Quaternary International 426 (2016) 141–144

Contents lists available at ScienceDirect

Quaternary International

journal homepage: www.elsevier.com/locate/quaint

Point count estimation of articulated husk phytoliths of foxtail millet and its prospective use in agricultural archaeology

Xue Shang [a, b, *], Xin Wang [a, b], Pengfei Sheng [a, b], Changsui Wang [a, b]

[a] Department of Archaeology and Anthropology, University of Chinese Academy of Sciences, Beijing 100049, China
[b] Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China

ARTICLE INFO

Article history:
Available online 27 April 2016

Keywords:
Point count
Husk phytolith
Millet
Agricultural archaeology

ABSTRACT

Many researchers have focused on the precise identification of phytoliths extracted from archaeological samples, and on the extraction methods of phytolith from all kinds of samples as well. Additionally, it is important to perfect the scientific method of phytolith quantification. Phytoliths, especially from the husk, are easily broken after being long-buried or during extraction treatment. These can result in uncertainties about quantification of grain numbers and varies under different conditions. Here we introduce a method of point count estimation of articulated millet husk phytoliths, and evaluate its accuracy. As a result, we successfully used the point count method into estimating the area of husk phytolith in unit weight of modern millet samples, which will broaden the available data in archaeobiological research. We suggest precise estimations can therefore be done with this method.

1. Introduction

Phytolith analysis now plays a significant role in archaeobotanical research for its unique advantages in easier preservation and identification than any other plant macro-/micro-remains (Pearsall et al., 1995; Iriarte, 2003; Crawford, 2005; Harvey and Fuller, 2005; Lu et al., 2009a; Ryan, 2014). Recently, scientists have applied phytoliths to explore the origin and spread of crops (Lu et al., 2009a; Zhang et al., 2012; Ball et al., in press). First domesticated in the Yellow River valley, foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*) are regarded as staple foods in Northern China during the Neolithic period (Zhao, 2005; Crawford, 2005; Hunt et al., 2008; Barton et al., 2009). To date, with the relatively specific anatomical characters (Lu et al., 2009b; Zhang et al., 2011), husk phytoliths of millets extracted from archaeological samples have been frequently used to reconstruct ancient agricultural patterns (Harvey et al., 2005; Lu et al., 2009), especially where macrofossils were not well preserved (Crawford et al., 2005; Harvey et al., 2005; Ryan, 2014).

The now widespread use of phytolith analysis in archaeobotanical studies requires attention to be paid to the accuracy of the results, and there is little attention paid to this. The husk phytolith of millets with undulated extremities tend to be fragmented after long burial and then during the extraction processes (Wang et al., 2014). The statistical results of millet husk phytolith are thus influenced by many factors, and makes comparison of results difficult compared to proxies of seeds or pollen. A helpful attempt to estimate the total amount of phytolith fragments of millets has been made, which showed that foxtail millet and common millet produce almost the same number of fragments after using the same procedures (Zhang et al., 2010a,b). Nevertheless, it still depends on the characteristics of samples and methods, which make results difficult to compare between different sites or samples, or even different labs. So a scientific-based estimation method needed.

The point count method provides a most efficient way of quantifying the total area of a phase in a plane surface (Inversen, 1964, 1969; Clark, 1982; Whitlock and Larsen, 2001), which involves selecting random points on the microscope slide and determining the percentage of points that overlie charcoal fragments in the eyepieces (Whitelock et al., 2011). It was Robin Clark (1982) who firstly used this method on charcoal analysis, and she also implied this method can be used for other constituents of sediments, such as diatoms, phytoliths, fungal spores or mineral grains. After that, this method became a standardized methodology for estimating the area of charcoal in pollen preparations and was widely used on reconstruction of fire history all over the world

* Corresponding author. Department of Archaeology and Anthropology, University of Chinese Academy of Sciences, 19A, Yuquan Road, Beijing 100049, China.
E-mail address: shangxue@ucas.ac.cn (X. Shang).

http://dx.doi.org/10.1016/j.quaint.2016.04.017
1040-6182/

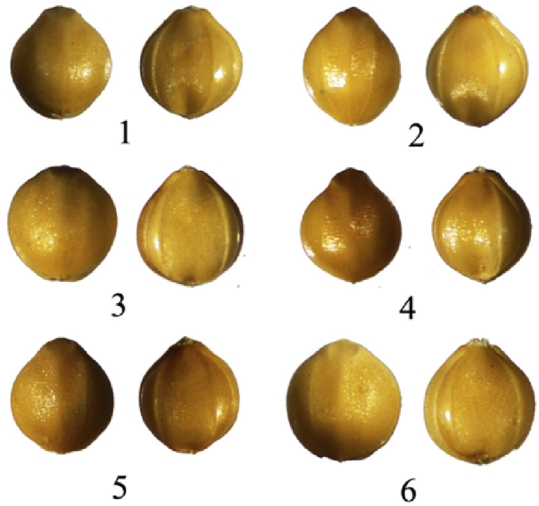

Fig. 1. Images of foxtail millet modern samples. 1. Cishangu; 2. Jigu No.14; 3. Zhuyeqing; 4. Donghuigu; 5. Cixuan No.6; 6. Jinzagu No.3.

(Clark, 1983; Odgaard, 1992; Maenza-Gmelch, 1997; Kershaw et al., 2002; Li et al., 2009; Ballard et al., 2016). Like charcoal particles, articulated husk phytolith of millets are visually recognizable as angular and usually planar fragments, which make it possible to use the similar method to estimate millet husk phytolith abundance.

Here, we introduce the method of point count into quantifying analysis of articulated millet husk phytoliths. In order to test its accuracy, the results of different methods will be compared by using six cultivars of modern samples of foxtail millet (*Setaria italica*) (Fig. 1, Table 1).

Table 1
Information of modern samples

Sample no.	Name of cultivars	Producing area
FM1	Cishangu	Hebei province
FM2	Jigu No.14	Hebei province
FM3	Zhuyeqing	Hebei province
FM4	Donhuigu	Hebei province
FM5	Cixuan No.6	Hebei province
FM6	Jinzagu No.3	Shanxi province

2. Materials and methods

Each sample was weighed to about 1 g. After traditional dry ashing process for modern plant phytolith extraction (Sun et al., 2012), a spike of *Lycopodium* spores was added to each sample in order to estimate the concentration of phytolith abundance (Stockmarr, 1971). After pretreatment, microscope slides were made for phytolith analysis by the point count method. In the present study, phytolith abundance was estimated by counting the proportion of 5500 points of husk phytoliths that were touching an 11-point ocular scale in 500 different eyepiece views, as described by Clark (1982). Only points falling on particles greater than 5 μm were counted in order to reduce potential error from small particles that are difficult to assign as millet phytolith. By using this method we can get the total area per gram (S1, cm^2/g) of articulated husk phytoliths in our samples. Fig. 2.

The area concentration of millet husk phytoliths per gram was then calculated as follows:

$$\text{Formula 1:}\quad S1 = \frac{PL}{(M \times l \times W)}$$

S1: the area in unit of weight of sample (cm^2/g).
P: total point counted that phytolith overlies.
L: the concentration of the *Lycopodium* spores.
l: the counted number of *Lycopodium* spores.
W: the weight of sample (g).
M: the counted number of coordinate points of micrometer in the field of vision (usually 11*500 = 5500).

In order to test the accuracy of point count method in phytolith analysis, we select 100 grains of foxtail millet at random, and then measured the length, width and thickness of each grain. We made the calculation and statistics on the premise that these tiny grains were approximately oblate spheroids with length, width and thickness (Fig. 1). The following formula is used to calculate the approximate area of 100 millet grains. Then 100 millet grains were weighed. Finally, we can get the approximately total area of the husk phytolith for 1 g (S2, cm^2/g).

The approximate area concentration of millet husk per gram was then calculated as follows:

$$\text{Formula 2 :}\quad S2 = \frac{\sum_{i=1}^{100} 4\pi(a_i b_i + b_i c_i + a_i c_i)/3}{w}$$

S2: approximately total husk area in unit of weight of sample (cm^2/g).
a: half value of length of one grain.
b: half value of width of one grain.
c: half value of thickness of one grain.
w: the weight of 100 grains (g).

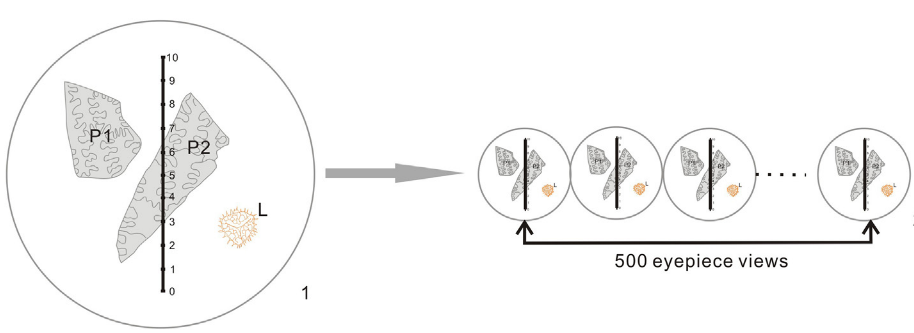

Fig. 2. Diagram of point count method in this paper. 1. A field of view with a standard eyepiece micrometer. In this case, of the eleven points defined by the cross on reticles nearest the numbers, four "touch" phytolith. 2. Then repeating estimation in 500 different eyepiece views.

3. Results

Husk phytoliths of six cultivars modern samples of *Setaria italica*, were treated by dry ashing method, and counted by using point count estimation method, and the statistical results are listed in Table 2. All six cultivars of foxtail millet grains were measured by their length, width and thickness, then the area of husk was counted, and the results are shown in Tables 3 and 4.

Here the mean values of the area concentration of millet husk phytolith per gram varied from 20.67 cm^2 (FM5) to 40.49 cm^2 (FM3) for different cultivars. Whereas, the mean values of the area of millet seed with husk varies from 30.71 cm^2 (FM6) to 34.70 cm^2 (FM1). The differences between S1 and S2 for the same cultivar showed from the minimum of 0.2 cm^2 (FM6) to the maximum of 11.62 cm^2 (FM5) per gram, and the proportion of average values of the difference is about 14.77%.

Table 2
Statistical results of measured values of the area concentration of millet husk phytolith

No.of samples	Mean values of S1 (cm^2/g)	Standard deviation (cm^2/g, n = 3)
FM1	30.18	1.88
FM2	30.55	7.33
FM3	40.49	6.33
FM4	31.70	8.85
FM5	20.67	5.36
FM6	30.51	3.72

Table 3
Statistical results of length, width and thickness of millet seed, and the weight of 100 grains for each cultivar

No.of samples	Length (mm)	Width (mm)	Thickness (mm)	Count number	Weight of 100 grains (g)
FM1	1.74-(1.91)-2.17	1.14-(1.61)-1.83	0.27-(1.24)-1.72	100	0.22
FM2	1.74-(1.94)-2.18	1.21-(1.65)-1.78	0.83-(1.37)-1.60	100	0.27
FM3	1.74-(1.91)-2.29	1.41-(1.75)-1.91	0.68-(1.40)-1.61	100	0.29
FM4	1.72-(1.94)-2.44	1.16-(1.63)-1.83	0.78-(1.24)-1.50	100	0.24
FM5	1.63-(1.93)-2.40	1.10-(1.61)-1.88	0.82-(1.27)-1.52	100	0.25
FM6	1.92-(2.09)-2.37	1.53-(1.85)-2.03	1.03-(1.39)-1.60	100	0.32

Table 4
Comparison between measured values of S1 and S2

No.of samples	Value of S1 (cm^2/g)	Value of S2 (cm^2/g)	Absolute value of differences (cm^2/g)	Proportion of differences (%)
FM1	30.18 ± 1.88	34.70 ± 4.90	4.53	13.05
FM2	30.55 ± 7.33	31.02 ± 2.60	0.48	1.54
FM3	40.49 ± 6.33	30.81 ± 2.33	9.68	31.43
FM4	31.70 ± 8.85	33.74 ± 0.80	2.03	6.02
FM5	20.67 ± 5.36	32.29 ± 0.76	11.62	35.99
FM6	30.51 ± 3.72	30.71 ± 0.56	0.20	0.64

4. Discussion and conclusion

Seed flotation and phytolith analysis are the most important two archaeobotanical methods for exploring agricultural characteristics in rain-fed crops of the Neolithic in China. One of the controversial issues is the relative balance of foxtail and common millet usage. Most charred seed records demonstrated that common millet was the first rain-fed crop in northern China. Then the use of foxtail millets arrived later in the mid-Yangshao culture period, and took the dominant position by the end of Yangshao culture period (Zhao, 2004; Zhou et al., 2011; Shang et al., 2012; Wang et al., 2015). Records from phytolith analysis suggest an early appearance of common millet, but suggest the domination of common millet during the late-Yangshao period (Zhang et al., 2010a,b, 2012). The reasons of this are probably complicated. The different compressive strength of high temperature between foxtail millet and common millet may cause the underestimation the amount of common millet in archaeological samples (Tanja and Manfred, 2008). On the other hand, the approaches may have underestimated relative phytolith abundance, especially for husk samples which are easily broken after long period burying or experimental treatment.

In this paper, we introduce the point count estimate method to quantify the area abundant of husk phytoliths of different cultivars of foxtail millets. As is shown in the Fig. 3, in most of samples, values of S1 and S2 have been basically consistent. The difference between those data might be due to the seeds whose husk has been broken off, or samples with extra husk, or to the of overlap between upper lemma and palea at the junctions among different cultivars. Nevertheless, the point count estimate method can be used to evaluate the area of husk phytolith abundant of foxtail millet.

If we use the point count method in archaeological samples, one can estimate the amount of husk phytolith of millet, which broadens the available data in archaeobiological research. We suggest more precise estimations should be done in the future, such as the differences between foxtail millet and common millet, and the relationship between the area of phytolith and the harvest abundance.

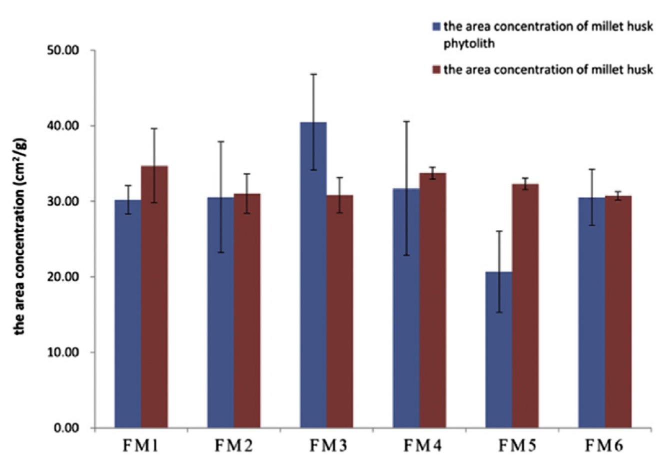

Fig. 3. Comparison between measured values of S1 and S2.

Acknowledgement

This study was supported by the National Natural Science Foundation of China (Grant No. 41471167). We are grateful to Prof. John Dodson from Institute of Earth Environment, Chinese Academy of Sciences for method guidance and modification of manuscript, and to Dr. Jianping Zhang from Institute of Geology and Geophysics, Chinese Academy of Sciences for sample supply and helpful discussions.

References

Ball, T., Chandler-Ezell, K., Dickau, R., Duncan, N., Hart, C.T., Iriarte, J., Lentfer, C., Logan, A., Lu, H.Y., Madella, M., Pearsall, M.D., Piperno, R.D., Rosen, M.A., Vrydaghs, L., Weisskopf, A., Zhang, J.P., 2015. Phytoliths as a tool for investigations of agricultural origins and dispersals around the world. Journal of Archaeological Science. (in press). http://dx.doi.org/10.1016/j.jas.2015.08.010.

Ballard, J.P., Horn, S.P., Li, Z.H., 2016. A 23,000-year microscopic charcoal record from Anderson pond, Tennessee, USA (just-accepted). Palynology 1–53.

Barton, L., Newsome, S.D., Chen, F.H., Wang, H., Guilderson, T.P., Bettinger, R.L., 2009. Agricultural origins and the isotopic identity of domestication in northern China. Proceedings of Natlional Academy of Sciences U.S.A 106, 5523–5528.

Clark, R.L., 1982. Point count estimation of charcoal in pollen preparations and thin section of sediments. Pollen Spore 24, 523–535.

Clark, R.L., 1983. Pollen and charcoal evidence for the effects of aboriginal burning on the vegetation of Australia. Archaeology in Oceania 18 (1), 32–37.

Crawford, G.W., 2005. East asian plant domestication. In: Stark, M.T. (Ed.), Archaeology of Asia. Blackwell Press, Oxford, pp. 77–95.

Harvey, E.L., Fuller, D.Q., 2005. Investigating crop processing using phytolith analysis: the example of rice and millets. Journal of Archaeological Science 32, 739–752.

Hunt, H.V., Linden, M.V., Liu, X.Y., Motuzaite-Matuzeviciute, G., Colledge, S., Jones, M.K., 2008. Millets across Eurasia: chronology and context of early records of the genera Panicum and Setaria from archaeological sites in the Old World. Vegetation History and Archaeobotany 17, S5–S18.

Iriarte, J., 2003. Assessing the feasibility of identifying maize through the analysis of cross-shaped size and three-dimensional morphology of phytoliths in the grasslands of southeastern South America. Journal of Archaeological Science 30 (9), 1085–1094.

Kershaw, A.P., Clark, J.S., Gill, A.M., et al., 2002. A history of fire in Australia. Flammable Australia: The Fire Regimes And Biodiversity Of A Continent 3–25.

Li, X., Shang, X., Dodson, J., et al., 2009. Holocene agriculture in the Guanzhong Basin in NW China indicated by pollen and charcoal evidence. The Holocene 19 (8), 1213–1220.

Lu, H.Y., Zhang, J.P., Liu, K., Wu, N.Q., Li, Y.M., Zhou, K.S., Ye, M.L., Zhang, T.Y., Zhang, H.J., Yang, X.Y., Shen, L.C., Xu, D.K., Li, Q., 2009a. Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. PNAS 106 (18), 7367–7372.

Lu, H.Y., Zhang, J.P., Wu, N.Q., Liu, K.B., Xu, D.K., Li, Q., 2009b. Phytolith analysis for the discrimination of foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*). PLoS ONE 4, e4448.

Maenza-Gmelch, T.E., 1997. Holocene vegetation, climate, and fire history of the Hudson Highlands, southeastern New York, USA. The Holocene 7 (1), 25–37.

Odgaard, B.V., 1992. The fire history of Danish heathland areas as reflected by pollen and charred particles in lake sediments. The Holocene 2 (3), 218–226.

Pearsall, D.M., Piperno, D.R., Dinan, H.E., Umlauf, M., Zhao, Z., Benfer Jr., A.R., 1995. Distinguishing rice (*Oryza sativa* Poaceae) from wild oryza species through phytolith analysis: results of preliminary research. Economic Botany 49 (2), 183–196.

Ryan, P., 2014. Phytolith studies in archaeology. In: Smith, C. (Ed.), Encyclopedia of Global Archaeology. Springer, New York, pp. 5920–5931.

Shang, X., Zhang, P.C., Zhou, X.Y., Qiu, Z.W., Qu, Y.T., Wang, W.L., Wang, C.S., 2012. Primary study of early agriculture during the Neolithic in Xiahe site Shaanxi province. Archaeology and Relics 4, 55–59 (in Chinese with English Abstract).

Stockmarr, J., 1971. Tablets with spores used in absolute pollen analysis. Pollen Spores 13, 615–621.

Sun, X.P., Wu, Y., Wang, C.S., Hill, D.V., 2012. Comparing dry ashing and wet oxidation methods. The case of the rice husk (*Oryza sativa* L.). Microsc. Res. Tech 75, 1272–1276.

Tanja, M., Manfred, R., 2008. Experiments on the effects of carbonization on some cultivated plant seeds. Vegetation History and Archaeobotany 17 (Suppl. 1), S257–S263.

Wang, X., Shang, X., Jiang, H.E., Zhang, P.C., Wang, W.L., Wang, C.S., 2015. Primary analysis of flotation of two site in Baishuihe river valley Shaanxi province. Archaeology and Relics 2, 100–104 (in Chinese with English Abstract).

Wang, X., Jiang, H.E., Shang, X., Wang, T., Wu, Y., Zhang, P.C., Wang, W.L., Wang, C.S., 2014. Comparison of dry ashing and wet oxidation methods for recovering articulated husk phytoliths of foxtail millet and common millet from archaeological soil. Journal of Archaeological Science 45 (3), 234–239.

Whitlock, C., Larsen, C., 2001. Charcoal as a Fire Proxy. Developments in Paleoenvironmental Research, pp. 75–97.

Zhang, J.P., Lu, H.Y., Gu, W.F., Wu, N.Q., Zhou, K.S., Hu, Y.Y., Xin, Y.J., Wang, C., 2012. Early mixed farming of millet and rice 7800 years ago in the middle Yellow River region, China. Plos One 7 (12), e52146.

Zhang, J.P., Lu, H.Y., Wu, N.Q., Li, F.J., Yang, X.Y., Wang, W.L., Ma, M.Z., Zhang, X.H., 2010a. Phytolith evidence for rice cultivation and spread in mid-late Neolithic archaeological sites in central north China. Boreas 39 (3), 592–602.

Zhang, J.P., Lu, H.Y., Wu, N.Q., Li, F.J., Yang, X.Y., Wang, W.L., Ma, M.Z., Zhang, X.H., 2010b. Phytolith evidence of millet agriculture during about 6000-2100 a BP in the Guanzhong Basin, China. Quaternary Sciences 30 (2), 288–297 (in Chinese with English Abstract).

Zhang, J.P., Lu, H.Y., Wu, N.Q., Yang, X.Y., Diao, X.M., 2011. Phytolith analysis for differentiating between foxtail millet (*Setaria italica*) and green foxtail (*Setaria viridis*). PLoS ONE 6, e19726.

Zhao, Z.J., 2004. The elementary analysis of the floating sieving result from the Zhouyuan site (Wangjiazui site). Cultural Relic 10, 89–96 (in Chinese).

Zhao, Z.J., 2005. Palaeoethnobotany and its new achievements in China. Kaogu Archaeology 7, 42–49 (in Chinese).

Zhou, X.Y., Li, X.Q., Zhao, K.L., Dodson, J., Sun, N., Yang, Q., 2011. Early agricultural development and environmental effects in the Neolithic Longdong basin (eastern Gansu). Chinese Science Bulletin 56 (8), 762–771.