기, 가시돌기와 납질 등의 모양과 배열을 관찰하였다. 특히 주사현미경에 의한 미세구조의 특징도 종류에 따라 차이가 있어 사초과 식물의 유연관계를 밝히는데 중요한 형질이 될 수가 있다고 보는 바이다. 그리고 아울러 검색표도 만들었다.

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Plate I. LM and SEM photomicrographs of the leaf surfaces of *Carex*. (ab = abaxial surface, ad = adaxial surface)

1. <i>Carex rostrata</i> (ab, LM × 500)	7. <i>C. atrata</i> (ab, SEM × 500)
2. <i>C. rostrata</i> (ab, SEM × 500)	8. <i>C. buxabaumii</i> (ad, LM × 500)
3. <i>C. saxatilis</i> (ad, LM × 500)	9. <i>C. buxabaumii</i> (ab, SEM × 200)
4. <i>C. saxatilis</i> (ad, SEM × 500)	10. <i>C. buxabaumii</i> (ab, LM × 500)
5. <i>C. atrata</i> (ab, LM × 500)	11. <i>C. buxabaumii</i> (ad, SEM × 1,000)
6. <i>C. atrata</i> (ad, SEM × 500)	

Plate II. LM and SEM photomicrographs of the leaf surfaces of *Carex*. (ab = abaxial surface, ad = adaxial surface)

1. <i>Carex aquatilis</i> (ab, LM × 500)	7. <i>C. limosa</i> (ad, LM × 500)
2. <i>C. aquatilis</i> (ab, SEM × 200)	8. <i>C. limosa</i> (ad, SEM × 500)
3. <i>C. pseudocyperus</i> (ab, LM × 500)	9. <i>C. maculata</i> (ab, LM × 500)
4. <i>C. aquatilis</i> (ad, SEM × 200)	10. <i>C. maculata</i> (ab, SEM × 1,000)
5. <i>C. limosa</i> (ab, LM × 500)	11. <i>C. disperma</i> (ad, LM × 500)
6. <i>C. limosa</i> (ab, SEM × 500)	12. <i>C. disperma</i> (ad, SEM × 500)

Plate III. LM and SEM photomicrographs of the leaf surfaces of *Carex*. (ab = abaxial surface, ad = adaxial surface)

1. <i>Carex disperma</i> (ab, LM × 500)	7. <i>C. norvegica</i> (ad, LM × 500)
2. <i>C. disperma</i> (ab, SEM × 1,000)	8. <i>C. norvegica</i> (ad, SEM × 500)
3. <i>C. brunnescens</i> (ad, LM × 500)	9. <i>C. norvegica</i> (ab, LM × 500)
4. <i>C. brunnescens</i> (ad, SEM × 200)	10. <i>C. norvegica</i> (ab, SEM × 500)
5. <i>C. brunnescens</i> (ab, LM × 500)	11. <i>C. macloviana</i> (ad, LM × 500)
6. <i>C. brunnescens</i> (ab, SEM × 1,000)	12. <i>C. macloviana</i> (ad, SEM × 1,000)

Plate I

Plate II

Plate III