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Phytolith Morphology of Leaf Epidermal Cells of *Oryza* L.

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Epidermal leaf cell phytoliths of 17 species of *Oryza* were examined with backscattered electron imaging with scanning electron microscopy to determine the usefulness of phytolith morphology for systematic studies. Many kinds of identifiable phytolith morphology are observed in the leaf blades of *Oryza*. They are different among species as well as between adaxial and abaxial surface and costal and intercostal strip portions of leaf blades. Distinguishing phytolith characters are useful at the level of the section, such as *Angustifoliae*, *Ridleyanae*, and *Granulatae*. The results of a cluster analysis using 21 qualitative characters of phytoliths reveal many taxonomic characteristics which are compatible with current taxonomy.

Keywords: phytolith, morphology, taxonomy, *Oryza*

Phytolith formed by silicon deposition are known to be produced in almost all the plant parts (Whang and Kim, 1994), and they can have important systematic implications because of their various morphologies (e.g. Rovner, 1971; Rapp and Mulholland, 1992; Whang and Hill, 1995). In addition, silicon deposition is not universal because many taxa have little or no identifiable phytoliths. In general, monocots form more phytolith than the dicots (Piperno, 1988). Because of their distinctive characteristics such as development of silica cells for silica deposition as well as the differing amounts of opaline silica deposites compared to other taxa, the grass plants have received much attention for phytolith analysis (Prat, 1932, 1936; Metcalfe, 1960; Parry and Smithson, 1964; Twiss *et al.*, 1969; Ellis, 1976, 1979; Brown, 1984; Piperno, 1984; Mulholland, 1987; Pearsall, 1989; Whang, 1993). However phytolith systematics, which can be useful in interdisciplinary research, is still far from complete. The process necessary to establish the phytolith systematics would likely include firstly investigating all the types of phytoliths produced by the taxa; secondly, setting up a taxonomic key using these morphological features; and finally comparing this new system with current plant taxonomies to determine its utility. Up to the present there are few studies which even investigate all the types of phytolith produced by all the species in a taxa.

As a silicon accumulating plant, the genus *Oryza* is a representative taxa. Much phytolith research has only focused on rice (*Oryza sativa* L.) because it is an important economic plant (Soni *et al.*, 1972; Soni and Parry, 1973; Kunoh and Akai, 1977; Fujiwara and Sasaki, 1978; Whang and Kim, 1994). In the case of rice, it is possible to observe almost all types of phytoliths produced by the whole plant by examining only leaf phytoliths (Whang and Kim, 1994). However, no studies have been conducted with all the types of phytoliths found in all species within the genus *Oryza,* nor has the taxonomic significance been tested.

The aims of this study were to examine the types of phytoliths produced by 17 species of *Oryza*, to provide a taxonomic key using these morphological features, and to compare this system with current taxonomic treatment of *Oryza* to evaluate the taxonomic significance of phytolith morphology.

MATERIALS AND METHODS

Plant materials used for this study were 17 species of *Oryza* (Table 1). From 1991 to 1992, *Oryza sativa* L. was collected from around Chonju City and also obtained from Honam Crop Experiment Station in Korea. Others were obtained from IRGC (Herbarium, International Rice Germplasm Center) in the Philippines.

Leaf squares of about 5 mm were excised from

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the middle portion of mature leaves and were observed with scanning electron microscopy (SEM). The leaf squares were sonicated in hexane-chloroform solutions (give the concentration here such as 1 : 5) for 5 min. The tissues were then dried and mounted on aluminum stubs using double-sided adhesive tape. The samples were observed with SEM, using backscattered electron imaging (BSI) at 30 Kv with a Robinson detector.

Cluster analysis was used for statistical analysis. The analysis involved squared Euclidean distance (distance $(X,Y)=\Sigma_i (X_i-Y_i)^2$) using average linkage and UPGMA. All analyses were performed using an SPSS-X Release 2.2 Program and CYBER 932-31 computer at Chonbuk National University.

The phytolith terminology used for these studies were cited from the articles of Metcalfe (1960), Twiss *et al.* (1969), and Whang (1993).

Table 1. Specimens used for the study of *Oryza* phytoliths

OTU	scientific name	accession number	distribution
	Sect. *Oryzae*		
1	*O. alta* Swallen	IRGC 88-807	America
2	*O. australiensis* Domin	Acc. 105272 100882	Austraila
3	*O. eichingeri* Peter	Acc. 101426 105407	Africa
4	*O. grandiglumis* Prod.	Acc. 105669	America
5	*O. barthii* Chev.	W1232	Africa
6	*O. minuta* J.S. Presl	P90-18a Acc. 103878	Asia
7	*O. officinalis* Wall	LME-Zamboanya, NS 32	Asia
8	*O. punctata* Kotschy	Acc. 105158 104975	Africa
9	*O. rhizomatis* Vaughan	Acc. 103410 103417	Asia
10	*O. rufipogon Griff.*	IRGC 88-1044,	Asia,
11	*O. rufipogon* (intermediate type by D.A. Vaughan)	VN90-W29 P90-1A	America
12	*O. sativa* L.	Acc. 64508 JNU 91.9.15, 91.10.10, 91.10.15, 91.11.5, 91.11.7, 92.5.14, 92.6.10, 92.7.9,	Asia World wide
	Sect. *Granulatae*		
13	*O. granulata* Nees	W5 89-23 SMV 9	Asia
14	*O. meyeriana* Baill.	P90-5	Asia
	Sect. *Ridleyanae*		
15	*O. longiglumis* Jansen	Acc. 101254 105662	New Guinea
16	*O. ridleyi* Hook	Acc. 100820 100821	Asia
	Sect. *Angustifoliae*		
17	*O. brachyantha* Chev.	Acc. 1012340 101234	Africa

RESULTS

Many kinds of identifiable phytolith morphologies developed on leaf blades were observed in the 17 species of *Oryza* (Table 2, 3). These morphologies were different from not only from various leaf blade areas but also among species.

The morphologies and distribution of phytoliths on the adaxial surfaces of leaf blades are as follows. Silica bodies on the costal strip had three shapes (Figs. 1-6); cross was only found in *O. brachyantha*, bilobate in 10 such as *O. alta, O. australiensis, O. grandiglumis, O. barthii, O. minuta, O. officinalis, O. rhizomatis, O. rufipogon, O. rufipogon* intermediate

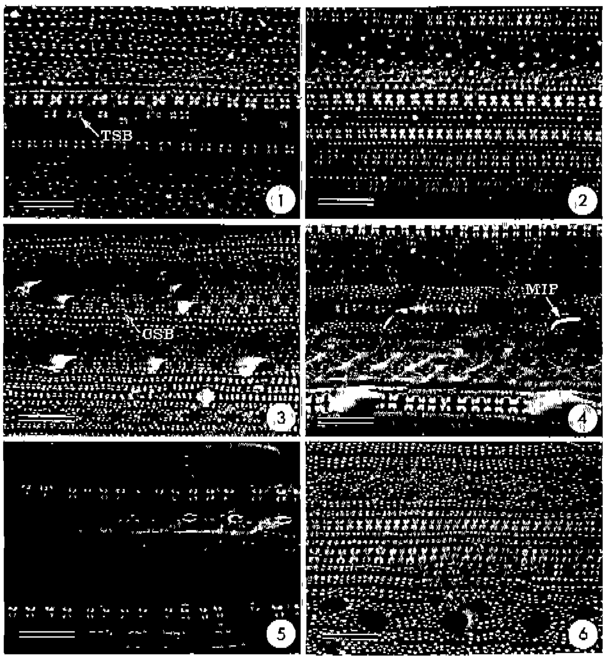
Table 2. Qualitative characters used for cluster analysis of phytoliths in *Oryza* leaf blades

character	character coding
Adaxial side of leaf blade	
A: types of silica bodies	cross only(1), cross and bilobate(3), bilobate only(5)
B: papillae	absent(1), present(5)
C: prickle-hair on costal strip	absent(1), present(5)
D: macro-hair	present(1), absent(5)
E: prickle-hair on intercostal strip	present(1), absent(5)
F: micro-hair	present(1), absent(5)
G: silica bodies on intercostal strip	present(1), absent(5)
H: number of the band of silica body on midrib	1(1), 1 or 2(2), 2(3), 2 or 3(4), >3(5)
I: number of the band of silica body on large vein	1(1), 1 or 2(2), 2(3), >3(5)
J: number of the band of silica body on small vein	1(1), 1 or 2(5)
K: number of the band of silica body on leaf margin	1(1), 1 or 2(2), 2(3), >3(5)
L: middle size papillae	present(1), absent(5)
Abaxial side of leaf blade	
M: large papillae	present(1), absent(5)
N: prickle-hair on costal strip	present(1), absent(5)
O: prickle-hair on intercostal strip	present(1), absent(5)
P: macro-hair	present(1), absent(5)
Q: micro-hair	present(1), absent(5)
R: silica bodies on intercostal strip	present(1), absent(5)
S: number of the band of silica body on midrib	1(1), 1+scattered(3), scattered(5)
T: number of the band of silica body on large vein	2(1), 2 or 3(3), >3(5)
U: number of the band of silica body on leaf margin	1(1), 2(2), 2 or 3(3), >3(5)

Table 3. Data matrix used for cluster analysis of phytoliths on leaf of *Oryza* (OTU numbers correspond to those in Table 1. For character numbers, see Table 2)

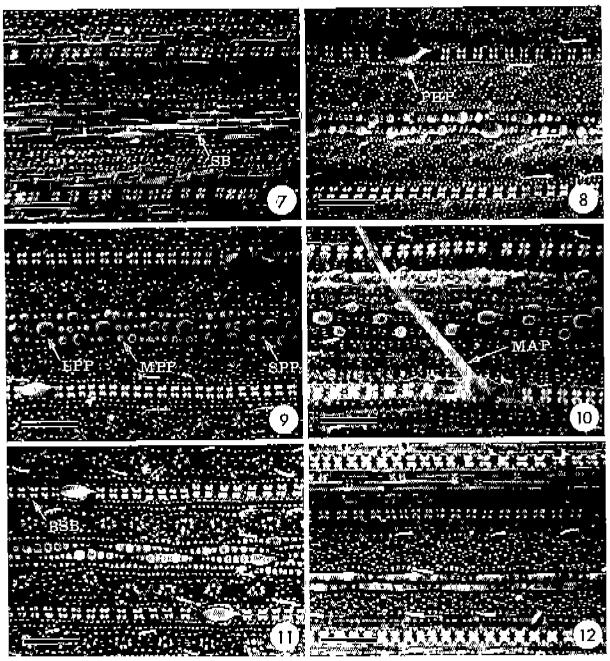
OTU	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	5	5	1	5	5	1	5	3	2	1	2	5	1	1	5	5	1	5	3	1	2
2	5	5	5	5	1	1	1	5	5	5	5	5	5	5	5	5	1	1	3	1	5
3	3	5	1	5	1	1	1	1	1	1	1	5	5	1	5	5	1	1	3	1	5
4	5	5	5	5	1	1	5	5	3	1	5	5	1	1	5	5	1	5	5	1	2
5	5	5	5	5	1	1	5	5	1	1	5	1	5	1	5	5	1	5	3	1	2
6	5	5	5	5	1	1	1	3	1	1	1	5	5	1	1	5	1	1	3	1	2
7	5	5	5	5	1	1	5	4	1	1	1	5	1	1	1	5	1	1	1	1	2
8	3	5	5	5	1	1	1	4	5	1	1	5	5	1	1	5	1	5	5	1	2
9	5	5	5	1	1	1	1	3	2	5	1	1	1	1	5	1	1	1	5	1	2
10	5	5	5	5	1	1	5	1	1	1	1	5	1	1	1	5	1	5	1	1	2
11	5	5	5	1	1	1	5	5	5	1	1	1	1	5	5	1	1	5	1	1	2
12	5	5	5	1	5	1	5	5	3	1	3	1	1	1	5	1	1	5	5	1	1
13	3	5	1	5	5	1	5	3	3	1	3	5	1	1	5	5	1	5	3	5	5
14	3	5	1	5	5	1	5	3	5	1	5	5	1	1	5	5	1	5	3	5	5
15	3	1	5	5	5	1	1	2	3	1	1	5	5	1	1	5	1	1	5	1	2
16	3	1	1	5	5	1	1	3	3	1	2	5	5	1	1	5	1	1	5	1	2
17	1	5	5	5	1	5	5	3	3	5	3	5	1	1	5	5	5	5	1	3	3

type, and *O. sativa*, and cross and/or bilobate in six such as *O. eichingeri*, *O. punctata*, *O. granulata*, *O. meyeriana*, *O. longiglumis*, and *O. ridleyi*. Silica bodies from the intercostal strip were produced by seven species (Fig. 4) such as *O. australiensis*, *O. eichingeri*, *O. minuta*, *O. punctata*, *O. rhizomatis*, *O. longiglumis*, and *O. ridleyi*, but by 10 (Figs. 1, 2, 3, 5, 6) such as *O. alta*, *O. grandiglumis*, *O. barthii*, *O. officinalis*, *O. rufipogon*, *O. rufipogon* intermediate type, *O. sativa*, *O. granulata*, and *O. meyeriana*. The papillae phytoliths were produced by all species (Figs. 1, 2, 3, 4, 6) except for *O. longiglumis* and *O. ridleyi* (Fig. 5). The middle-papillae phytoliths which have their origin in the middle sized papillae are produced by four species (Fig. 2) such as *O. barthii*, *O. rhizomatis*, *O. rufipogon* intermediate type and O. sativa out of the 17 (Figs. 1, 3, 4, 6). The prickle-hair phytoliths from both the costal strip and the intercostal strips were produced by many species (Figs. 3, 4), except five or six (Figs. 5, 6) such as *O. alta*, *O. eichingeri*, *O. granulata*, *O. meyeriana*, and *O. ridleyi*. Macro-hair phytoliths were absent from many species, except for three such as *O. rhizomatis*, *O. rufipogon* intermediate type, and *O. sativa*. But micro-hair phytoliths were produced by most species (Fig. 4) except for *O. brachyantha* (Fig. 3). The band number of silica bodies varied both among species and among costal strips (Figs. 1-6).



Figs. 1-6. BSI (Back-scattered Electron Imanging) photographs of phytoliths on adaxial surface of leaf blade. Bars=66 μm. 1. *O. punctata* showing the costal strip on midrib (TSB: trilobate silica body). 2. *O. sativa* showing the costal strip on midrib. Note the poly-bands of silica body. 3. *O. brachyantha* showing the costal strip on midrib and the intercostal strip on between midrib and large veins (CSB: cross-shape silica body). 4. *O. minuta* showing the costal strip on large veins and the intercostal strip on between large veins and small veins (MIP: micro-hair phytolith). 5. *O. ridleyi* showing the costal strip on small veins and the intercostal strip on between small veins. Note the absence of papillae phytoliths. 6. *O. barthii* showing the costal strip on leaf margin and the intercostal strip on near the leaf margin.

The morphologies and distributions of phytoliths on the abaxial surface of leaf blades were as follows. Silica bodies on intercostal strips were produced by seven species (Figs. 7, 8) such as *O. australiensis*, *O. eichingeri*, *O. minuta*, *O. officinalis*, *O. rhizomatis*, *O. longiglumis*, and *O. ridleyi* out of the 17 (Figs. 9-12). The large-papillae phytoloths, which have their origin in large papillae, were produced by 10 species (Figs. 8-10) such as *O. alta*, *O. grandiglumis*, *O. officinalis*, *O. rhizomatis*, *O. rufipogon*, *O. rufipogon* intermediate type, *O. sativa*, *O. granulata*, *O. meyeriana*, and *O. brachyantha* but not by seven (Figs. 7, 12) such as *O. australiensis*, *O. eichingeri*, *O. barthii*, *O. minuta*, *O. punctata*, *O. longiglumis*, and *O. ridleyi*. Prickle-hair phytoliths



Figs. 7-12. BSI (Back-scattered Electron Imanging) photographs of phytoliths on abaxial surface of leaf blade. Bars=66 μm. 7. *O. australiensis* showing the costal strip on small veins and the intercostal strip on between small veins. Note the silica bodies developed on intercostal strip (SB: silica body). 8. *O. minuta* showing the costal strip of small veins and the intercostal strip on between small veins. Note the absence of silica body on intercostal strip (PHP: prickle-hair phytolith). 9. *O. officinalis* showing the costal strip on small veins and the intercostal strip on between small veins. Note the papillae phytoliths (LPP: large papillae phytolith; MPP: middle papillae phytolith; SPP: small papillae phytolith). 10. *O. rufipogon* (intermediate type) showing the costal strip on small veins and the intercostal strip on between small veins (MAP: macro-hair phytolith). 11. *O. meyeriana* showing the costal strip on small veins and the intercostal strip on between small veins (BSB: bilobate silica body). 12. *O. eichingeri* showing the costal strip of large veins and intercostal strip on between large veins and small veins. Note the absence of large papillae phytolith.

from costal strips were produced by all species (Figs. 8, 9, 11) except for two (Figs. 7, 10) such as *O. australiensis* and *O. rufipogon* intermediate type, but not in intercostal strips in many species (Figs. 7, 10, 11, 12) such as *O. alta, O. australiensis, O. eichingeri, O. grandiglumis, O. barthii, O. rhizomatis, O. rufipogon* intermediate type, *O. sativa, O. granulata, O. meyeriana,* and *O. brachyantha.* Micro-hair phytoliths were produced by most species (Figs. 8-12) except for *O. brachyantha*. Macro-hair phytoliths were observed in three (Fig. 10) such as *O. rhizomatis, O. rufipogon* intermediate type, and *O. sativa.* The band numbers and distributions of silica bodies varied both among species and among costal strips (Figs. 7-12).

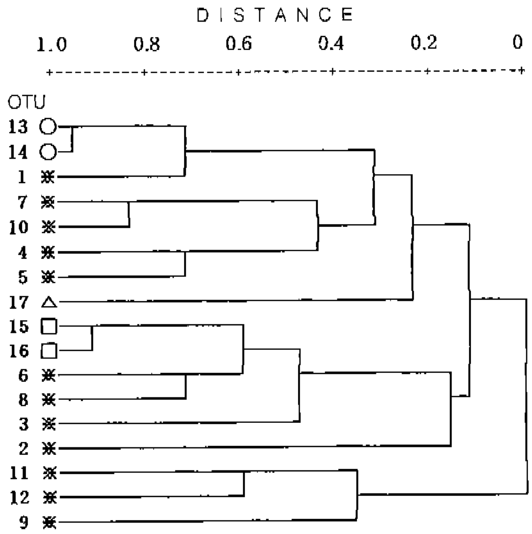


Fig. 13. Phenogram of *Oryza* species by UPGMA based upon twenty-one qualitative characters of phytoliths. OTU numbers correspond to those in Table 1 (※: *Oryzae*; ○: *Granulatae*; □: *Ridleyanae*; △: *Angustifoliae*).

Table 2 is 21 qualitative characters obseved and their character coding for cluster analysis of phytoliths from *Oryza* leaf blades. A data matrix was also constructed using the above 21 qualitative characters of *Oryza* phytoliths to do a cluster analysis by UPGMA (Table 3, Fig. 13).

According to the agglomeration schedule, *O. granulata* and *O. meyeriana* both belong to the section *Granulatae* which was clustered by a cluster coefficient of 8.000, and secondly *O. longiglumis* and *O. ridleyi* both belong to the section *Ridleyanae* by a cluster coefficient of 18.000. However the species belonging to the section *Oryzae* were combined with each other by several cluster coefficient values of agglomeration schedules. *Oryza brachyantha* belongs to the section *Angustifoliae* and had a high cluster coefficient value in combining with other clusters (Fig. 13).

At the level of similarity distance 0.3 (Fig. 13), the largest group was determined by combining the sections *Oryzae* and *Granulatae*. Based upon these data, the group of *Oryzae* includes the species of *O. alta, O. barthii, O. grandiglumis, O. officinalis,* and *O. rufipogon,* and these species were characterized by the non-production of silica bodies on intercostal strips (Table 2). The second biggest group was determined by combining of the sections *Oryzae* and

Ridleyanae. In this case, the group of *Oryzae* includes the species of *O. australiensis, O. minuta, O. eichingeri*, and *O. punctata*, and these species were characterized by the production of silica bodies on intercostal strips except for *O. punctata* (Table 2). The third group is *Oryzae* including the species of *O. rhizomatis, O. rufipogon* (intermediate type), and *O. sativa*, and these species did not produce the macro-hair phytoliths (Table 2). The smallest group is *Angustifoliae* consisting of only *O. brachyantha*.

DISCUSSION

Oryza belongs to the Gramineae, and its infrageneric classifications differ with interpretations of different researchers. The four section taxonomic system (Roschevicz, 1931; Tateoka, 1965; Vaughan, 1989), based upon the features of spikelet and glume, was used for this study to compare with current phytolith data. The number of *Oryza* species is over 20 (Launert, 1965; Tateoka, 1965), but now some of them are either already extinct or are hard to collect (Vaughan, 1989). Therefore for this study 17 species of *Oryza* were examined which are possible to obtain at present. Backscattered electron imaging was employed in this study, because it had an advantage of making it possible to observe the superficial siliceous structures of grass leaves (Brandenburg *et al.*, 1985).

Cluster analysis of *Oryza*, based upon 21 qualitative characters of phytoliths in leaf blades, likely implies some taxonomic significance. Namely, *O. granulata* and *O. meyeriana* are considered to be closely related to each other because the first cluster analysis yielded a low cluster coefficient; and currently both are characterized by having the subulate of sterile lemma and the lack of awn as a single section (Tateoka, 1963; Vaughan, 1989). *Oryza longiglumis* and *O. ridleyi* also yielded a low cluster coefficient, and currently both are characterized by having the subulate of sterile lemma, the development of awns, and are perennial as a single section (Tateoka, 1963; Vaughan, 1989). Therefore, these findings clearly support the current infrageneric classification. The species of *O. brachyantha* belonging to the section *Angustifoliae* is seperated by 0. 3 of overall similarity as an independent taxa, and currently this species is characterized by having the subulate of sterile lemma, the development of awns, and are annuals (Tateoka, 1963; Vaughan, 1989). This species is also distinctive because it produces the cross shaped silica body on the costal strip compared to others. In the case of *Oryzae*, the results of the cluster test are more or less different from the current taxonomy based upon the features of inflorescence. However, it is interesting that this section could be divided into two groups; one produces the silica body on intercostal strips, the other develops the macro-hair phytoliths. These findings suggest that phytoliths can be a significant factor for taxonomic studies and need to be studied further to confirm these observations.

On the other hand, this study has made it obvious that many types of phytoliths are present among species and by different portions of leaf blades. In particular the species of *O. brachyantha* belonging to the section *Angustifoliae* is characterized by both cross shaped silica bodies, and having no micro-hair phytoliths on either adaxial and abaxial surfaces of leaf blades. These distinctive features seem to reflect the fact that it is most closely related to the genus *Leersia* of all *Oryza* species (Vaughan, 1989). Most species definitely produce pappillae phytoliths on both adaxial and abaxial leaf surfaces whether they origined from the small-, middle-, and large-size papillae. However it is interesting that only two species, *O. longiglumis* and *O. ridleyi* belong to the section *Ridleyanae*, but do not produce them. It could be useful as a diagnostic character in dividing the section; this finding coincided with the current taxonomy based upon spiket features (Tateoka, 1965). Most species form one and/or two band(s) of silica bodies on the large veins. But two species *O. granulata* and *O. meyeriana*, which belong to the section *Granulatae* are distinctive, forming more than three bands. It is therefore possible that this feature could also be useful in dividing the section to the genus level, because it coincides with current taxonomy (Tateoka, 1965; Vaughan, 1989). In addition, the trilobate silica bodies seem to be a species specific character within the genus, for which *O. punctata* is an example.

There were many representative phytoliths which were found during these studies; silica bodies, macro-hair, micro-hair, prickle-hair, and papillae. Stomata phytoliths and long-cell phytoliths were also found to have important morphological and taxonomical useful characteristics. We know that the descriptions of their features are absent in this study. We will discuss these more fully in a later paper.

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