Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago

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Edited by Dolores R. Piperno, Smithsonian Tropical Research Institute and National Museum of Natural History, Washington, DC, and approved March 17, 2009 (received for review January 8, 2009)

The origin of millet from Neolithic China has generally been accepted, but it remains unknown whether common millet (*Panicum miliaceum*) or foxtail millet (*Setaria italica*) was the first species domesticated. Nor do we know the timing of their domestication and their routes of dispersal. Here, we report the discovery of husk phytoliths and biomolecular components identifiable solely as common millet from newly excavated storage pits at the Neolithic Cishan site, China, dated to between ca. 10,300 and ca. 8,700 calibrated years before present (cal yr BP). After ca. 8,700 cal yr BP, the grain crops began to contain a small quantity of foxtail millet. Our research reveals that the common millet was the earliest dry-farming crop in East Asia, which is probably attributed to its excellent resistance to drought.

Holocene | origins of agriculture | phytoliths | Neolithic | Cishan

Foxtail millet (*Setaria italica*) and common millet (or broom-corn millet; *Panicum miliaceum*) were among the world’s most important and ancient domesticated crops. They were staple foods in the semi-arid regions of East Asia (China, Japan, Russia, India, and Korea) and even in the entire Eurasian continent before the popularity of rice and wheat (1–4), and are still important foods in these regions today (5, 6).

Thirty years ago, the world’s oldest millet remains, dating to ca. 8,200 calibrated years before present (cal yr BP), were discovered at the Early Neolithic site of Cishan, northern China. The site contained >50,000 kg of grain crops stored in the storage pits (7–9). Until now, the importance of these findings has been constrained by limited taxonomic identification with regard to whether they are from foxtail millet (*S. italica*) or common millet (*P. miliaceum*), because the early reported *S. italica* identifications are not all accepted (4, 9–12). This article presents the phytoliths, biomolecular records, and new radiocarbon dating from newly excavated grain crop storage pits at the Cishan site. Large modern reference collections are used to compare and contrast microfossil morphology and biomolecular components in different millets and related grass species (13). The renewed investigations show that common millet agriculture arose independently in the semi-arid regions of China by 10,000 cal yr BP. Our findings contribute to our knowledge of agricultural origins across the globe and have broader implications for understanding the development of human societies.

The Cishan site (36°34′51″ N, 114°06′72″ E) is located near the junction between the Loess Plateau and the North China Plain at an elevation of 260–270 m above sea level (Fig. 1). The archaeological site, containing a total of 88 storage pits with significant quantities (~109 m³) of grain crop remains, was excavated from 1976 to 1978 (7, 8). Each storage pit included 0.3- to 2-mm-thick grain crops, which were well preserved and found in situ in the 3- to 5-m-deep loess layer (9). All grain remains have been oxidized to ashes soon after they were exposed to air. Archaeological excavations also revealed the remains of houses and numerous millstones (Fig. S1), stone shovels, grind rollers, potteries, rich faunal remains, and plant assemblages including charred fruits of walnut (*Juglans regia*), hazel (*Corylus heterophylla*), and hackberry (*Celtis bungeana*) (7–9). Only 214C dates of charcoal from previously excavated H145 and H48 storage pits yielded uncalibrated ages of 7355 ± 100 yr BP and 7235 ± 105 yr BP, respectively (8). These remains represent the earliest evidence for the significant use of dry-farming crop plants in the human diet in East Asia. They also suggest that by this time agriculture had already been relatively well developed here.

Early identification assumed these grain crop remains to be foxtail millet (*S. italica*). This preliminary identification was mainly based on a characteristic of very small sizes of grain crop ash (no charred grains)—often <2–3 mm in length—that resembled the foxtail millets (Fig. S2), but without any spodogram evidence for the grain crop remains in the Cishan site (7–10). These have been reported as the world’s oldest foxtail millet in the literature (6, 9, 10, 14). However, the millet identification has been questionable (4, 11, 12, 15), because the macro (ash) remains were too friable to be observed under the microscope. Furthermore, very little study has been conducted on the spodograms or phytoliths of modern millets, so no clear diagnostic feature has been used to distinguish foxtail millet from common millet (12, 16). Previous study has also considered at some length how the charred dehusked grains of various native millet species might have been systematically misidentified (3). Thus, questions remain regarding whether the Cishan grain crops are from foxtail millet or common millet, or both, because they cannot be distinguished from the ash vestige (11, 12).

Results

Phytoliths and biomolecular components have provided substantial empirical evidence demonstrating the considerable antiquity of food production and crop dispersals in many regions of the world (17–20). Based on our observation and statistics of the variation of anatomy and silicon structure patterns in the glumes, lemmas, and paleas occurring in modern cultivated millets and related grass species collected from different regions in China (13), we found that 5 key diagnostic characteristics in phytolith morphology could be used to distinguish between foxtail millet and common millet (13): (i) a cross-shaped type phytolith is formed in the lower lemma and glume of *S. italica*, (i) a cross-shaped type phytolith is formed in the lower lemma and glume of *S. italica*,...
whereas a bilobe-shaped type is formed in those of *P. miliaceum*; (ii) regularly arranged papillae on the surface of the upper lemma and paleas are peculiar to *S. italica*; (iii) the epidermal long cell walls are Ω-undulated in *S. italica* (Fig. 2H), and η-undulated in *P. miliaceum* (Fig. 2B, D, and F); (iv) the endings of epidermal long cells are cross-wavy type in *S. italica* and cross-finger type in *P. miliaceum*; the R value (ratio of the width of endings interdigitation to the amplitude of undulations) is higher (0.79 ± 0.12; *n* = 3,303) in *P. miliaceum* than in *S. italica* (0.33 ± 0.11; *n* = 2,774); and (v) surface ridgy line sculpture of the upper lemma is peculiar to *S. italica*. These 5 diagnostic characteristics used together give the only reliable way of distinguishing foxtail millet from common millet when only powder remains are available. In addition, a species-specific identification of phytoliths is possible for *S. italica* and *P. miliaceum* because they have typically well-defined silica skele-

![Locality map.](https://www.pnas.org/cgi/doi/10.1073/pnas.0900158106)

Fig. 1. Locality map. (A) Map showing the location of Cishan and other important Early Neolithic millet agricultural sites in the semi-arid region of northeastern China. (B) The Cishan site is located on a terrace of the Ming River (Inset). A detailed plan of the west area of Cishan site excavated in 1976–1978, showing the outlines of the serried storage pits and the 5 newly excavated storage pits, CS-I to CS-V (numbers in brackets), in an area to the northwest of the earlier excavation is presented. (C) A photograph of the newly excavated storage pit CS-V, found on the cliff of the northern terrace. (D) Close-up photograph of the loose layer of grain crop remains in storage pit CS-III found in situ in the loess layer.

![Scanning microscopic interferometer photographs.](https://www.pnas.org/cgi/doi/10.1073/pnas.0900158106)

Fig. 2. Scanning microscopic interferometer photographs. (A and B) Phytoliths from CS-I storage pit (A), compared with modern η-I type husk phytoliths from *P. miliaceum* (B). (C and D) Phytoliths from CS-II storage pit (C), compared with modern η-II type husk phytoliths from *P. miliaceum* (D). (E and F) Phytoliths from BWG (E), compared with modern Ω-II type husk phytoliths from *P. miliaceum* (F). (G and H) Phytoliths from CS-II storage pit (G), compared with modern Ω-II type husk phytoliths from *S. italica* (H). (I) Bivariate biplot showing coordinates of the 3,303 measurements from epidermal long cells of *P. miliaceum* and 2,774 measurements from those of *S. italica*, plotted along axis W (width of endings interdigitation of dendriform epidermal long cells) and axis R (ratios of W to undulations amplitude of dendriform epidermal long cells), and their classification into 2 groups corresponding to 2 species (*P. miliaceum* and *S. italica*) (13). Also plotted are the fossil samples of husk phytoliths from CS-I-V and BWG, interpreted to be of *P. miliaceum* origin, dated between ca. 10,300 and ca. 7,500 yr BP. The CS-II, V, and BWG samples contained 0.4–2.83% Ω-type husk phytoliths, interpreted to be of *S. italica* origin, dated to less than ca. 8,700 yr BP.
tons that are distinguishable from those in *Panicum bisulcatum*, *Setaria viridis*, and *Setaria plicata*, which have no such demonstrable patterns (13).

To determine the taxa of foxtail millet and common millet, we analyzed the phytoliths of 46 grain crop samples stored in 5 newly excavated storage pits (CS-I to CS-V) at the Cishan site, and 1 sample (BWG) preserved in a storage bottle from the Culture Museum of Cishan. These grain crop samples are dated between ca. 10,300 and ca. 7,500 cal yr BP based on new 14C dating measurements (Fig. 3, Table S1).

All 47 archaeological samples we analyzed contained abundant diagnostic husk phytoliths that can be divided into 2 groups according to their phytolith assemblages and 14C dating results. The first group, including 27 samples from CS-I, III, and IV, is dated between ca. 10,300 and ca. 8,700 cal yr BP. All of the husk phytoliths present are diagnostic of common millet based on their shapes, η-patterns (Fig. 2A, C, and E), and average R value (>0.7) (Fig. 2I). The second group, including 20 samples from CS-II, V, and BWG, is dated between ca. 8,700 and ca. 7,500 cal yr BP. More than 97% of the husk phytoliths are also diagnostic of the common millet (97.2% for CS-II, n = 1,273; 97.5% for CS-V, n = 1,000; 99.6% for BWG, n = 1,000), but a small quantity (0.4–2.8%) of the husk phytoliths in the second group can be attributed to *S. italica* (Fig. 2 G and I).

Fig. 4 shows that the CS-I storage pit contains ~1.5-m-thick grain crop remains composed of 3 prominent layers of lemma and palea from common millets alternating with 3 glume plus reed layers. This storage manner indicates that prehistoric humans had known how to preserve large volumes of grains in secure storage. They did so by digging deep storage pits in the dry loess strata and by covering the floors with thick mats of millet glumes and reed (*Phragmites australis*) leaves.

Identification of biomolecular components was mainly based on our modern reference collection. A recent study has used the biomolecular components of *P. miliaceum*, the only miliacin-exclusive producer reported, as a basis for identification (20). However, because of the lack of comparable data derived from *S. italica*, previous investigators are still not clear how to use biomarkers to distinguish *P. miliaceum* from *S. italica*. In this study, we examined biomolecular components in 6 samples of modern Paniceae (see Materials and Methods). The results show that biomolecular components can be used to distinguish between *P. miliaceum* and *S. italica* based on the presence or absence of 5 biomarkers—miliacin, α-amyrin methyl ether (ME), and 3 pentacyclic triterpene methyl ethers (PTMEs), although the structures of the 3 PTMEs have yet to be confirmed (Fig. 5, Fig. S3). The total ion current trace shows the whole distribution of aromatic hydrocarbons and ethers extracted from *P. miliaceum* (Fig. 5A) and *S. italica* (Fig. 5B) to be in the 54- to 62-min analysis time range. The significant relative abundance of miliacin (compound 2) (Fig. 5D) was found in both modern species (89.0 ± 1.64% for *P. miliaceum* and 33.8 ± 22.2% for *S. italica*); the miliacin relative abundances in the aromatic hydrocarbons and ethers were estimated by measuring the area of the miliacin peak on the m/z 189 + 204 + 218 ion-specific chromatogram. However, compounds 1, 4, and 5 are peculiar to *P. miliaceum*, and compound 3 is peculiar to *S. italica*.

Fig. 5 shows that the prominent compound products of the archaeological samples (BWG, CS-V-03) are compounds 1, 2, 4, and 5, which are also present in *P. miliaceum*. However, compounds 1, 4, and 5 are notably absent from *S. italica*, which contain compounds 2 and 3 only. Thus, the distribution pattern of major biomarkers of BWG and CS-V-03 grain crop remains is similar to that of modern common millet (Fig. 5A). In 2 archeological samples, including BWG and CS-V-03, the relative abundance of miliacin reaches 88.5% and 88.2%, respectively, which is also very similar to that of common millet. These results provide further support to our conclusion and suggest that common millet is an important source of grain crops stored in the storage pits.

**Discussion**

According to archeobotanical research, the early charred grains of common millet occurred during the initial stages of various Early Neolithic sites (Fig. 1), including Dadiwan (ca. 7.8–7.35 cal kyr BP) (21), Xinglonggou (ca. 8.0–7.5 cal kyr BP) (22), and...
Yuezhuang (ca. 7.87 cal kyr BP) (23) in North China, but foxtail millet was barely present during these stages. Lee et al. (24) have speculated that the Early Neolithic predominance of broomcorn over foxtail millet at Xinglonggou and Yuezhuang ca. 6000 cal B.C. might be a regional phenomenon, implying that broomcorn millet might have been domesticated earlier than foxtail millet. Our analytical results of both phytoliths and biomolecular components have established that the earliest cereal remains stored in the Cishan Neolithic sites, during ca. 10,300–8,700 cal yr BP, are not foxtail millet, but only common millet. After 8,700 cal yr BP, the grain crops gradually contained 0.4–2.8% foxtail millet. Our study also suggests that common millet was used as a staple food significantly earlier than foxtail millet in northern China. It provides direct evidence to show that, by 10,000 cal yr BP, the early people in northern China had developed various methods of maintenance and multiplication of millet seeds for

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Fig. 5. Total ion chromatogram of extracted aromatic hydrocarbons and ethers from common millet (A), foxtail millet (B), and grain crops from BWG and CS-V-03 (C). Peaks labeled 1, 2, 3, 4, and 5 correspond to PTME-1 \([M^+ 440, m/z, 425, 393, 257, 218, 204, 189, 161, 135, 109(100%), 95, 69]\) (Fig. S3A), PTME-2 [miliacin, olean-18-en-3β-ol ME, \([M^+ 440, m/z, 425, 393, 257, 218, 204, 189(100%), 177, 161, 135, 109, 95, 69]\), PTME-3 \([M^+ 440, m/z, 425, 397(100%), 365, 261, 229, 218, 204, 189, 175, 161, 135]\) (Fig. S3B), PTME-4 \([\alpha\text{-amyrin ME, urs-12-en-3β-ol ME, } M^+ 440, m/z, 393, 259, 218(100%), 203, 189, 161, 135, 109, 95]\) (Fig. S3C), and PTME-5 \([M^+ 440, m/z, 425, 408, 393, 257, 221, 203, 189(100%), 147, 135, 121, 109, 95]\) (Fig. S3D), respectively. Mass spectra of PTME-2 (miliacin, olean-18-en-3β-ol ME) are presented in D.
the next generation, and had known how to store crops of staple food in secure, dry places of storage pits during the Early Neolithic epoch.

Common millet has the lowest water requirement among all grain crops; it is also a relatively short-season crop, and could grow well in poor soils (5, 6, 25). The geographical distribution of both foxtail millet and common millet in China (Fig. S4) shows that foxtail millet is more common in the semidry eastern areas, and its optimal growth occurs at mean annual temperature (MAT) from 8 to 10 °C and mean annual precipitation (MAP) from 450 to 550 mm. However, common millet is more adapted to the drier interior areas, and its optimal growing conditions occur at MAT from 6 to 8 °C and MAP from 350 to 450 mm (5, 6). The origin and dispersal of millet agriculture is a key problem closely related to the history of human impact on the environment and transformation of natural vegetation.

Paleoenvironmental data from the Weinan section (26–29) (Fig. 1) in the southern part of the Loess Plateau between the Cishan and Dadiwan sites are crucial for understanding the early stage of the forager–cultivator transition. The early Holocene was a period of significant environmental change marked by dry climate conditions as inferred from sediment texture (26, 28), magnetic susceptibility (26, 28), pollen (27), phytoliths (28), and mollusk assemblages (29). These proxy records show an environmental transition from cold–dry (ca. 11,000–8,700 cal yr BP) to warm–wet (ca. 8,700–5,500 cal yr BP) conditions. Many lacustrine and loess records from the Chinese Loess Plateau to Central Asia also support the scenario of a dry climate during the early Holocene (30–34). Under the drier climate conditions, soil development was slowed, and the soil developed on the underlying older and coarser loess of the glacial period was poor in nutrients (28). This raises the possibility that common millet was more significant than foxtail millet in the early stages of food production in North China because it was more adaptable than foxtail millet to the dry condition prevailing during the early Holocene. The common millet cultivation may involve complex selection by natural forces and human activities, although no clear evidence has been documented in this region for the transitions from gathering to cultivation and/or from a wild ancestor to domesticated common millet (1, 2, 5).

Conclusions

Our research indicates that the earliest significant common millet cultivation system was established in the semiarid regions of China by 10,000 cal yr BP, and that the relatively dry condition in the early Holocene may have been favorable for the domestication of common millet over foxtail millet. Our study shows that common millet appeared as a staple crop in northern China ≈10,000 years ago, suggesting that common millet might have been domesticated independently in this area and later spread to Russia, India, the Middle East, and Europe. Nevertheless, like Mesopotamia, where the spread of wheat and barley to the fertile floodplains of the Lower Tigris and Euphrates was a key factor in the emergence of civilization, the spread of common millet to the more productive regions of the Yellow River and its tributaries provided the essential food surplus that later permitted the development of social complexity in the Chinese civilization.

Materials and Methods

Phytolith Extraction from Archaeological Material. The Cishan site is located on the north terrace of the Ming River (Fig. 1). The geography of this region consists of alluvial terraces covered by loess deposits. Five storage pits (CS-I to CS-V) are situated on the edge of the terrace. The terrace is composed of thick sediments (4–5 m thick for CS-I to CS-IV, 11 m thick for CS-V) that consist of alternating layers of loess and clay. Each of the storage pits (CS-I to CS-V) contains ~1- to 2-m-thick grain crop remains, being well preserved in the 3- to 5-m-deep loess layer (Table S1).

A total of 46 samples from 5 storage pits (CS-I to CS-V) at the Cishan site and 1 sample (BWG) from the Culture Museum of Cishan were analyzed. Of these, 16 continuous samples were taken at 10-cm intervals from CS-I storage pit. The samples were prepared according to the procedure slightly modified from Piperno (35, 36) and Lu et al. (37). It consists of sodium pyrophosphate (Na2P2O7) deflocculation, treatment with 30% hydrogen peroxide (H2O2) and cold 15% hydrochloric acid (HCl), zinc bromide (ZnBr2; density, 2.35 g/cm3) heavy liquid separation, and mounting on a slide with Canada balsam. Phytolith counting and identification were performed by using a Leica microscope with phase-contrast and microscopic interferometer at 400× magnification.

Millet Molecule Extractions from Modern Plants and Archaeological Material. We examined modern millet molecules from 6 domesticated samples of Paniciceae, including 3 samples of Setaria italica L. Beauv. (foxtail millet) and 3 samples of Panicum miliaceum L. (common millet). Two archaeological samples of grain crop remains, including 1 sample from CS-V storage pit and 1 sample (BWG) in a storage bottle from the Culture Museum of Cishan, were analyzed.

One gram of each dry sample (smaller than 100 mesh) was ultrasonically extracted for 3 times with a mixture of acetone and pentane at 1:1 (vol/vol). After filtration, the mixture was eluted with n-hexane to give aliphatic hydrocarbons (N). Using dichloromethane in n-hexane passing through the column resulted in satisfactory separation of aromatic hydrocarbons and ethers (A). The extracted subfractions (A) were then analyzed by gas chromatography coupled with mass spectrometry (GC/MS).

GC-MS was conducted by using an Agilent 6890 N GC-5973 N MSD mass selective detector system (EI 70 eV; ion source temperature, 230 °C) fitted with a fused silica chemically bonded capillary column (J&W DB-5; 0.25 mm in diameter, 30 m long, 0.25 μm film thickness). Each sample was injected onto the column at 280 °C in the splitless mode. After a 1-min isothermal hold at 50 °C, the column temperature was increased by 30 °C/min to 120 °C; and then 3 °C/min to 290 °C, successively, with a 30-min isothermal hold at 290 °C. The flow rate of the helium carrier gas was 1.2 mL/min (40 cm/sec).

Compounds were identified by their retention time within the gas chromatograph, their fragmentation pattern within the mass spectrometer, and by matching their mass spectra to reference spectra from the literature or from libraries (NIST02L). The miliacin relative abundances were estimated by measuring the area of the miliacin peak on the ms2 189 + 204 + 218 ion-specific chromatogram. Note that this value may vary under different GC/MS conditions, and there will be a small change in the abundance of this ion.

ACKNOWLEDGMENTS. We are grateful to Dolores R. Piperno for critically reading the original manuscript and her helpful comments in improving this manuscript. We also greatly appreciate the valuable comments from two anonymous reviewers. We are grateful to Jacques Jérémie and A. Gerasimenko for their help on biomolecular analyses, and D. Q. Fuller and L. Qin for their discussions on phytolith analyses. We thank C. D. Shen, Z. Y. Gu, and B. Xu for discussions on phytolith analyses. We thank C. D. Shen, Z. Y. Gu, and B. Xu for their help in radiocarbon age measurement. This work was supported by the Chinese Academy of Sciences (100 Talents Program, kzx2-yw-117), the National Natural Science Foundation of China (40771216, 40325002), Chinese Civilization Origin projects (2006BAK21B02), and the U.S. National Science Foundation (BCS-0623514, ATM-0402475).


