# DISTINGUISHING RICE (*ORYZA SATIVA* POACEAE) FROM WILD *ORYZA* SPECIES THROUGH PHYTOLITH ANALYSIS: RESULTS OF PRELIMINARY RESEARCH[1]

DEBORAH M. PEARSALL, DOLORES R. PIPERNO, ELIZABETH H. DINAN, MARCELLE UMLAUF, ZHIJUN ZHAO, AND ROBERT A. BENFER, JR.

**Pearsall, Deborah M.** *(Department of Anthropology, 107 Swallow Hall, University of Missouri, Columbia, MO 65211),* **Dolores R. Piperno** *(Smithsonian Tropical Research Institute, A.P.O Miami, FL 34002 and MASCA, University of Pennsylvania Museum, 33rd and Spruce Sts., Philadelphia, PA 19104),* **Elizabeth H. Dinan** *(Department of Anthropology, 109 Davenport Hall, University of Illinois, Urbana, IL 65201),* **Marcelle Umlauf** *(Bilby Research Center, Northern Arizona University, Flagstaff, AZ 86011),* **Zhijun Zhao,** *and* **Robert A. Benfer, Jr.** *(University of Missouri).* DISTINGUISHING RICE (*ORYZA SATIVA* POACEAE) FROM WILD *ORYZA* SPECIES THROUGH PHYTOLITH ANALYSIS: RESULTS OF PRELIMINARY RESEARCH. *Economic Botany 49(2):183–196. 1995.* *Asian rice is an important grain, not only in its homeland but in many areas of the world. Identifying rice in the archaeological record is a challenge, especially in the moist tropics, where organic materials preserve only when charred. Phytolith analysis, the identification of opaline silica bodies, provides an alternative method for identifying this important crop. Results of our research suggest that* Oryza *contributes phytoliths that are genus-specific, that bulliform characteristics alone do not permit separation of wild and domesticated* Oryza *in regions where species overlap, and that a number of phytolith types, especially silicified glumes, show promise for separating wild from domesticated forms. With further research it should be possible to identify rice through its phytolith assemblage in archaeological soils in the heartland of its domestication and use.*

Discerner entre le riz (*Oryza sativa* Poaceae;) et les espèces sauvages D'*Oryza* par l'analyze phytolithique: Résultats de recherches préliminaires. *Le riz d'Asie est une céréale importante, non seulement dans son pays d'origine, mais à travers le monde. L'identification du riz dand les données archaéologiques présente des problèmes, surtout dans les tropiques humides où les restes organiques ne se conservent qu'à l'état brulé. L'analyze phytolitique-identification de particules de silica opalisé-fournit une méthode alternative qui permet l'étude de cette céréale importante. Nos recherches suggèrent que* Oryza *produit des phytolithes qui sont identifiables au niveau du genre, que les charactéristiques bulliformes seules ne permettent pas de faire la distinction entre* l'Oryza *sauvage et domestiqué dans les régions où les espèces se chevauchent, et que plusieurs sortes de phytolithes, surtout les glumes silicifiées promettent de pouvoir séparer les formes sauvages des formes domestiques. Avec des recherches supplémentaires, il devrait être possible d'identifier le riz grâce à son assemblage phytolithique obtenu de sols archaéologiques provenant du centre de sa domestication et de son usage.*

〈利用植物硅体细胞分析方法对稻谷(Oryza sativa)的鉴定〉 亚洲稻谷，不仅在其原生地而且在世界许多地区，均为一种重要的谷物。 在考古发现中鉴定稻谷是一个难题，尤其在潮湿的热带地区，有机物经过炭化方可保存下来。然而，植物硅体细胞分析为这种重要的谷物的鉴定提供了另一种方法。我们的研究结果揭示了某些产生于稻属(Oryza)植物的硅体细胞具有属级鉴定的特点；所谓bulliform类型硅体细胞，其特点不足以将栽培稻与野生稻区分开，尤其在那些不同种杂生的地区；但某些硅体细胞类型，特别是一种产生于稻类植

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物颖壳上的细胞类型和某些小细胞，却显示了将栽培稻从野生稻中鉴别出来的能力。在今后的研究中，利用从考古土样中提取的植物硅体细胞鉴定稻谷遗存应是可能的。

**Key Words:** *Oryza sativa*; Asia; China; Phytolith.

Rice is one of the most important grain crops in the world today, a mainstay of diet not only in eastern Asia, its place of origin, but throughout much of the Old World and New World topics. It is an ancient crop, appearing in the archaeological record as early as 6200–7100 B.C. at the Pengtoushan site in Hunan Province, Central China (Institute of Archaeology of Hunan Province and the Administration of Antiquities of Li County 1990), at a number of sites in eastern and south central China by 4000–5000 B.C. (Chen 1989), and was probably brought under cultivation millenia earlier.

In this paper we report our preliminary results on developing a method to distinguish domesticated rice from wild *Oryza* species through phytolith analysis. Our ultimate goal is to develop a method which can be applied in archaeological settings in the homeland of rice, and be used to document its domestication and spread. Phytolith analysis has been shown to be a valuable tool in identifying the presence of crops in archaeological sites, especially useful in areas of poor preservation of organic macroremains (Pearsall 1989; Pearsall and Piperno 1993; Piperno 1988). Fujiwara's success in identifying rice phytoliths in Japan, outside the native distribution of related or ancestral *Oryza* species (Fujiwara 1993), encouraged us to test the feasibility of identifying domesticated rice in its heartland. We present the results of our research to date, beginning with an overview of phytolith analysis and the issues surrounding the origin of rice. Among our preliminary findings are: (1) that *Oryza* contributes phytoliths which appear to be genus-specific, (2) that bulliform characteristics (Fujiwara's identification method) alone do not permit separation of wild and domesticated *Oryza* in regions where species overlap, and (3) that a number of phytolith types, especially silicified glumes, in combination with bulliform, short cell, and long cell phytoliths, do show promise for separating wild from domesticated forms. In fact, our preliminary results suggest that ultimately it will be possible to identify rice through its phytolith assemblage in archaeological soils in the heartland of its domestication and use.

## Background

### Phytolith Analysis

Study of phytoliths to identify archaeological plant material began early in this century in the Old World. Application of phytolith analysis in archaeology in the New World dates from the 1960s with investigations at the Kotosh site in Peru (Matsutani 1972), but it was not until the mid-1970s that interest in the technique among American archaeologists began to grow (for reviews of recent work see Pearsall 1982, 1989; Pearsall and Piperno 1993; Piperno 1988; Rapp and Mulholland 1992; Rovner 1983).

Phytoliths occur in stems, leaves, inflorescence bracts, and seeds of many plants. Silica that forms phytoliths is carried up from ground water as monosilicic acid (Jones and Handreck 1967) and is deposited in cells. In some taxa, distinctively shaped bodies are formed when silica completely fills the cell, solidifies, and retains cell shape after organic tissue has decayed or burned (Blackman 1971). The taxonomic value of phytoliths produced by grasses, other monocotyledons, and dicotyledons varies. However, recent work has shown that many families outside of the Poaceae produce phytoliths diagnostic at the family, genus, and even species levels [see Piperno (1985, 1988, 1989, 1991) and papers in Rapp and Mulholland (1992) and Pearsall and Piperno (1993) for reviews of phytolith production patterns; these publications and Pearsall (1989) for illustrations of major types].

Phytoliths occurring in grasses, one of the highest silica accumulating groups, can be divided into two broad morphological classes: bodies in long cells and bodies in short cells (Metcalfe 1960). Phytoliths produced by forbs and woody plants are very diverse. Some are irregular in appearance, such as the honeycomb and platelike masses produced by many plants, and are of limited taxonomic value. Others, like the spinulose spheres produced by palms, trough-like bodies produced by *Heliconia,* and distinctive seed phytoliths produced by many tropical tree taxa, to name just a few, are diagnostic at the family, genus, and sometimes species levels.

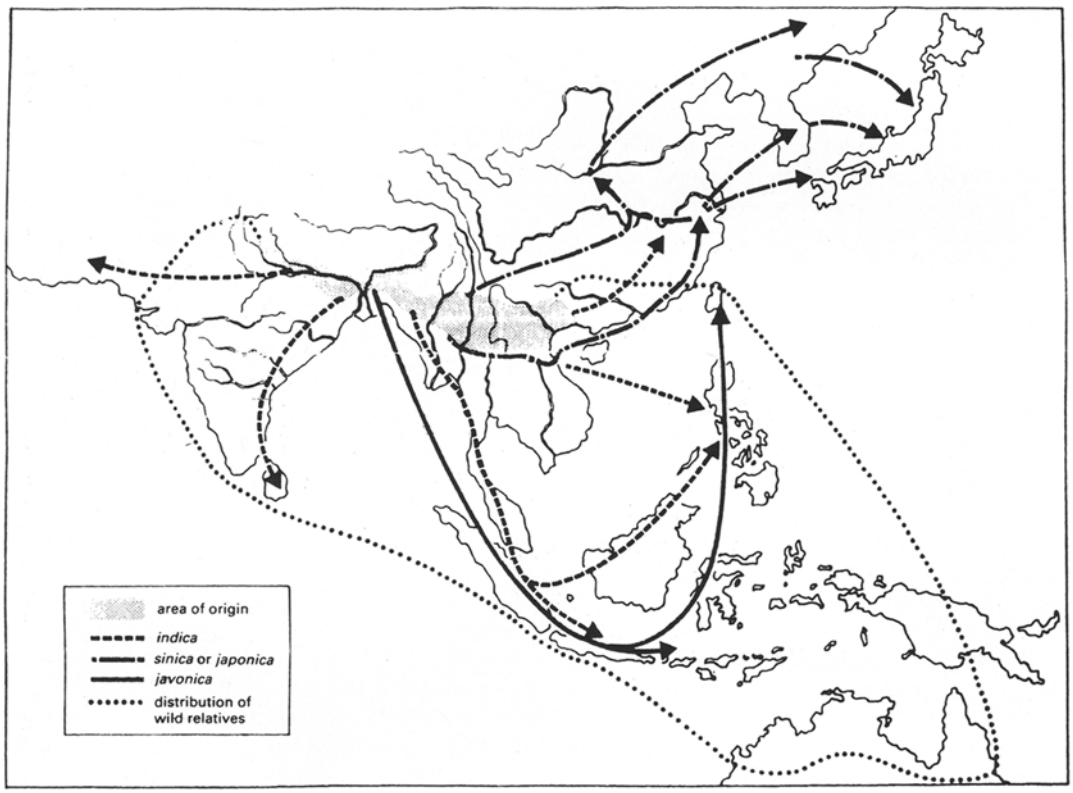


**Fig. 1.** Distribution of rice and related wild species. Adapted with permission from Chang 1989.

## Issues in Rice Domestication and Identification

The question of the center of origin of *Oryza sativa* is an area of active research. Chang (1976a,b, 1989) has presented useful overviews of the domestication and spread of Asian rice. Fig. 1 shows the distribution of the wild relatives of *Oryza sativa,* as well as the routes of spread of the major land races from the proposed area of origin, a broad belt extending from the southern foothills of the Himalayas, across northern Thailand, Laos, and Vietnam, and into south China. However, the earliest archaeological sites with rice remains occur to the north of this area, in the lower and middle reaches of the Yangtze River, China.

The early remains of rice, chaff incorporated into early Neolithic pottery, occur at the Pengtoushan site, dated between 6200 and 7100 B.C. These remains were badly crushed and could not be identified to species. Another early site, Hemodu, in Zheijian Province, dates to 5000 B.C., and contains thick deposits of rice remains, including fruits, leaves, and stalks, identified as *Oryza sativa* (Administration of Antiquities of Zhejiang Province and the Zhejiang Museum 1978; Hemudu Archaeological Team 1980). The earliest sites with rice remains in southern China date only to 2000 to 2500 B.C. The lack of early dates in this important region is likely a product of preservation, since south China, like much of southeast Asia, is characterized by moist, warm climate. Only charred remains would be commonly preserved, and recovery would be minimal without application of water flotation (Pearsall 1989). Rice remains dating to 2000–4000 B.C. have been recovered from a cave in northwest Vietnam, and the Ban Chiang site in Thailand produced remains dating to 3500 B.C. (Chang 1989).

There are many unknowns in the question of the antiquity and area of origin of Asian rice (this paper will not discuss the domestication of the African species, *Oryza glaberrima*). Three different phylogenetic pathways have been pro-

posed for the development of domesticated rice. In a recent summary, Thompson (1992) describes these as follows:

(1) a pathway from wild perennial rice (*Oryza rufipogon*), through development of a wild annual species (*Oryza nivara*), then to annual domesticated *Oryza sativa* (proposed by Chang 1976a),
(2) multiple domestications directly from wild perennial rice (proposed by Oka 1974 and Oka and Morishima 1982), and
(3) multiple domestications from wild perennial rice, with distinctive varieties of that species, *Oryza rufipogon,* being derived from a common ancestor (proposed by Second 1985).

The geographical distributions of the various wild *Oryza* species, including *O. rufipogon,* need further investigation, both to confirm the limits of the probable area or areas of origin and to guide application of techniques for identifying rice archaeologically.

Identifying the presence of rice in archaeological sites is a paleoethnobotanical problem of some complexity, and one that has not been systematically addressed. It may be useful to consider this as two problems: (1) distinguishing domesticated rice from other *Oryza* species in regions where they overlap, i.e., the area enclosed by the dotted line in Fig. 1, and (2) identifying rice outside this range, i.e., in areas to which the domesticated species was carried.

The first identification problem is by far the more difficult. Many archaeological reports do not detail how identifications of seed, glume, or other materials were made, and what criteria were used to identify the species present. Identifying rice outside the distribution of related wild *Oryza* species is not as complex a problem, since glume and seed characteristics can usually be used to distinguish grasses at the genus level. This is also the identification problem most easily addressed through phytolith analysis, as the research of Fujiwara and his co-workers in Japan has shown (Fujiwara 1993).

Fujiwara's phytolith method for identifying rice in agricultural sites in Japan is based on the presence of distinctive thick, multiple-ridged, keystone bulliform cells. His research indicates that bulliform measurements will separate the subspecies *Oryza sativa japonica* and *Oryza sativa indica,* permitting very precise identification of the type of rice grown at sites in Japan. Fujiwara's success in identifying rice outside its area of origin using bulliform characteristics suggests a useful starting point for the potentially more difficult problem of separating domesticated rice from closely related species using phytolith characteristics.

## Methods and Materials

### Distinguishing Rice from Wild *Oryza* Species

In order to evaluate the potential for identifying rice archaeologically through phytolith analysis in regions where both domesticated rice and related wild taxa occur, we conducted a preliminary study of phytolith characteristics of rice and related grasses. Our overall strategy may be described as follows: (1) to examine a number of specimens of cultivated rice to determine phytolith production patterns and assess variability among collections, (2) to examine wild Asian species in the genus *Oryza,* (3) to study phytolith characteristics of the other Asian genera of the tribe Oryzeae, and (4) to examine grass taxa in related tribes on a regional basis. Our major goals were to develop sets of criteria for characterizing the Oryzeae tribe, for distinguishing the genus *Oryza* from other genera in the tribe and *Oryza sativa* from other species in the genus, and to check for "rice confusers" by examining grasses in other tribes selected for their common occurrence in the native flora of regions in which we plan to apply the identification method. Here we report on our progress on all but the final goal; we have only completed preliminary scans of bamboos and have not yet initiated the regional grass study.

We utilized the Clayton and Renvoize (1986) grass classification, which places *Oryza* in the subfamily Bambusoideae and tribe Oryzeae, to select materials for analysis. Table 1 presents the species and herbarium specimens examined in our preliminary study. With a few exceptions we examined two or more specimens of each species to determine overall phytolith production patterns.

Classification within *Oryza* is complex. There are some 20–22 accepted species in six sections (Chang 1976c; Oka 1964). In reviewing herbarium materials for this project, we encountered a number of specimens whose names are no longer accepted. We were also unable to obtain specimens of all Asian *Oryza* species, but did test all Asian species in the section Oryzae (*O. sativa, O. rufipogon, O. officinalis, O. minuta*) (sensu

TABLE 1. SPECIMENS EXAMINED.

| | |
|---|---|
| Oryzeae (tribe): | |
| *Oryza sativa* L. (27)[1] | MBG 2974557, MBG 860758, MBG 860967, MBG 860896, MBG 865166, MBG 832796, MBG 1639785, MBG 1741209, MBG 2721747, MBG 837500, NH 2683283, NH 1130064,[2] NH 1130065, NH 1094074, NH 712749, NH 712729, NH 1962598, NH 1865665, NH 2479714, NH 2479712, NH 2479740, NH 2479707, NH 775933, NH 904154, NH 2240030, NH 1108744, NH 1108746. |
| *Oryza granulata* Nees et Arn. ex Hook f. (1) | MBG 3487373. |
| *Oryza longiglumis* Jansen (2) | NH 2636084, NH 3001417. |
| *Oryza meyeriana* (Zoll. et Mor. ex Steud.) Baill (6) | MBG 2587527, MBG 776312, NH 2589347, NH 1937607, NH 1238730, NH 1526492. |
| *Oryza minuta* J.S. Presl ex C.B. Presl (3) | MBG 2383278, NH 1109775, NH 1259282. |
| *Oryza officinalis* Wall. et Watt (2) | MBG 1824753, MBG 1702248. |
| *Oryza ridleyi* Hook f. (2) | NH 1761831, NH 1718552. |
| *Oryza rufipogon* Griff. (4) | MBG 2317554, NH 3005004, NH 3087113, MBG 2317820. |
| *Chikusichloa aquatica* Koidzumi (1) | NH 1964457. |
| *Chikusichloa brachyathera* Ohwi (1) | NH 1447800. |
| *Hygroryza aristata* (Retz.) Nees (1) | MBG 3010863. |
| *Leersia ciliata* (Retz.) Roxb. (= L. hexandra) (2) | MBG 864694, NH 1649069. |
| *Leersia oryzoides* (L.) Sw. (4) | MBG 45261, MBG 1144681, NH 1962586, NH ?. |
| *Zizania aquatica* L. (1) | MBG 2780512. |
| *Zizania latifolia* (Griseb.) Turcz. ex Stapf. (4) | MBG 1700476, MBG 2366723, NH 1964343, NH 3087122. |

[1] Numbers in parentheses indicate the numbers of specimens examined in the general study of phytolith production patterns.
[2] Specimens used in the glume study (cluster and multiple linear discriminant function analyses) are underlined.
NH: National Herbarium; MBG: Missouri Botanical Garden Herbarium.

Oka 1964). We have not examined *O. nivara,* considered by Chang (1976c) to be a member of the "sativa complex," i.e., carrying the "A" genome (we will include specimens of this and related spontanea forms of *O. sativa* in our final study). We did not examine African, American, or Australian *Oryza* species.

## TRIBAL LEVEL PHYTOLITH CHARACTERISTICS

Members of the Oryzeae tribe are characterized by production of distinctive, rather weakly silicified, leaf epidermal cells with regular, rounded projections (Type 10IIEf, Fig. 2d). [Phytolith types are numbered using the hierarchical system developed at the University of Missouri phytolith laboratory (Pearsall and Dinan 1992).] Such cells are moderate to abundantly produced in *Oryza sativa* and also occur in many of the wild species examined. Other leaf epidermal cells, more three-dimensional, or "blocky," in appearance, also occur widely in the tribe (11IIICa–11IIICc, Fig. 2e,f,g), as do keystone bulliform cells with multiple ridges (50IIIAa, b, and c, Fig. 2h,i,j). In addition to epidermal and bulliform cells, three short cell types are also commonly produced in leaf tissue in the tribe: a dumbbell with scooped ends, thicker than typical panicoid dumbbells (Type 1, Fig. 2a), which grades into a cross-like form; a true cross with raised corners

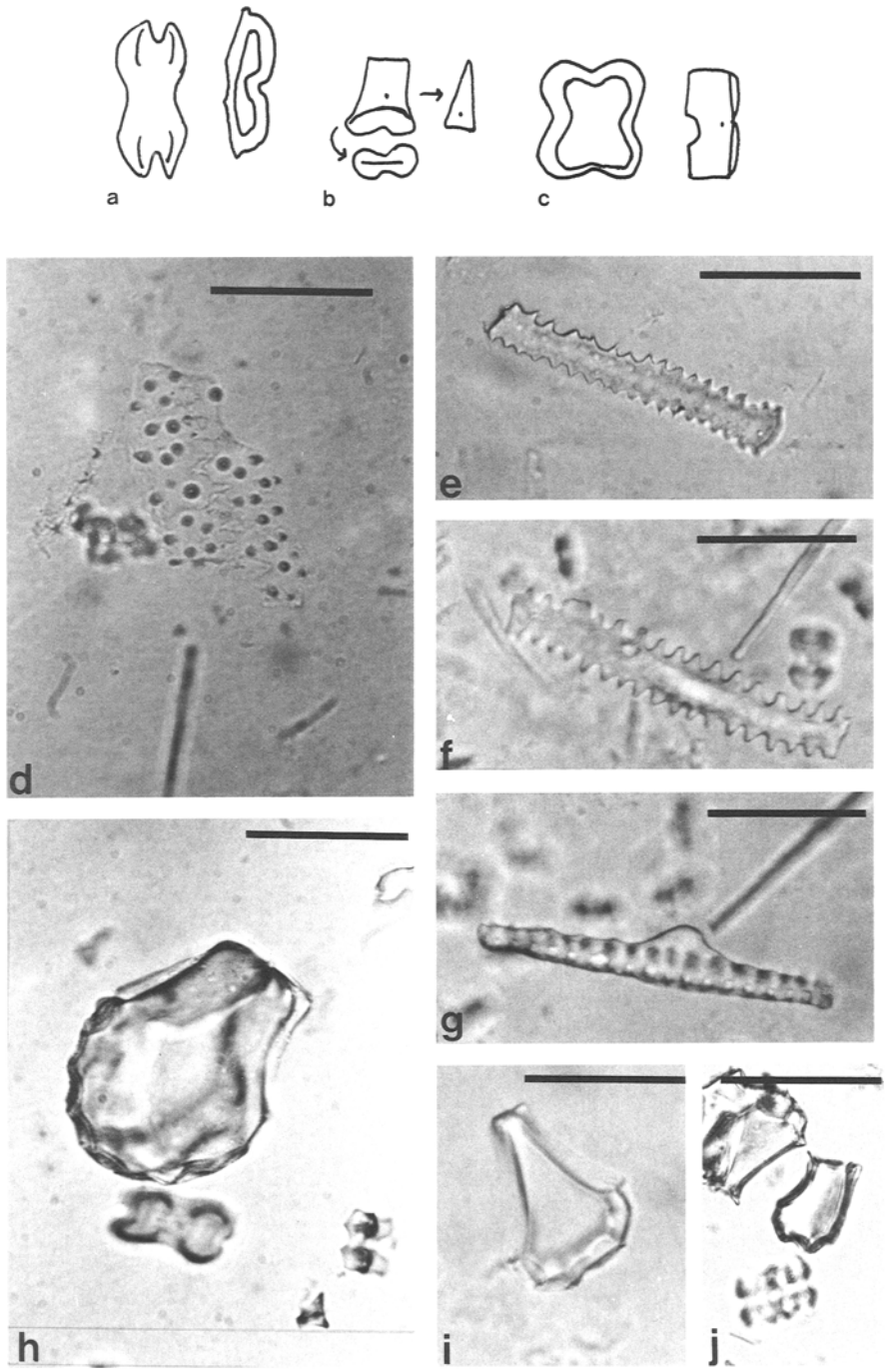


**Fig. 2.** Phytolith types distinguishing the Oryzeae tribe. a: Type 1 dumbbell with scooped ends, curved and lobed in side view (right), thick. The most common short cell in many taxa in the tribe. Grades into a scooped cross-like form; b: Type 8a short cell with a dumbbell on one tier (bottom view, lower), and a thin plate extending perpendicular to the dumbbell-type tier (side view, right); c: Type 5 thick cross, lobed in side view (right), with raised corners. Common in two genera (a–c short cells not drawn to scale. Size 15–25 micrometers); d: 10IIEf epidermal long cell, weakly silicified, with regular, well-silicified, rounded projections (example from *Oryza sativa*); e: 11IIICa. 11IIICa–Cc are blockly epidermal cells with numerous projections of variable size, and

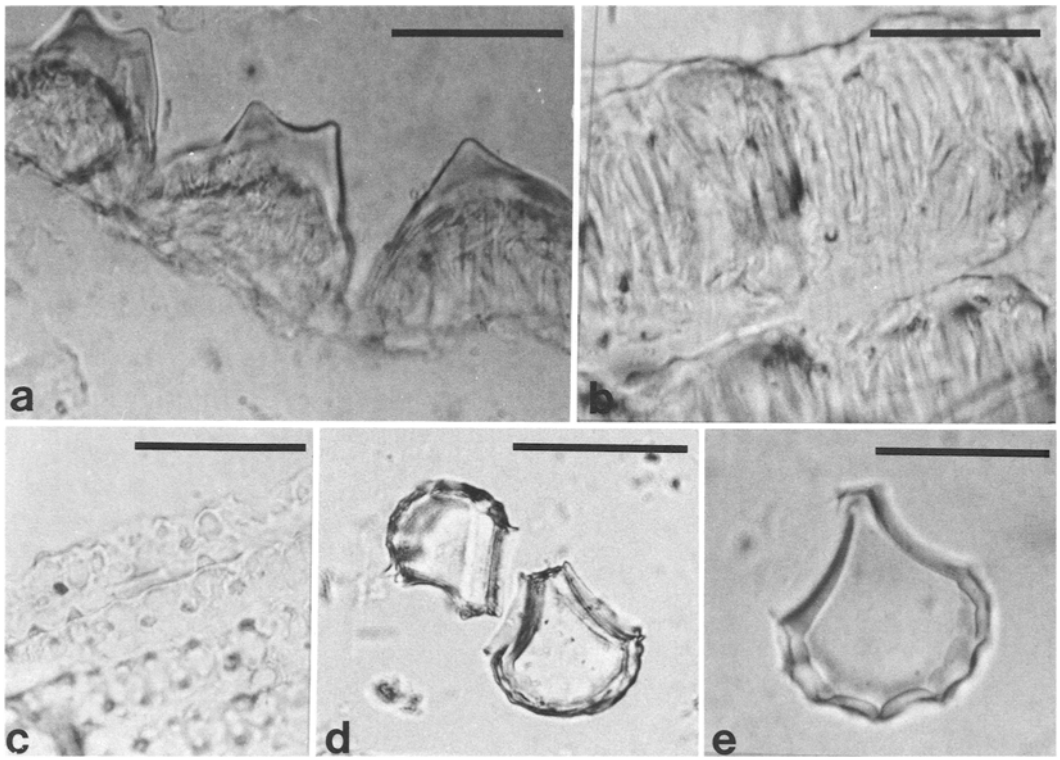


**Fig. 3.** Epidermal and bulliform types distinguishing the genus *Oryza*. a: 22IIIAa and 22IIIAb. Seed epidermis with large conical hairs (22IIIAa: 1 peak, 22IIIAb: two peaks) arising from very deeply serrated cells. Large hairs may be flanked by smaller projections (example from *Oryza sativa*); b: 22IVA. Seed epidermis, very deeply serrated, with pointed serrations (example from *Oryza sativa*); c: 22IVB. Seed epidermis, very deeply serrated, with sinuous-edged serrations (example from *Oryza sativa*); d: 50IIIAc1 and 50IIIAc2. Very widely flared keystone bulliform cells, a number of forms, varying by the extent of flaring and width of top. Bases symmetrical (example from *Oryza sativa*); e: 50IIIAb301. Small, moderately flared keystone bulliform cells (example from *Oryza minuta*). Scale bar = 20 micrometers.

(Type 5, Fig. 2c); and a short cell with a dumbbell on one tier, and a thin plate extending perpendicular to the dumbbell-type tier (Type 8a, Fig. 2b), which also occurs in the inflorescence.

## Genus Level Phytolith Characteristics

The genus *Oryza* is distinguished by several glume epidermal phytoliths and bulliform cells. The glume types (22IIIAa, 22IIIAb, 22IVA, 22IVB, Fig. 3a,b,c), are produced abundantly in nearly all the species of *Oryza* studied, and were not encountered in other genera in the tribe. One type, a glume cell with a single "peak" (hair) (22IIIAa, Fig. 3a, right) was observed in *Setaria italica,* however. Because size and shape characteristics of glume epidermal cells have been found to be useful for identifying articulated glume tissue by R. Savithri and A. Sharma (cited in Thompson 1992:219–225), we studied these types further to see if species-level identification criteria could be developed (see below). Two keystone bulliform cells (50IIIAc1,2 and 50IIIAb301, Fig. 3d,e) are also common in the genus *Oryza.*

Fujiwara's research indicates that keystone bulliform measurements can be used to distin-

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sometimes with one prominent projection (example from *Oryza manilensis*); f: 11IIICc, top view (example from *Oryza sativa*); g: same as f, side view; h: 50IIIAc. 50IIIAa, b, and c are keystone bulliform types, sometimes thick and with multiple ridges (example from *Oryza meyeriana*); i: 50IIIAb (example from *Oryza minuta*); j: 50IIIAa (example from *Oryza manilensis*). Scale bar = 20 micrometers.

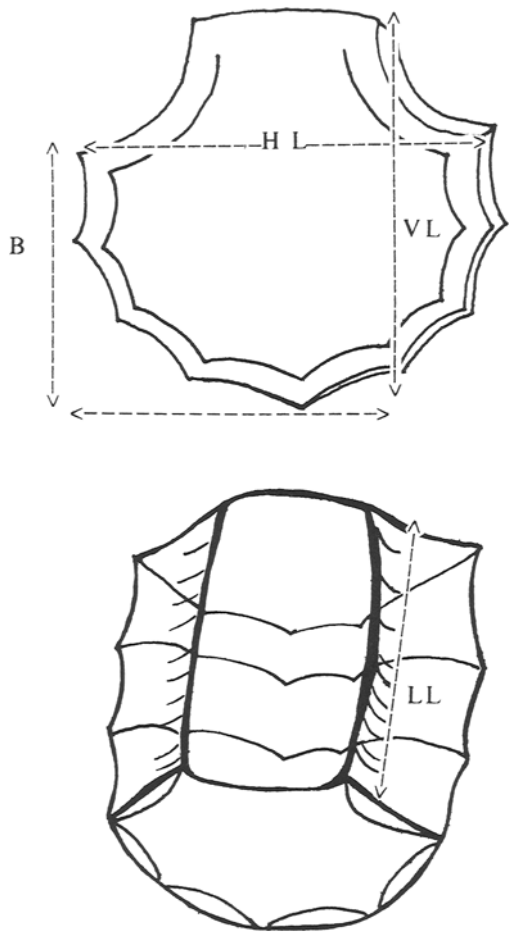


**Fig. 4.** Measurements of keystone bulliforms, shown from side view (upper) and top view (lower). HL: horizontal length (length of base); LL: lateral length (width of side); VL: vertical length; B: length of base portion.

guish between domesticated rice subspecies *indica* and *japonica* (Fujiwara 1993). To investigate whether Fujiwara's method, which uses a series of bulliform measurements (vertical length, horizontal length, lateral length, ratio of the base length and length of the non-base portion) (Fig. 4), would separate domesticated rice from wild grasses, we measured bulliforms from five species in the Oryzeae and Bambusinae tribes: *Oryza sativa* (two examples), *O. minuta, Leersia oryzoides, Melocanna baccifera,* and *Phyllosta bambusoides.*

We duplicated Fujiwara's measurements and utilized multiple linear discriminant function analysis (programs SPSS, 4.0 for the MacIntosh, and Systat, version 5.0 for the MacIntosh) to analyze the data. Table 2 presents the results of the multiple linear discriminant analysis on the five species listed above. In Table 2A the results based on equal prior probabilities are presented; Table 2B shows the results with prior probabilities set according to relative sample size. The data in Table 2B illustrate that *O. sativa* will be correctly assigned to *O. sativa* only 52.33% of the time using bulliform measurements. Additionally, *O. minuta, Leersia oryzoides,* and *Melocanna baccifera* may each be misclassified as domesticated rice more than 25% of the time. These results, although based on a small number of species, indicate that multiple linear discriminant analysis is unsuccessful in predicting group membership of keystone-bulliform cells accurately among species of *Oryza.*

To summarize, the results of our study of bulliform phytoliths suggest that this phytolith type cannot be used alone to identify rice archaeologically in geographic areas where rice relatives exist. In areas outside the distribution of related species, bulliforms are a useful indicator for the presence of rice, as demonstrated by Fujiwara's research in Japan.

A number of commonly produced short cell phytoliths (making up about 20% of short cell assemblages) appear to have promise for separating the genera of the Oryzeae. Because the ultimate usefulness of these forms will depend on whether they occur in other, unrelated, grasses in the region of study, they will not be discussed in detail at this time. The genus *Oryza,* with the greatest number of species, produces the greatest variety of short cell types. Potentially useful shapes include various types of lobed bodies, including dumbbells with distinctively shaped bodies or projections (spikes) on the nontype tier (nondumbbell side), as well as polylobate forms. Distinctive short cells were also observed in *Chikusichola, Hygroryza,* and *Leersia.*

## Species Level Phytolith Characteristics

### *Glume Cells*

As discussed above, some commonly occurring, well silicified phytoliths that characterize the genus *Oryza* are glume epidermal cells. We studied one common type, a glume cell with two "peaks" (hairs) (22IIIAb, Fig. 3a), in detail to determine if the size of these cells could be used to separate species of *Oryza,* specifically, to identify *O. sativa.* Glume epidermal cells, like short cells, are well silicified and preserve well in ancient soils. Recent analysis of geological deposits

TABLE 2. CLASSIFICATION RESULTS OF MULTIPLE LINEAR DISCRIMINANT FUNCTION ANALYSIS USING FUJIWARA'S BULLIFORM MEASUREMENTS. A. RESULTS WITH PRIOR PROBABILITIES ASSUMED TO BE EQUAL. B. RESULTS WITH PRIOR PROBABILITIES SET ACCORDING TO RELATIVE SAMPLE SIZE.

| Actual group | No. of cases | Predicted group membership (%) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| A. | | | | | | |
| Group 1 | | | | | | |
| *Oryza sativa* | 170 | 26.47 | 25.25 | 18.82 | 19.41 | 10 |
| Group 2 | | | | | | |
| *Oryza minuta* | 97 | 14.43 | 56.7 | 10.31 | 3.09 | 15.46 |
| Group 3 | | | | | | |
| *Leersia oryzoides* | 105 | 11.43 | 21.9 | 54.29 | 4.76 | 7.62 |
| Group 4 | | | | | | |
| *Mecolcanna baccifera* | 105 | 7.62 | 3.81 | 9.52 | 60.95 | 18.1 |
| Group 5 | | | | | | |
| *Phyllosta bambusoides* | 90 | 7.78 | 14.44 | 14.44 | 23.33 | 40 |
| B. | | | | | | |
| Group 1 | | | | | | |
| *Oryza sativa* | 170 | 52.33 | 16.28 | 13.95 | 9.3 | 8.14 |
| Group 2 | | | | | | |
| *Oryza minuta* | 97 | 31.16 | 39.86 | 16.67 | 2.9 | 9.42 |
| Group 3 | | | | | | |
| *Leersia oryzoides* | 105 | 26.23 | 8.2 | 46.72 | 8.2 | 10.66 |
| Group 4 | | | | | | |
| *Mecolcanna baccifera* | 105 | 26.19 | 2.38 | 3.97 | 50.79 | 16.67 |
| Group 5 | | | | | | |
| *Phyllosta bambusoides* | 90 | 17.89 | 15.79 | 8.42 | 20 | 37.89 |

from Thailand, for example, has revealed that *Oryza* glumes and scooped bilobates (Type 1, Fig. 2a) are well-preserved in sediments dating to at least 7000 years ago (Kealhofer and Piperno, unpublished data). In this case *Oryza* glume phytoliths recovered consisted of large pieces of articulated epidermis containing the epidermis proper and the attached hair cells, making identification a direct and easy endeavor.

One specimen each of six wild species of *Oryza* and five collections of cultivated rice were included in the glume cell study (Table 1). Each inflorescence slide was scanned at 400× under light microscopy until 25 individual two-peaked glume epidermal cells were encountered. Four measurements (Fig. 5) were taken for each glume cell: width of the top (TW; the distance between the two peaks of the projecting hairs), width at the middle (MW; width at the point where the glume projection attaches to the base), and the height of each hair (H1, H2, measured from the tip to the base of the hair. H2 is defined as the smaller measurement).

Measurements of glumes within each specimen varied considerably for all four characteristics, making it impossible to judge differences or similarities among species on the basis of simple observations of means and ranges of measurements. Although each individual measurement was too variable to permit classification to species, it was possible that they could be used in combination to achieve this goal. Multivariate statistical analyses of the data were therefore carried out in order to create functions of the original measurements optimized for classification.

To determine whether there was a significant difference among measurements of glumes of cultivated and wild species, a complete linkage hierarchical agglomerative cluster analysis (using SYSTAT) was performed for each of the four measurements. We hoped that the cultivated specimens would cluster together and separate


**Fig. 5.** Measurements used to characterize the two-peaked glume cell, type 22IIIAb. TW: top width; MW: width at the point where the glume projection (hair) attaches to the base; H: height of each hair.

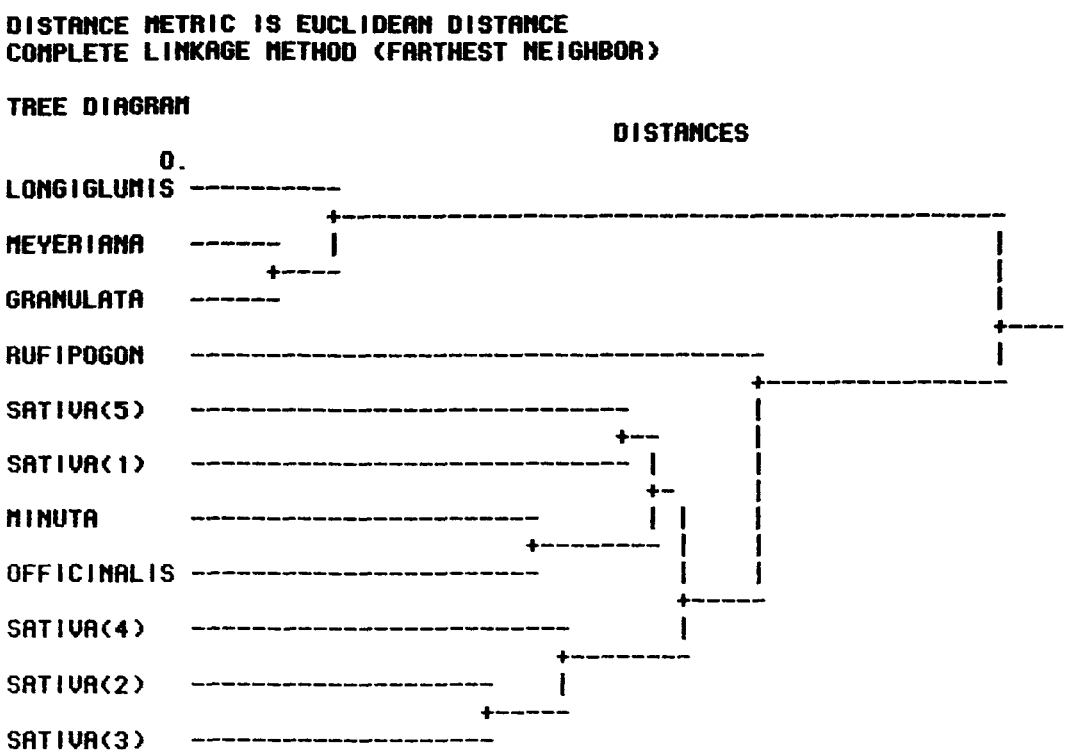


**Fig. 6.** Cluster analysis for top width (TW) of two-peaked glume cells for *Oryza* species. Euclidean distance; complete linkage method.

from all wild species. The results of the cluster analysis using top width are representative, and since this measurement is the one most readily made, only this analysis will be discussed here. At the lowest level in the tree (Fig. 6), three wild species separated from the other eight specimens. Those eight specimens separated into two groups: (1) *O. rufipogon* and (2) the other seven specimens, including all five domestic rice specimens. These results were encouraging in suggesting that there were significant differences among glume cells of domesticated rice and four of the six wild rice species in our sample. The analysis also indicates that two wild species, *O. minuta* and *O. officinalis,* resemble rice in glume characteristics.

To determine to what extent the two-peaked glume cell could be used in practice to separate rice from wild *Oryza* species, we then performed a series of multiple linear discriminant function analyses. As samples sizes were equal among the specimens (n = 25 glume cells), the linear model will not be biased due to differing underlying distributions of the different species. The best separation was achieved by an analysis in which all wild species (six) made up one group and the five rice specimens the other; the results of this analysis are presented here. All four measurements, TW, MW, H1, and H2, were used as predictor variables in the analysis. SYSTAT was used for the multiple linear discriminant function analysis.

In order to determine reliability, we randomly divided the data into a training set (80% of the data) and test set (20%). Using the group classification function coefficient and group classification constants from the training set, we then calculated the predictions for the test set (Table 3, frequency; Table 4, percentage). The results showed that 81.5% of rice glumes were correctly classified, and only 14.3% of glumes from wild species were misclassified. This indicates that the results of our multiple linear discriminant function analysis are able to predict accurately glume cells not used to develop the model.

The results of using the full data set are presented in Tables 5 and 6. A total of 81.6% of glumes from cultivated rice were correctly classified in the multiple linear discriminant function

TABLE 3. FREQUENCIES OF INDIVIDUALS IN PREDICTED GROUPS OF TEST SET, MULTIPLE LINEAR DISCRIMINANT FUNCTION ANALYSIS OF TWO-PEAKED GLUME CELLS.

| | Predicted | | |
|---|---|---|---|
| Actual | Wild | Domestic | Total |
| Wild | 24 | 4 | 28 |
| Domestic | 5 | 22 | 27 |
| Total | 29 | 26 | 55 |

TABLE 5. FREQUENCIES OF INDIVIDUALS IN PREDICTED GROUPS, MULTIPLE LINEAR DISCRIMINANT FUNCTION ANALYSIS OF TWO-PEAKED GLUME CELLS.

| | Predicted | | |
|---|---|---|---|
| Actual | Wild | Domestic | Total |
| Wild | 124 | 26 | 150 |
| Domestic | 23 | 102 | 125 |
| Total | 147 | 128 | 275 |

analysis, and only 17.3% of glumes from wild species were incorrectly classified as domesticated. A degree of misclassification of glume cells from wild species as domesticated was expected, given the results of the cluster analysis. However, using a formula derived from the analysis to calculate the probability of correct classification allows the two-peaked glume cell to be used to evaluate the presence of domesticated rice in archaeological sediments. The following formulae are based on the group classification function coefficients and group classification constants:

Prediction of *O. sativa*
$$= -9.403 - 0.066(TW) + 0.457(MW) - 0.248(H1) + 0.389(H2)$$

Prediction of wild rice
$$= -4.275 - 0.098(TW) + 0.356(MW) + 0.035(H1) - 0.074(H2)$$

To apply these formulae, measure unclassified glume cells and apply each formula. If the score from the prediction of cultivated is larger than that of the prediction of wild, then there is an 81.6% probability that the glume is derived from domesticated rice.

In practice, if the number of individual glume cells from an archaeological sample predicted into the domestic group is significantly larger than that predicted into the wild group using the formulae presented above, common sense suggests that the probability of correct classification should be much higher than 81.6%. Bayesian statistical inference, for which a good simple introduction is available (Iverson 1984), provides the mechanism to estimate the likelihood of joint findings of rice. Here we present findings for the case where exactly five phytoliths are to be studied. Unless the exact number is specified in advance and adhered to, the probabilities are unreliable. For example, if we set the probability of correct classification from the multiple linear discriminant function as 85% for purposes of illustration (Table 7), then we find that a single correct classification could result by chance 55% of the time in a sample of five phytoliths when there in fact were no phytoliths of the target species in the group; two would be less likely, but could still occur 16% of the time. However, if three or more correct classifications occurred, the analyst would be justified in concluding that the target species was present with some confidence ($P < .03$). These probabilities could be in error if the study population did not contain the target species and also had a different proportion of the diagnostic phytolith form than did the training set from which the multiple linear discriminant function was derived (leading to incorrect calculation of

TABLE 4. PERCENTAGES OF INDIVIDUALS IN PREDICTED GROUPS OF TEST SET, MULTIPLE LINEAR DISCRIMINANT FUNCTION ANALYSIS OF TWO-PEAKED GLUME CELLS.

| | Predicted | | |
|---|---|---|---|
| Actual | Wild | Domestic | Total |
| Wild | 85.71 | 14.29 | 100 |
| Domestic | 18.52 | 81.48 | 100 |
| Total | 52.73 | 47.27 | 100 |

TABLE 6. PERCENTAGES OF INDIVIDUALS IN PREDICTED GROUPS, MULTIPLE LINEAR DISCRIMINANT FUNCTION ANALYSIS OF TWO-PEAKED GLUME CELLS.

| | Predicted | | |
|---|---|---|---|
| Actual | Wild | Domestic | Total |
| Wild | 82.67 | 17.33 | 100 |
| Domestic | 18.40 | 81.60 | 100 |
| Total | 53.45 | 46.55 | 100 |

TABLE 7. APPLICATION OF BAYESIAN STATISTICAL PROCEDURE TO ESTIMATE THE LIKELIHOOD OF JOINT FINDINGS OF A TARGET SPECIES.

| # of positive classifications from exactly five examinations | Likelihood target species is NOT present |
|---|---|
| 1 | .55 |
| 2 | .16 |
| 3 | .03 |
| 4 | <.01 |
| 5 | <.01 |

the inverse probability). Thus, it is important to understand the texture of the assemblage. Work in progress will establish summary statistics for other properties of the training set used to develop the formula presented here so that an investigator can match her or his sample against it in order to decide whether to use this approach.

The results of multiple linear discriminant function analysis of two-peaked glume cells (22IIIAb) suggest that this type will be a useful marker for domesticated rice. The glume cells are large, readily recognizable, well silicified, and commonly produced. Measurements can be easily taken under light microscopy using an eyepiece micrometer. H2 (the shorter height) is the most significant variable for discriminating between wild and cultivated *Oryza.*

### *Short Cells*

During this study we observed a number of short cells that distinguish *Oryza sativa* from the other species we have studied. Most of these short cells are low frequency forms produced in the rice inflorescence (bases bilobate to polylobate, nonlobed tiers with 2–3 spikes). Because these short cells are not abundantly produced, they are probably of little practical use. However, as is the case for short cells distinguishing the genus *Oryza,* the ultimate usefulness of these species-level indicators will depend on whether they occur in unrelated grasses in our study region.

## SEPARATING RICE AND MILLETS

It is not the focus of this paper to discuss in detail how phytoliths can be used to distinguish rice from other cultivated Asian grasses. It is useful, however, to point out a few general trends in phytolith occurrence that permit easy separation of the Oryzeae group from two domesticated grasses we have examined thus far, the *Setaria italica* (L.) Pal. and *Panicum miliaceum* L. millets.

As discussed by Baenziger and Zhao (1992), the *Setaria* and *Panicum* domesticated millets can be separated from one another using a combination of short cell and long cell characteristics. This is also the case for distinguishing these grasses from rice and related species. Looking at short cells, the most common dumbbell shape in the Oryzeae, a scooped dumbbell very thick and curved in side view (Type 1, Fig. 2a) does not occur in the millets. Many Oryzeae dumbbells are thicker than *Setaria* or *Panicum* types. *Panicum* millet produces many types of crosses; this may make it difficult to use cross morphology to separate this millet from *Oryza,* although a very thick cross with raised corners (Type 5, Fig. 2c) may be an Oryzeae marker. Spiked panicoid forms, especially those with dumbbell bases, occur in both *Panicum* and Oryzeae, but the rice types are 3-spiked, whereas the millet forms are 1- or 2-spiked. *Setaria* spiked bodies tend to have constricted rather than dumbbell bases. Among long cells, only the Oryzeae group is characterized by thick keystone bulliform cells and long cells with rounded projections (10IIEf, Fig. 2d). The two-peaked glume epidermal cell discussed above has *not* been observed in the millets, further illustrating its potential as a rice diagnostic.

Although the phytolith production patterns revealed in our preliminary research give encouraging results concerning separating the major cultivated grains of southeast Asia using phytoliths, more research is needed, especially study of more wild species and landraces of domesticated millet species.

## CONCLUSIONS

The ultimate goal of our research into phytolith production in rice and its relatives is to establish phytolith criteria for identifying domesticated rice in archaeological contexts in regions where wild and domesticated *Oryza* species overlap, so that phytoliths can be used to investigate the origin and early spread of this important crop. As part of this effort, we also investigated general patterns of phytolith production in the Oryzeae tribe and have initiated work looking for rice "confusers" in related groups, such as the bamboos, and other domesticated and utilized grasses, such as the millets.

Our results so far are encouraging and suggest this is a productive direction for further research. A number of phytolith types were found that characterize the members of the tribe Oryzeae. We have not yet finished our study of bamboos, however, to see if these types are more widely produced in the subfamily Bambusoideae. The genus *Oryza* is distinguishable from related genera on a number of grounds, including glume epidermal cells, bulliform shape, and some short cell types. At the species level, the two-peaked glume cell looks the most promising for separating domesticated rice from wild species. Measuring a population of glume cells allows the probability of the presence of domesticated rice to be assessed.

It is likely that an assemblage of phytolith types, including glume epidermis cells, bulliforms, and perhaps short cells, will be needed to identify rice at the species level within its homeland. Applying such a "rice assemblage" in the archaeological setting will require study of regional grass communities, local races of rice and spontanea forms, other domesticated grasses, and replicate studies of wild *Oryza* species. Once these data are available, Bayesian calculation of joint probability obtained from multiple linear discriminant function classification will allow relatively unambiguous judgement as to whether domesticated rice is present in an assemblage. This is because while any individual glume cell might be misclassified as rice, the likelihood that many would be is very small. We hope to focus on these issues in our future research.

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# Book Reviews

**Food Phytochemicals for Cancer Prevention II. Teas, Spices and Herbs.** Chi-Tang Ho, Toshihiko Osawa, Mou-Tuan Huang, and Robert T. Rosen. (eds.). 1994. American Chemical Society, P.O. Box 57136, Washington, DC 20037-0136. xii + 370 pp. (hardcover). $89.95. ISBN 0-8412-2769-1.

This volume is a well-edited reference for those interested in naturally occurring anticarcinogens. The book specifically targets biologists, chemists, biochemists, pharmacologists, oncologists, molecular biologists and food science researchers. *Food Phytochemicals for Cancer Prevention* contains 35 papers that deal with: 1) perspectives; 2) phytochemicals from tea; 3) antioxidants; 4) phytochemicals in turmeric and ginger; 5) lignins; and 6) licorice, ginseng and other medicinal plants.

Each paper contains a brief account of the biological activities attributed to the plants being studied. Each topic has a wide range of research papers dealing with the same biochemical compound by using different research approaches. The valuable information presented provides a stimulus for further investigation into the use of these plants for the modulation of the carcinogenic process. Most of the papers conclude that the plants and the chemical they contain aided in cancer prevention.

There is an index divided into three sections: 1) author; 2) affiliation; and 3) subject. The subject index in particular affords the reader easy reference to the particular compounds under investigation. This book will help a wide variety of people understand the relationship between science and the use of herbal medicine. The editors deserve the gratitude of those who are only beginning to understand the contribution plants make in stemming the tide of the carcinogenic process.

Cynthia Butler
Medical Technology
Florida Atlantic University
Boca Raton, FL 33431