Characterization of Silica Distribution in Rice Husk Using Synchrotron Radiation μCT and its Implications for Archaeological Interpretation

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ABSTRACT This article reports the results of a pilot project using Synchrotron Radiation μCT (computer-aided tomography) to examine the distribution of silica within phytoliths from rice husks. Experiments indicate that computed tomography can be used to show how silica accumulates and is distributed in a distinctive zigzag pattern of long epidermal cells that are characteristic of phytoliths from rice husks. This method will help us to understand why the dry ashing method produced much more zigzag pattern of long cells phytoliths from rice husk than did the acid extraction method. Besides, the zigzag morphological pattern exhibited by long epidermal cells is characteristic of this species which makes it useful in the identification of rice husks from archaeological contexts and indicating heating process. *Microsc. Res. Tech. 77:785–789, 2014.*

INTRODUCTION

The origin of rice agriculture has made a significant contribution not only to the development of complex civilization in southern China and but also a major development in the world history. China is the largest rice producer and consumer of rice in the world today. In recent research regarding the origins of rice agriculture in China, new methods to distinguish wild from domesticated rice have been developed (Crawford, 2006; Fuller, 2007; Khush, 1997; Liu, 2007). Because of poor preservation of plant macrofossils from archaeological contexts in southern China, opal phytoliths have become a major indicator of rice cultivation. Several kinds of rice phytoliths can be identified to genus on the basis of their morphological characteristics (Fujiwara, 1993; Lu et al., 2009; Pearsall et al., 1995; Piperno, 2006; Zhang, 1996; Zhao et al., 1998). Specially, silica is the most abundant element in rice husks (Stroeven et al., 1999), which are a by-product of the rice milling process. Thus rice processing activity would produce a large amount of phytoliths. Thus, the identification of phytoliths from rice husks has contributed greatly to the research of early rice cultivation in China.

Rice husks produce large phytoliths characterized by single or double "peaks." Discriminate function analysis of the double-peaked glume phytoliths can be used to separate domestic rice from its wild relatives (Zhao et al., 1998). The presence of double-peaked glume phytoliths derived from rice husks (*Oryza sativa* L.) recovered from archaeological contexts provides strong evidence for the cultivation of rice in ancient times (Zhao et al., 1998).

In the previous study, it was found that different sample processing methods could affect the morphology of phytoliths from rice husk. The dry ashing method produced much more zigzag pattern of long cells phytoliths from rice husk than did the acid extraction method (Sun et al., 2012). The zigzag pattern in phytoliths forms the base of double peaked glume cells, which are joined row by row in long cell phytoliths as shown in Figure 1 (Sun et al., 2012). Further experimentation is required to explore the mechanisms that govern the formation of zigzag pattern of long cell phytoliths. Therefore, it is necessary to examine the silica distribution and internal structure of long cell phytoliths from rice husks.

The distribution of silica within rice husk using scanning electron microscopy with energy dispersive X-ray (SEM–EDX) has been reviewed (Parka et al.,

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2003). However, it is impossible to obtain a three dimensional distribution of silica and microstructure of rice husk using SEM–EDX. Therefore, it is necessary to select an appropriate method to examine the distribution of silica within the internal structure of rice husks.

Synchrotron Radiation Micro-Computed Tomography (SR-μCT) possesses many significant advantages over industry μCT. The high flux available at SR sources allows rapid CT data acquisition with high spatial resolution, which can result in precise mapping of internal structures even at the microscopic level (Trtik et al., 2007). SR-μCT has been used in archeology such as the analysis of faience (Yang et al., 2013). More importantly, SR-μCT is a suitable technique for the characterization of biological samples (Mizun et al., 2010), because SR-μCT possesses implementation of phase-sensitive imaging techniques based on the high degree of (lateral) coherence of the SR sources (Cloetens et al., 1996). Accordingly, this paper used SR-μCT to reflect the distribution of silica and the organization of the internal structure of a rice husk.

MATERIALS AND METHODS

The rice husk from *O. sativa* L. subsp. *indica Kato* was provided by the Agricultural Research Institute of Anhui Province. The SR-μCT analysis was conducted at the Shanghai Synchrotron Radiation Facility. The rice husk was set in a plastic tube, which was placed on an open sample platform and scanned. SR-μCT enabled the in situ nondestructive investigation of the microstructures of rice husk. The rice husk sample was irradiated with SR parallel X-rays at 13 keV. The CCD detector (2,048 × 2,048 pixels) has a spatial resolution of 0.74 μm. The distance between the sample platform and CCD is 8 cm while the exposure time is 4.5 s with 180 degree's scanning angle. In each scan, 1,650 slices were obtained. The scan data were then analyzed using Mimics 12 image processing software (Materialise Company). Besides, we applied the PITRE (Phase-sensitive X-ray Image processing and Tomography RE construction, version 3) and PITRE-BM (PITRE Batch Manager) software to reconstruct the CT slices with phase retrieval using PAD-BA algorithm. According to X-ray imaging principles, heavy elements have higher rate of X-ray absorption than light elements and present a brighter CT image. Thus, the variation of brightness on the slice would reflect the variation of density and chemical composition of the sample.

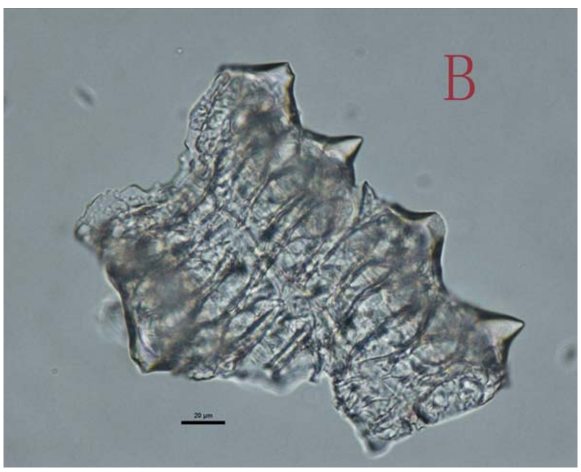

Fig. 1. The zigzag pattern in phytoliths forms the base of double peaked glume cells, which are joined row by row in long cell phytoliths (Scale bar, 20 μm) (Sun et al., 2012). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

Rice husks are composed of the lemma and palea, which tightly interlock with each other. Since the surface morphology of lemma and palea are similar to each other, only the features of lemma will be described in this paper. A part of the lemma was cut for analysis. The CT image of the lemma is presented in Figures 2 and 3. The image was constructed from three directions: cross-section, tangential section, and radial section.

In the cross-section (Fig. 2a), the outer epidermis displays an undulating surface due to the presence of regularly spaced protrusions. In the tangential slice (Fig. 2b), besides the above mentioned characteristics, the internal tissues consist of vascular bundle cells. Finally and more importantly, the radial slice (Fig. 3a) clearly discloses the dentate contours on the epidermis of long cells with regular intervals on the rice lemma.

The SR micro-CT images show differences in brightness reflecting the variation in density and chemical composition of the rice phytoliths. As presented in Figure 2a, the outer epidermis appears to be double-peaked with an undulating surface due to the presence of regularly spaced protrusions where silica was deposited. Figure 2a appears bright due to the high concentration of silica in the prominent twin peaks. Due to the high concentrations of silica in rice husks phytoliths from the rice lemma can withstand 1,100°C without deforming (Wu et al., 2012, 2014).

The general features of Figure 3a indicate that the dentate contour on the epidermis of long cells occurs at regular intervals in the phytolith of rich husks. Figure 3b is produced from Figure 3a by image processing. In Figure 3b, it is interesting that there is little or no brightness. The lack of bright areas shows that silica is present but in much smaller amounts in the regions

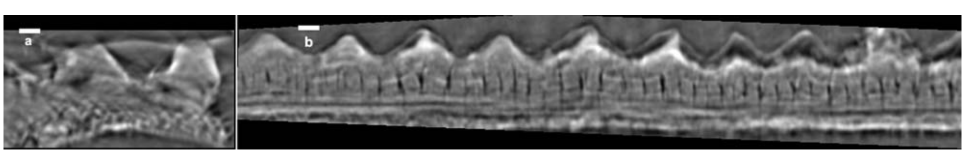

Fig. 2. A part of lemma reconstructed by Synchrotron Radiation MCT (**a**) cross-section, (**b**) tangential section (scale bar, 20 μm).

displayed in the image. It appears that the brightness is most intense in the outline regions of dentate contours of epidermal cells. So the variation of brightness likely reflects where silica accumulates and where phytoliths are located in the plant body. More importantly, the bright outline appears as a zigzag pattern (Fig. 3b). So there is no doubt that the zigzag pattern of long cells phytoliths corresponds to the dentate contours of long cells on the epidermis of the rice husk. It is clear that the zigzag pattern observed in long cells

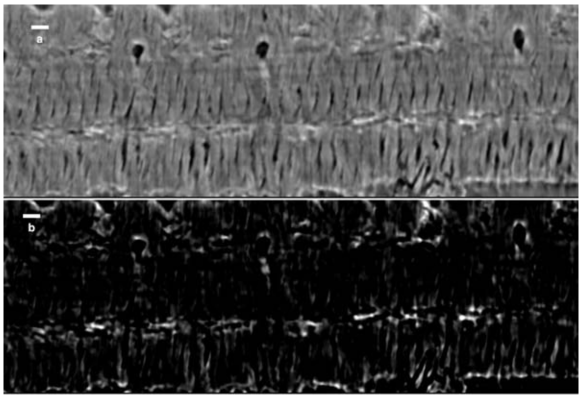

Fig. 3. Dentate contours long cells reconstructed by (**a**) radial section, (**b**) bright outline appears as a zigzag pattern in dentate contours long cells (scale bar, 20 μm).

Fig. 4. Zigzag pattern of long cells phytoliths of rice from dry-ashing method (scale bar, 20 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

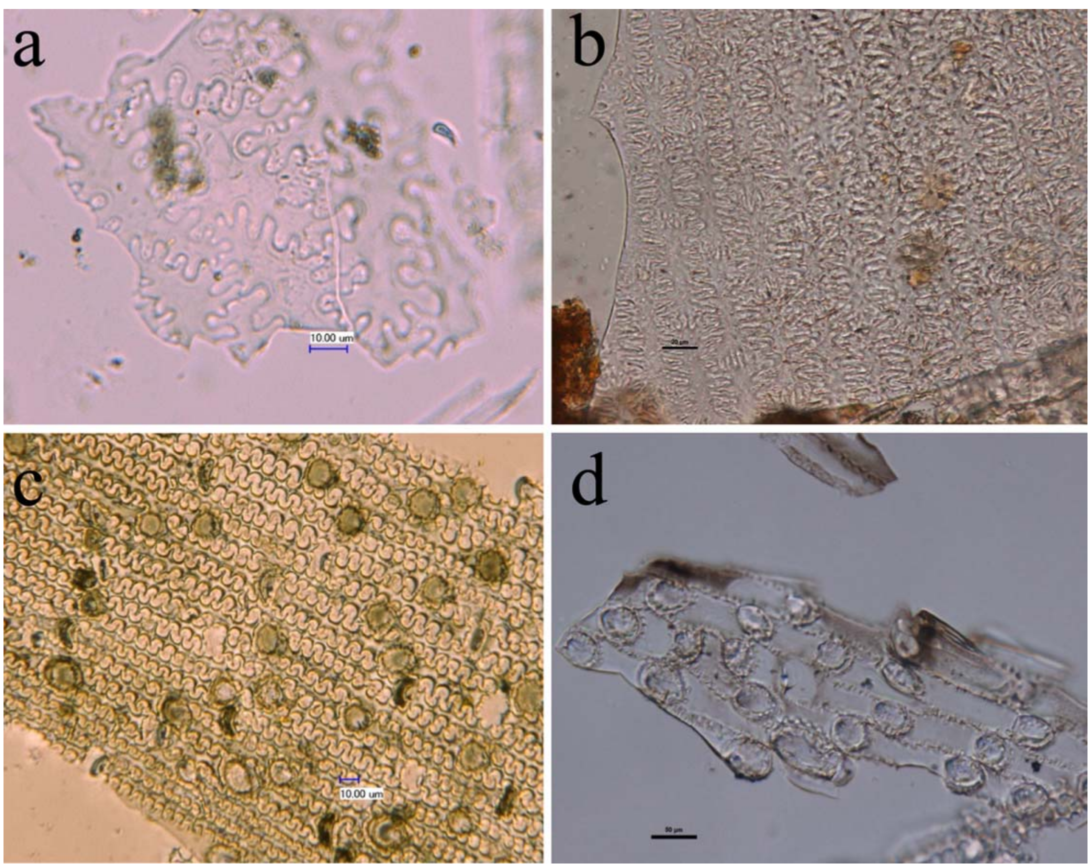

Fig. 5. Epidermal long cell phytolith from foxtail millet (**a**, scale bar, 10 μm), common millet (**b**, scale bar, 20 μm), wheat (**c** scale bar, 10 μm), and barley (**d**, scale bar, 50 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Fig. 6. Zigzag pattern of a long cell phytolith from ancient Heying Site (scale bar, 20 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

phytoliths from rice husks are determined by two factors: the type of cell where silica accumulates and its location in the plant body. The zigzag pattern of long cells phytoliths (Fig. 4) is produced by the dentate contours of long cells on the epidermis of rice husks.

In our previous study, the acid extraction method used to prepare the phytolith samples weakens and dissolves zigzag pattern of the long cell phytoliths. It is worth noting that the distribution of silica in a zigzag pattern of long cells phytoliths is associated with a lesser amount of silica within the rice husk. This is the reason that dry ashing method produced the zigzag pattern of long cells with double peaked glume. The zigzag pattern is much less visible when the phytoliths were produced using the acid extraction method. The zigzag pattern of long cells phytoliths is based on conjoined long cell phytoliths, which was previously identified as characteristic of rice phytoliths that had been processed by the dry-ashing method. More interestingly, Parka et al. reported that other inorganic elements, such as calcium (Ca), are significantly higher in the regions where the domes are broken including the zigzag pattern of long cells (Parka et al., 2003). Since the concentration of calcium in the area of the zigzag pattern of long cells phytoliths are higher, the dry ashing method with high temperature are ready to produce more conjoined long cell phytoliths.

A great deal of research concentrates on the identification of epidermal cells phytoliths from cereal plants such as Foxtail millet, Common millet, wheat, and barley on a taxonomic level. Phytoliths of long epidermal cells could produce distinctive morphological characteristics such as elongate echinate wavy long cells with papillae from wheat and barley (Ball et al., 1999); Ω-undulated with cross-wavy type from Foxtail millet; and η-undulated with a cross-finger type morphology from Common millet (Lu et al., 2009). As compared to these characteristic traits, phytoliths are the same feature from the dry ashing method as shown in Figure 5. It is apparent that the zigzag pattern of long cells phytoliths from rice husks are unique features. Moreover, this characteristic trait in the epidermal microstructure of rice husks is an efficient key for distinguishing rice from wheat, barley, Foxtail millet, and Common millet. Thus, the zigzag pattern of long cells phytoliths from rice husks can be identified in archaeological assemblages.

In our previous study, we had observed that the zigzag pattern of long cells phytoliths was identified in samples from archaeological contexts. Rice husk phytoliths including the zigzag pattern long cells have been identified at a number of Chinese archaeological sites including Heying site (Fig. 6) (Wu et al., 2011). Since high temperature easily results in the formation of conjoined cells, zigzag pattern long cell phytoliths in archaeological sites may indicate heating processes, e.g., cooking or in situ burning.

CONCLUSION

In this article, we describe our experiments to characterize the distribution of silica and its relationship to the morphology of rice phytoliths using SR-μCT. Through using SR-μCT, we can clearly see the shape and arrangement of long cells in the epidermis of a rice husk. This article confirms the location of the zigzag pattern characteristic of long epidermal cell phytoliths in a rice husk (*O. sativa* L.). This method will help us to understand why the dry ashing method produced much more zigzag pattern of long cells phytoliths from rice husk than did the acid extraction method. The morphology of this kind of phytolith is characteristic of all rice husks making the recognition of the zigzag pattern useful for the identification of rice husks in archaeological contexts. We believe that through the use of SR-μCT to characterize the variability in plant phytoliths it will be possible to more reliably identifiable plants to genus, or when comparative studies have been made even species.

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