

## Phytoliths as indicators of plant water availability: The case of millets cultivation in the Indus Valley civilization

F. D'Agostini<sup>a,b,\*</sup>, J. Ruiz-Pérez<sup>a</sup>, M. Madella<sup>a,c,d</sup>, V. Vadez<sup>b,e</sup>, J. Kholova<sup>e,f</sup>, C. Lancelotti<sup>a,c</sup>

<sup>a</sup> CaSeS Research Group, Department of Humanities, University Pompeu Fabra, C/ Ramon Trias Fargas 25-27, Barcelona 08005, Spain

<sup>b</sup> DIADE Unit, IRD, University of Montpellier, Av. Agropolis 911, Montpellier 34394, France

<sup>c</sup> ICREA-Catalan Institution for Research and Advanced Studies, Pg. Lluís Companys 23, 08010 Barcelona, Spain

<sup>d</sup> Department of Geography, Archaeology and Environmental Studies, University of Witwatersrand, 1 Jan Smuts Avenue, Braamfontein, Johannesburg 2000, South Africa

<sup>e</sup> Crop Physiology Laboratory, ICRISAT, Patancheru 502324, Telangana, India

<sup>f</sup> Department of Information Technologies, Faculty of Economics and Management, Czech University of Life Sciences, Kamýcká 129, Prague 165 00, Czech Republic

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### ABSTRACT

The interpretation of crop water management practices has been central to the archeological debate on agricultural strategies and is crucial where the type of water strategy can provide fundamental explanations for the adoption and use of specific crops. Traces of water administration are difficult to detect and are mostly indirect, in the form of water harvesting or distribution structures. Attempts have been made to infer plant water availability directly from archaeobotanical remains. Current evidence suggests that the ratio of sensitive to fixed phytolith morphotypes can be used as a proxy for water availability in C<sub>3</sub> crops, as well as in sorghum and maize. Nevertheless, the controversy on whether genetically and environmentally controlled mechanisms of biosilica deposition are directly connected to water availability in C<sub>4</sub> crops is open, and several species remain to be tested for their phytolith production in relation to water levels. This research aims at clarifying whether leaf phytolith assemblages and concentration, silica skeleton size and ratio of sensitive to fixed morphotypes can be related to different water regimes in *Eleusine coracana* Gaertn., *Pennisetum glaucum* (L.) R. Br., and *Sorghum bicolor* (L.) Moench. We cultivated 5 traditional landraces for each species in lysimeters, under different watering conditions and analyzed their phytolith content/production in leaves. Results show higher proportions of long cells, bulliforms and stomata produced in well watered conditions. The model built on the basis of phytolith composition has been then applied to interpret archeological phytolith assemblages recovered from a single phase at four different sites of the Indus Civilisation: Harappa, Kanmer, Shikarpur and Alamgirpur. The results show that most probably C<sub>4</sub> crops grew under water stress conditions, providing new data on the interpretation of ancient agricultural management in the Indus Valley.

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### 1. Introduction

Archeologists have long appreciated the importance of identifying past water management systems, which marked a key change in the socio-ecological trajectory of human societies and their land use. Indeed, most ancient civilizations were dependent upon sophisticated techniques of water management for agricultural intensification and technological development (Mithen, 2010). Research on water management techniques for agriculture has been crucially contributing to our understanding of the evolution of land use (Beckers et al., 2013). In arid regions the subject of crop water management in the

past is rather controversial and difficult to untangle. In pre-industrial drylands pastoralism is often considered to be the primary livelihood strategy, with irrigated and floodplain agriculture as main cultivation strategies, while rain-fed agriculture tends to be considered a secondary activity due to water scarcity (Giosan et al., 2012). Lancelotti et al. (2019), on the other hand, argued that rain-fed cultivation might have played a so-far under recognized role in the development of food production in arid areas, even where rainfall is normally considered too scarce for crop cultivation. Despite many modern examples testify to the existence of successful rain-fed systems (Salmon et al., 2015), such practices have received little attention in archaeology, possibly for the difficulties in identifying them from the archeological record. Water management is usually inferred from the presence of related technology, such as canals and tanks (see Madella and Lancelotti, 2022). However, in the last few decades, water management practices

\* Corresponding author at: C/ Ramon Trias Fargas 25-27, office n° 24.504, 08005 Barcelona, Spain.

E-mail address: [francesca.dagostini@upf.edu](mailto:francesca.dagostini@upf.edu) (F. D'Agostini).

have been also inferred from archaeobotanical remains such as phytoliths (e.g. Jenkins et al., 2020), and charred seeds isotopic signatures (e.g. Lightfoot et al., 2019).

### 1.1. Plant micro-remains: Phytoliths

Translocation of Silicon in the plants structures is mediated by the water flux, with the transpiration stream acting as the main motive force (Ma and Yamaji, 2015). This opal silica deposition in the plant is related to water availability. Two types of Silicon deposition have been described so far (Hodson, 2019): one at the level of the cell wall, and one that occurs within the lumen. In both cases, the precipitation of Silicon to form bio-opals may depend on spontaneous dehydration phenomena or may be stimulated by molecules designed to activate the deposition process (Kumar et al., 2017). A recent phylogenetic analysis of Silicon transporters across the plant kingdom showed the extensive presence of the channel Lsi1 and a high level of conservation of the Lsi2 in embryophytes (Coskun et al., 2019), indicating an early evolution for such transporters and their possible presence in all Poaceae. The presence of these Silicon carrier channels has been tested in rice (*Oryza sativa* L.) and homologous transporters (Vatansver, 2017) have been found in maize (*Zea Mays* L. Sp. Pl.) (Mitani et al., 2009), and in several vegetables such as pumpkin (Mitani et al., 2011) and cucumber (Sun et al., 2017). This discovery suggests that the deposition of phytoliths may not depend entirely on environmental factors such as water abundance, and that genetics certainly plays a key role in the accumulation of Silicon in shoots. Since Silicon polymerization is also due to supersaturation by transpiration-driven water loss (Schaller et al., 2013), we hypothesize that in the tissues with photosynthetic activity, such as leaves, phytolith production, and indices based on their relative abundance, can be tested as proxies for water availability.

Several indicators based on phytoliths production have been developed to assess water availability. Miller Rosen and Weiner (1994) suggested using the dimension of silica skeletons, under the assumption that a greater water absorption by the plant led to a greater silica uptake and deposition, which allows for the formation of larger silica conjoined structures (Miller Rosen and Weiner, 1994). The methodology has been applied in archeological contexts by Katz et al. (2007) producing positive results (Katz et al., 2007). Madella et al. (2009) used the ratio of fixed versus sensitive morphotypes (elongates + stomata/ bilobates, crosses, polylobates, rondels, saddles) and tested it in bread wheat (*Triticum aestivum* L.), emmer wheat (*Triticum dicoccum* L.) and barley (*Hordeum vulgare* L.) (Madella et al., 2009). This method rests on the assumption that leaf phytolith assemblage is composed by genetically determined (short cells) and environmentally controlled morphotypes (stomata and elongates). The methodology was applied by Weisskopf et al. (2015) to detect water availability from cultivated rice (*Oryza sativa* L.) fields (Weisskopf et al., 2015), and by Jenkins et al. (2016) by cultivating barley (*Hordeum vulgare* L.) and durum wheat (*Triticum durum* Desf.) to prove the effectiveness of the ratio in these additional cereals (Jenkins et al., 2016). Both studies demonstrated distinctive phytolith patterns between plants cultivated in wet and dry conditions.

Jenkins et al. (2020) proposed for the first time to apply the same ratio to sorghum (*Sorghum bicolor* L. Moench), with positive results. The same ratio has been exploited by Ermish and Boomgarden (2022), who tested how sensitive to fixed phytoliths ratio and long-cells proportion of maize (*Zea Mays* L.) respond to wet-dry conditions (Ermish and Boomgarden, 2022). The results highlighted strong differences between well irrigated and less-irrigated C<sub>4</sub> crops, proving that the methodology is effective even in crops with reduced water availability. Additionally, the palaeoenvironmental study of Bremond et al. (2005) hypothesized that the more plants transpire and/or suffer water stress, the more silicified bulliform cells they would produce (Bremond et al., 2005). This research is noteworthy because it places for the first time the bulliforms flabellate of the Chloridoideae in the shortlist of possible morphotypes whose production is influenced by transpiration.

### 1.2. Finger millet, pearl millet and sorghum

Given these favorable examples, it was decided to expand the C<sub>4</sub> species tested by analyzing how finger millet, pearl millet and sorghum phytolith production and composition respond to different levels of irrigation. These three cereals belong to the C<sub>4</sub> species that today account for approximately 25% of the primary production of the entire planet (Sage and Zhu, 2011). C<sub>4</sub> are characterized by their physiological ability to withstand high temperatures and scarce and erratic rainfall patterns thanks to their specific photosynthetic pathway, which increases assimilation rate and reduces photorespiration by concentrating CO<sub>2</sub> at the site of the Rubisco (Bräutigam et al., 2014).

Finger millet, pearl millet and sorghum are native to Africa, but they probably followed different trajectories of domestication. Sorghum has been domesticated relatively late (c. around 50 BCE) even if it was probably cultivated in a wild form a few millennia earlier (Fuller and Stevens, 2018). The human population of east Sahel probably domesticated sorghum as a fodder grass once they adopted sedentarism and pastoral practices, abandoning hunting and gathering as a main economy (Winchell et al., 2018). Pearl millet is the oldest domesticated crop of Africa (c. 3000 BCE) (Manning et al., 2011), with a single domestication centre either in western Sahel or in the region included between eastern Mali and western Niger (Dussert et al., 2015). Presumably wild pearl millet attracted the attention of the local non sedentary pastoralists because of its prolific production of small grains, and its resilience to grazing (Mercuri et al., 2018). Finger millet, pearl millet and sorghum are traditionally considered to be among the first African millets to enter the Indian sub-continent along with Italian millet (*Setaria italica* (L.) P. Beauv.) (Pokharia et al., 2014).

In South Asia, domesticated pearl millet was present in the Saurashtra peninsula (Gujarat) by at least 1700 BCE and possibly arrived even earlier around 1900 BCE (Manning et al., 2011). While the evolution of big-grain pearl millet happened independently in India, it has been suggested that the small-grain varieties are a derivation of Sahelian forms, rapidly disseminated eastward (Winchell et al., 2018). The earliest evidence of sorghum in the Indian peninsula was found in Kunal, nearby Banawali and Rojdi, and is assumed to be as early as Late Harappan period (2000–1700 BCE) (Fuller and Boivin, 2009). Finger millet arrival inland is disputed but the most accepted hypothesis places its introduction in the Mature Harappan Rodji (Fuller, 2003). It has been hypothesized that in northwest South Asia, African millets eventually prevail on the local species because of their higher productivity under intensive cultivation as well as taste (Weber and Fuller, 2006).

### 1.3. The archeological application

Once it was verified that the methodological frame developed from phytolith assemblages of modern millets could work to indicate watering levels, it was decided to apply the model to archeological samples to test its validity and applicability and finally providing an answer to the primary archeological question on water management in past agricultural societies. To this purpose it was decided to exploit a C<sub>4</sub> phytolith dataset from layers dated to the Mature Harappan period (2500–1900 BCE) coming from four different archeological sites: Harappa, Kanmer, Shikarpur and Alamgirpur. Using millet phytolith samples from Mature Harappan period layers seemed interesting because, to date, there is an open debate on the consumption and spread of such crops throughout the Indus civilization. During the Mature Harappan period, small millets represented anywhere from 3 to over 50% of the recovered cereals (Pokharia et al., 2014). However, their use is difficult to justify since their grain processing is very labour intensive (Arunachalam et al., 2005), apart from the fact that their productivity is lower when compared to that of C<sub>3</sub> cereals (such as barley and wheat) (Percy and Ehleringer, 1984). We assume that a possible explanation could be directly related to water availability and management. On the one hand the adoption and increasing use of these cereals can be related to the implementation of an agricultural

system based on double cropping (*rabi* or winter & *kharif* or monsoon season) (Weber et al., 2010). While legumes and C<sub>3</sub> cereals such as barley or rice, could be cropped during the wet seasons or near water reservoirs, small millets could have been grown in the submarginal dry areas with limited rainfall (Weber and Fuller, 2006), or sown during summer, the warmest and driest season. An alternative theory sees millets involved in the process of adaptation to increasingly arid climates. Multi-proxy records indicate the onset of drier climate during the Late Harappan (Prasad et al., 2007). The progressive use of millets may have been in response to aridity (Pokharia et al., 2014). It is therefore of great interest to try to understand the water conditions under which this type of cereal survived during the late Harappan period, for adding crucial information in the reconstruction of land use.

#### 1.4. Aims

The work aims to reconstruct water availability of archeological C<sub>4</sub> cereals. To achieve this goal, a methodological framework that provides the tools to answer the archeological question needed to be built. In this work, we focused on trying to understand whether phytoliths could serve this purpose. First, it was necessary to check if phytolith production was related to plant water availability. Based on available literature, it was tested whether:

- 1) phytolith assemblage composition is related to water levels;
- 2) phytolith concentration is related to water levels;
- 3) ratio fixed to sensitive morphotypes is related to water levels;
- 4) silica skeletons size ratio is related to water levels.

Only in a second instance, once the methodology was confirmed, the model built on the modern assemblage was applied to see how likely it was that C<sub>4</sub> plants at Harappa, Kanmer, Shikarpur and Alamgirpur had grown under irrigated conditions.

## 2. Materials and methods

### 2.1. Modern samples

We selected five landraces of pearl millet (*Pennisetum glaucum* L.R. Br), four of finger millet (*Eleusine coracana* L.Gaertn) and five of sorghum (*Sorghum bicolor* L.Moench) from the gene bank ICRISAT collection (Hyderabad, India) (Table 1).

Traditional landraces were preferred to observe the variability of phytolith production in un-improved crops, which should exclude any possible recent modern change in the genetics of biosilica accumulation. Landraces were selected according to the area and climate of origin. The specific landraces were chosen using the Climatic Research Unit TS3.10 Dataset, a Google Earth Pro application that divides the entire world into high-resolution climate grids (Harris et al., 2013) combined with climate data from Climate-Data.org (<https://en.climate-data.org>). Thus, the landraces selected come either from the area of interest (Pakistan), or from African areas from which domestication might have started (Kenya and Tanzania) (Fuller and Boivin, 2009), or from areas in East Africa (Sudan and Ethiopia) where the climatic parameters are comparable to those in the Indus valley, covering a good range of variability.

The experimental cultivation took place at ICRISAT, Hyderabad, India (17°31' N 78°16' E) between February and May 2019. To reproduce field conditions while keeping a tight control on water-related parameters, the plants were cultivated in lysimeters (PVC tubes of 200 cm in length and 25 cm in diameter). Lysimeters, positioned in two parallel pits about half a meter apart, simulate real field conditions regarding plant spacing (11 plants/m<sup>2</sup>), soil availability for ground water exploration (2 m of soil available for each plant), and general growing conditions as the tubes are placed outdoors (but covered by a rain-out shelter in case of rain). The tubes were filled with a mixture of 1:1 Alfisol-Vertisol. Two different water managements were tested to simulate water availability in (a) rain-fed conditions in arid environments (water stressed, WS hereafter) and (b) irrigated conditions, which also acted as control (well watered, WW hereafter). WW plants have been watered weekly to maintain 80% of soil field capacity, which is the optimum for crops well adapted to dry climates (Zaman-Allah et al., 2011). WW sorghum plants received an average of 34.99 ± 0.93 L in total, WW pearl millet 35.80 ± 1.16 L and WW finger millet 48.93 ± 0.82 L. With WS replicas, the intention was to imitate a real rain-fed scenario where water is available at the beginning of the plant life cycle but then scarce/absent during the reproductive stage (Portmann et al., 2010). For this reason, considering the diameter of the cylinders (25 cm), and that the minimum rainfall average corresponds to approximately 150–155 mm of water (Climate-Data.org, <https://en.climate-data.org>), we calculated that the WS replicas should receive 11 l of water. So, WS cylinders received 11 L each which were administered gradually, every other day, during the first 2 months of plant growth, before the critical flowering time occurred (late stress imposition) and the reproductive stage began.

We cultivated five replications for each treatment per landrace (n = 70, total = 140 replicas). Genotype replicates were randomized in the two pits in order to prevent unintended environmental effects (e.g. heat gradient from the pit walls). To simulate real plant spacing in the field, two plants of the same genotype (same replication) were planted in each cylinder. When the plants had grown to c. 20 cm, about three weeks after planting, they were watered to field capacity and then the soil surface was covered with a plastic sheet and 2 cm of low-density polyethylene granules, which prevented about 90% of evaporation from the soil (Vadez et al., 2011b). After this, the lysimeters were weighed weekly to calculate plant water loss from only transpiration (Vadez et al., 2011a). Data on temperature and relative humidity were collected every 30 min by 2 recorders (Gemini Tinytag Ultra 2 TGU-4500 Datalogger) placed in the crop canopy. Temperature maintained at 32.28 ± 0.10 °C and relative humidity at 42.57 ± 0.23% RH. We harvested WW plants when the panicles were mature (when at least ⅓ replications reached maturity) and the WS plants when their transpiration rate dropped below 10% of the initial value, indicating stomatal closure (cuticular transpiration) (Schuster et al., 2017). Panicles, leaves and stems were first dried in ICRISAT at 60–70 °C for one week and then brought to the Laboratory for Environmental Archaeology of the University Pompeu Fabra (Barcelona, Spain) where samples were processed and phytoliths extracted. Further and more detailed information about the experimental cultivation can be found in D'Agostini et al. (2022).

**Table 1**

Selected landraces from the ICRISAT genebank with a) the acronym used to identify them and b) their accession number.

	Sudan	Ethiopia	Pakistan	Kenya	Tanzania
Sorghum	S2: IS23075	S5: IS11061	S8: IS35215		
		S6: IS38025	S9: IS35216		
Pearl millet	PM1: IP13327	PM5: IP2367	PM7: IP18019		
	PM2: IP9859		PM9: IP18021		
Finger millet				FM1: IE2511	FM6: IE4450
				FM2: IE3476	FM7: IE4456

## 2.2. The archeological sites

Sediment samples for phytolith analysis were recovered from layers dated to the Mature Harappan period (2500–1900 BCE) at four sites of the Indus Valley Civilization: Harappa, Kanmer, Shikarpur and Alamgirpur. The samples should be representative of the same level of agricultural technologies/practices even with significant differences on the type of occupation. Samples have been collected and processed by Carla Lancelotti during her PhD project. The results of her thesis along with more detailed information about the micro remains recovered from the sites are all available in Lancelotti (2010) and Lancelotti (2018).

The archeological site of Harappa (30°37' N 72°53' E) is situated in the Pakistan province of Punjab, on an elevated terrace (*doab*) on the left bank of a channel of the Ravi River. Today the main river flows 10 km north of Harappa, but it appears to have meandered in the vicinity of the site before and during the settlement occupation during the 3rd millennium BCE (Kenoyer, 1998). The samples have been recovered from deposits dated to the central part of the Urban period (phase 3B of mound E; Lancelotti, 2018). The archeological mound of Kanmer (23°23' N 70°52' E), locally known as Bakar Kot, stands to the north of the modern village of Kanmer in the Kachchh District (Gujarat). About 2 km away from the ancient settlement there is an ephemeral stream (*nullah*), known as Aludawaro Vokro. Rajaguru and Shushama (2008) suggested that the *nullah* was permanently active during the Harappan times. Presently the only source of water is a large natural tank located in the southeast of the mound, but there is no information on whether such a basin was in use during the Indus period. Shikarpur site (23°14' N 70°40' E), locally known as Valamiyo Timbo, is an archeological mound located on the southern part of the modern village of Shikarpur (Gujarat), along the margin of a narrow creek that runs eastwards towards the Rann of Kutch. Two water gullies cut the mound into three ridges. The archeological site of Alamgirpur (29°00.206' N 77°29.057' E), locally known as Parasuram-kakhera, is located in the east area of the modern town in Meerut district (Uttar Pradesh), 45 km northeast of New Delhi. It is situated in the plain between the river Ganga and Yamuna and sits on a consolidated sand dune.

## 2.3. Phytolith extraction, classification and counting

### 2.3.1. Modern phytolith samples

Leaves are the organs where transpiration rate is highest and therefore where the maximum production of phytoliths unfolds, including most of those related to hydration mechanisms, such as stomata and bulliforms. For each genotype we selected two replicas and one plant for each replica ( $n = 56$ ), in order to optimize sample size and physiological parameters variability observed during the fieldwork. Aiming at taking into account possible inter-replicate variability in one of the selected genotypes grown under WW conditions, as two plants were grown within each cylinder, we decided to analyze both plants corresponding to one replicate ( $+n = 6$ , total = 62). Therefore, we analyzed 18 samples of finger millet, 10 WW and 8 WS; and 22 samples each of sorghum and pearl millet with 12 WW and 10 WS each.

The protocol used for the extraction of phytoliths couples a dry ashing technique with a subsequent wet oxidation. From each sample 0.0001 g of silica residue was mounted on a microscope glass slide with Entellan New® mounting media. Phytoliths were observed using a Euromex light microscope (Euromex iScope + Euromex scientific camera sCMEX-6) at  $\times 400$  magnification. Since the extractions preserved the silica skeletons and most of the phytoliths were therefore embedded in silica sheets, we based the total count on a minimum number of silica skeletons (50), where all phytoliths in the resulting field of views were counted, whether articulated or disarticulated. The procedure for the extraction and counting of phytoliths, has been summarized in D'Agostini et al. (2022) and it can be fully consulted on protocols.io ([dx.doi.org/10.17504/protocols.io.q26g74mb8gwz/v2](https://dx.doi.org/10.17504/protocols.io.q26g74mb8gwz/v2)).

We counted an average of 619 cells for each slide (raw data is available in the Supplementary Information-File S1). The applied protocol ensures reaching the minimum statistically meaningful number of silica cells per sample by counting more than 300 phytoliths per slide, as suggested by Strömberg (2009). Additionally, it takes into consideration both disarticulated and conjoined cells, respecting richness and evenness distribution. Classification of morphotypes follows the available literature (especially Barboni and Bremond, 2009; Gu et al., 2016; Mercader et al., 2010). Nomenclature follows the International Code for Phytolith Nomenclature (ICPN) 2.0 (International Committee for Phytolith Taxonomy (ICPT) et al., 2019). Fig. 1 shows some examples, while descriptions and additional pictures of morphotypes are available in the Supplementary Information (File S2).

For each slide we evaluated and tested: the concentration of phytoliths in millions per gram of dry leaf, the concentration of each morphotype in millions per gram of dry leaf, the percentage of each morphotype, the sensitive/fixed ratio (following the indication of Jenkins et al. (2020)), and the silica skeleton size ratio. The formulas used in this work are:

$$\text{Phytoliths extracted} = \frac{(\text{total silica extracted} \times \text{total phytoliths per slide})}{\text{total silica mounted}}$$

$$\text{Concentration} = \frac{\left(\frac{\text{phytoliths extracted}}{\text{dry leaf weight}}\right)}{1000000}$$

$$\text{Sensitive/fixed ratio} = \frac{(\text{elongates} + \text{stomata})}{(\text{crosses} + \text{bilobates} + \text{polylobates} + \text{rondels} + \text{saddles})}$$

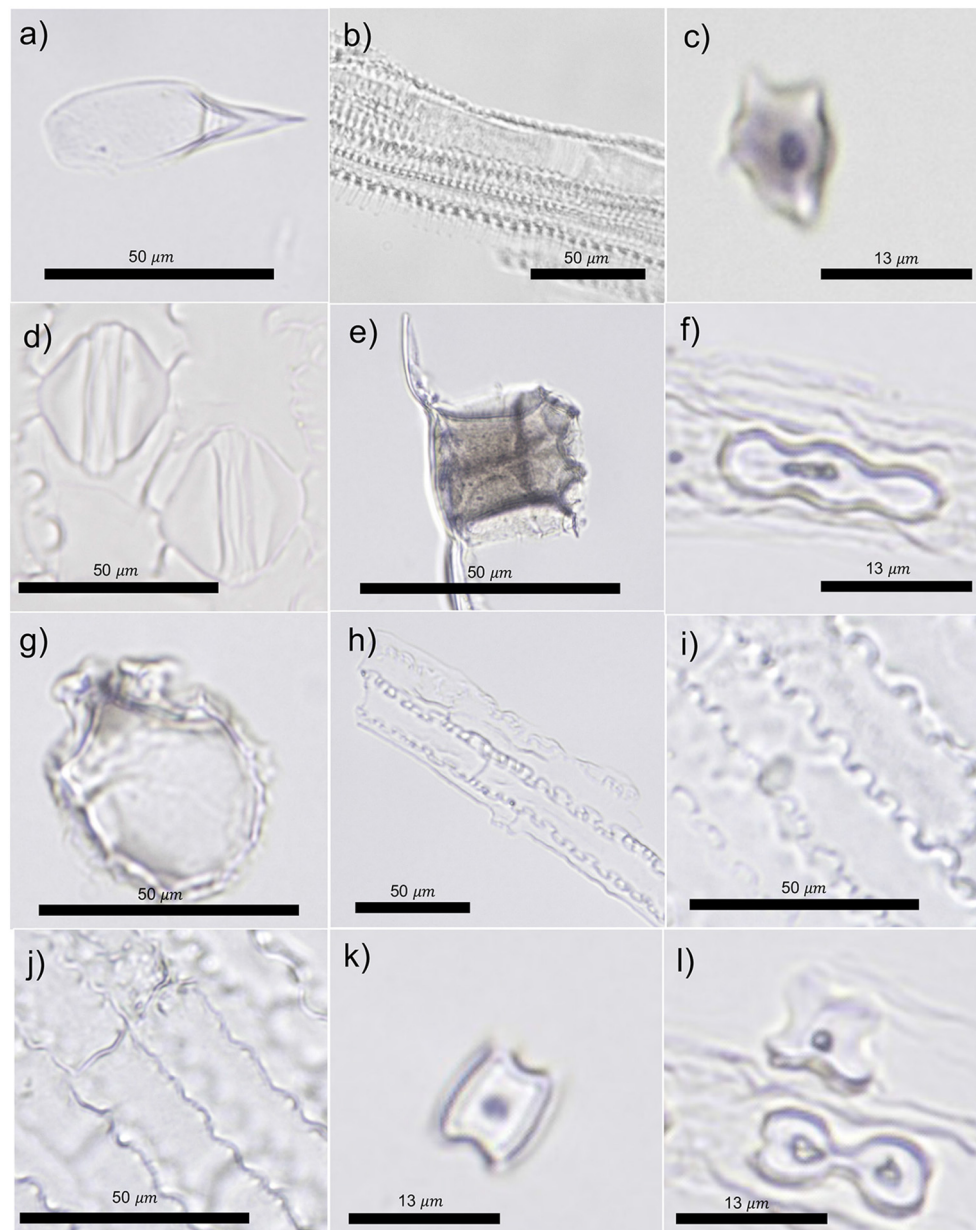
$$\text{Silica skeleton size ratio} = \frac{\text{number of cells in a silica skeleton}}{\text{total number of phytoliths}}$$

### 2.3.2. Archeological phytolith samples

A total of 16 contexts for each archeological site were sampled, giving a total of 64 phytolith assemblages. Phytoliths were extracted from sediment samples by following the procedure described in Lancelotti (2018) which is based on Madella et al. (1998). Phytoliths were identified through comparison with published material and a reference collection of phytoliths recovered from the leaves of local species (Lancelotti, 2010). A minimum of 350 single cell phytoliths were identified for each sample and silica skeletons were counted separately. The effect of taphonomy was tested using the methodology proposed by Madella and Lancelotti (2012). The phytolith assemblages were found to be representative and comparable with the modern ones (see Lancelotti, 2018). In order to obtain a dataset comparable with the experimental one, all morphotypes uniquely belonging to  $C_3$  species and all morphotypes produced in inflorescences were excluded from the archaeobotanical dataset. The remaining data include some redundant morphotypes that are present both in  $C_4$  and in  $C_3$ . Hence, the phytoliths selected are: elongate entire, elongate sinuate, bulliforms (both blocky and flabellate), stomata, and all the short cells (bilobates, crosses, polylobates, rondels, saddles) except for globulars. All silica skeletons formed by elongates entire and sinuate were also included for analysis. The full selection dataset used for this work is available in the Supplementary Information (File S1). Ubiquitous morphotypes were included because they are present in  $C_4$  and should they derive from  $C_3$  the analysis would not be compromised as this methodology has already been proved successful in  $C_3$  species.

## 2.4. Statistical analysis

Statistical analyses were performed with R (version 3.5.1) using standard functions of base, ggplot2 (version 3.3.5) (Wickham, 2016), vegan (version 2.5.6) (Oksanen et al., 2020) and MASS (version 7.3–



**Fig. 1.** Main phytolith morphotypes recovered from the leaf tissue of the three species finger millet, pearl millet and sorghum. Magnitude  $\times 400$  and  $\times 600$ . IPS: Inner Pericranial Surface. a) Acute bulbosus (finger millet) - IPS view; b) Tracheary anulate structures (finger millet) - IPS view; c) Rondel (pearl millet) with two spikes in the apex - side view; d) Two stomata (sorghum) in a silica skeleton - IPS view; e) Bulliform parallel (sorghum) attached to a silica skeleton - side view; f) Polylobate (pearl millet) in a silica skeleton - IPS view; g) Bulliform flabellate (finger millet) - side view; h) Silica skeleton of two elongates entire (pearl millet) - IPS view; i) Silica skeleton of elongates clavate (pearl millet) - IPS view; j) Silica skeleton of elongates crenate (sorghum) - IPS view; k) Saddle (finger millet) - side view; l) One cross (above) and one bilobate (below) in a silica skeleton (pearl millet) - IPS view.

51.5) (Ripley, 2022) packages. Scripts are available in Supplementary Information (File S3 and on GitHub [github.com](https://github.com)).

The tests conducted can be summarized in three steps: (1) phytolith assemblages of modern plants were tested by analyzing individual morphotypes separately to understand which morphotype is most efficient as a proxy for water availability; (2) concentration and ratios (sensitive/fix, skeleton size) were tested as proxies for water availability; (3) finally, once the model based on the phytolith assemblage of modern crops had been constructed, the archeological application was carried out.

(1) After calculating each morphotype concentration, generalized linear models (GLM) with Gaussian distribution (the dataset is composed of non-normal variables where the output is a continuous non-negative variable) were used to evaluate which explanatory variable

better predicts phytolith composition. Total phytolith and single morphotype concentration were tested as dependent variables while the treatment (WW, WS) and the species (finger millet, pearl millet and sorghum) were considered as independent variables. The p-value and the Akaike information criterion (AIC) were used to assess the validity of the models and to identify the best fitting model (Burnham et al., 2011).

(2) Total sample concentration, ratio of sensitive to fixed morphotypes and the silica skeleton size ratio were tested as possible proxies for water availability. The response variables (concentration and ratios) were normalized using natural logarithm to reduce skewness (Legendre and Legendre, 2012), then linear regressions were applied to model the relationship between phytolith ratios, concentration and transpiration, using ANOVA to test their significant

difference. In addition, GLMs were used to evaluate which explanatory environmental variable measured during the experiment (see D'Agostini et al., 2022) better predicts the phytolith concentration, the ratio of sensitive to fixed morphotypes or the silica skeleton size ratio. Although concentration and ratios were normalized, the GLM was still used as the remaining environmental variables are distributed according to a bimodal curve. The independent environmental variables tested were: total water transpired (sum of all the liters transpired by the plants during growth), transpiration efficiency (biomass/total water transpired), last liters of water transpired, genotypes and biomass.

(3) For comparing modern and archeological assemblages, we used phytolith percentage and not phytolith concentration. Although the concentration of phytoliths gives us a more precise measure of the accumulation of each morphotype in relation to the biomass analyzed, the percentage allows not only to compare the results with other studies, but above all, with the archeological assemblage, whose concentration does not correspond with the concentration of phytoliths derived from modern samples. To the percentages of the individual morphotypes, the ratio of sensitive to fixed phytoliths, which had yielded positive results in previous publications (Ermish and Boomgarden, 2022; Jenkins et al., 2020) was also added to the model. Logistic regressions were used to evaluate the chance that archeological samples grew in WW or WS conditions (Bruce et al., 2020). The dataset of modern crop phytoliths was trained by applying a stepwise selection to choose the best explanatory variables (phytolith morphotypes percentage and ratio sensitive/fixed morphotypes) to discriminate WW and WS treatment (dependent variable) in a GLM binomial model (Peduzzi et al., 1980).

### 3. Results

#### 3.1. Phytolith assemblage in modern species

The concentration of each morphotype is almost always higher in WW than in WS. Exceptions are polylobates, which have higher concentrations in WS for all the three species; and elongates clavate and bulliform flabellate, which have a higher concentration in WW only for pearl millet (Table 2). Sorghum is characterized by a high concentration of acute bulbous, bilobates, crosses, elongates clavate and stomata. Acute bulbous, crosses and elongates clavate have particularly high concentrations even in WS conditions. Finger millet is characterized by a high concentration of elongates dentate, entire and sinuate and saddles. Pearl millet has a high concentration of elongates clavate and entire and crosses, but compared to the remaining species, the concentrations of these three morphotypes are lower. Overall, morphotypes highlighted in previous studies as water sensitive (trichomes, bulliforms, stoma and elongates) are more abundant in WW conditions.

If we consider all three species together, water sensitive morphotypes (sum of bulliforms, stomata and elongates crenate, dentate, entire and sinuate) are predicted by the treatment (Table 3). Bulliforms are the morphotypes with the lower AIC and p-value. None of the fixed morphotypes (bilobates, crosses, polylobates, rondels, saddles) are predicted by the water treatments. However, species are good predictors of the morphotype, and the model demonstrates how different species produce morphotypes in different concentrations (Table 3). Sorghum is distinguished from the remaining species by the concentration of almost all morphotypes (apart from bulliforms flabellate, elongates crenate and sinuate). Concentration of elongates dentate and entire, rondels and saddles distinguish pearl millet from finger millet.

#### 3.2. Phytolith concentration and ratios

Total phytolith concentration between finger and pearl millet is not statistically different (p-adjusted value of 0.88), sorghum produces different (e.g. higher) concentrations of phytoliths if compared with finger millet and pearl millet (p-adjusted value of <0.05 in both cases). Sorghum shows to be a different phytolith producer in respect to the

millet, as highlighted also in Table 3, hence, it was decided to keep the three species separate with the aim of highlighting any possible difference among species and landraces.

#### 3.2.1. Concentration

Phytolith concentration is positively related to total water transpired in finger millet and pearl millet (Fig. 2). In sorghum the relationship between such variables is not statistically significant (p-value 0.33). The results of the ANOVA tests confirm the hypothesis that finger and pearl millets produced a higher and statistically different concentration of phytoliths in WW conditions both together (p-adjusted WW versus WS = 0.000378) or considered separately (Fig. 2), while in sorghum the two treatments are comparable (p-adjusted WW versus WS = 0.8304956). The GLM model, tested to understand which physiological parameter best explained the variability of the concentration of phytoliths identified the last transpiration value as the most significant explanatory variable (p-value = 0.00144), followed by the total water transpired (p-value 0.00359), and the transpiration efficiency (p-value 0.97779).

The variance for phytolith concentration within genotypes in sorghum is very high:  $\sigma^2$  is 196.511; while it is only 64.83 for pearl millet and 104.40 for finger millet. Some of the landraces appeared to be sensitive to the water treatment (S2, S5, S6) but with a very irregular response: in S5 phytolith concentration is higher in WS conditions while in S2 and S6 concentration is higher in WW conditions (Fig. 3).

#### 3.2.2. Sensitive to fixed morphotypes

The ratio of sensitive to fixed morphotypes is not related to total water transpired in any of the species (Fig. 4). The GLM model tested to understand which physiological parameter best explained the variability of the ratio of sensitive to fixed morphotypes identified the last transpiration value as the most significant explanatory variable (p-value 0.0305).

Sorghum varieties are less variable than the pearl millet and finger millet:  $\sigma^2$  of sorghum is 0.1439, 0.3360 for pearl millet and 0.4833 for finger millet. Most of the sorghum landraces (S2, S5, S6, S8) separate the two treatments (but not significantly) and only in S5 and in S9 the ratio has a higher value in WW conditions (Fig. 5).

#### 3.2.3. Silica skeleton dimensions

Silica skeleton size ratio (violin boxplots available in the Supplementary Information-Fig. S1) do not seem to relate to water availability in any of the species. The variability of the ratio is particularly high, especially in finger millet, where is higher than the variability within the treatments:  $\sigma^2$  of the three species in WW is 0.0068 and in WS is 0.0063;  $\sigma^2$  of sorghum replicas in WW is 0.00038 and in WS is 0.00157;  $\sigma^2$  of pearl millet replicas in WW is 0.00080 and in WS is 0.00165;  $\sigma^2$  of finger millet replicas in WW is 0.0088 and in WS is 0.00783.

#### 3.3. Comparison with the archeological samples

Fig. 6 shows the results of the stepwise model (stepwise AIC = 84.51 while full model AIC = 95.87) where the best explanatory variables (blockies, stomata and polylobates) chosen by the automatic procedure have been used as predictors to evaluate the possibility that the archeological phytolith assemblage derives from WW crops (1 means WW, 0 means WS). The probability that most of the samples derived from WW plants is around 40%, meaning that there is a 60–70% chance that they represent plants grown in water-stress conditions.

### 4. Discussion

We constructed a methodological framework to test whether different leaf phytolith indices (phytolith concentration, morphotypes concentration, and sensitive/fixed ratio), produced in a group of C<sub>4</sub>

**Table 2**

Mean and standard deviation of single morphotype concentration for WW and WS on the overall assemblage and for each species considered separately. The numbers underlined in gray correspond to the samples where the concentration increases in WS conditions, while all the remaining morphotypes have a higher concentration under well-watered conditions.

<b>Table 2 Morphotype</b>	<b>Well-watered (per g of dry leaf in millions)</b>	<b>Water-stress (per g of dry leaf in millions)</b>
<b>Phytolith concentration</b>	<b>21.6754 ± 13.8624</b>	<b>17.0006 ± 14.8692</b>
<b>Acute bulbosus</b>	<b>0.8243 ± 0.7500</b>	<b>0.7270 ± 0.8447</b>
Sorghum	1.3838 ± 0.9430	1.5061 ± 0.9292
Pearl millet	0.5579 ± 0.4276	0.3674 ± 0.3849
Finger millet	0.4726 ± 0.0847	0.2025 ± 0.4209
<b>Bilobates</b>	<b>0.5964 ± 0.8471</b>	<b>0.4687 ± 0.5322</b>
Sorghum	1.0723 ± 1.0455	0.8660 ± 0.6120
Pearl millet	0.6059 ± 0.7094	0.4304 ± 0.3442
Finger millet	0.0139 ± 0.0297	0.0200 ± 0.0286
<b>Blockies</b>	<b>0.1220 ± 0.1946</b>	<b>0.0481 ± 0.1110</b>
Sorghum	0.1826 ± 0.1645	0.1229 ± 0.2138
Pearl millet	0.1037 ± 0.0140	0.0073 ± 0.2337
Finger millet	0.0713 ± 0.0111	0.0058 ± 0.2138
<b>Bulliforms flabellate</b>	<b>0.0244 ± 0.0561</b>	<b>0.0085 ± 0.0168</b>
Sorghum	0.0432 ± 0.0854	0.0139 ± 0.0233
Pearl millet	0	<u>0.0053 ± 0.0114</u>
Finger millet	0.0310 ± 0.0366	0.0058 ± 0.0128
<b>Crosses</b>	<b>1.4729 ± 1.7005</b>	<b>1.3242 ± 1.5292</b>
Sorghum	2.6574 ± 1.2617	2.3420 ± 1.7253
Pearl millet	1.4714 ± 1.9281	1.3144 ± 1.2134
Finger millet	0.0531 ± 0.0605	0.0640 ± 0.0664
<b>Elongates clavate</b>	<b>6.7093 ± 10.2841</b>	<b>7.5099 ± 11.1163</b>
Sorghum	16.4743 ± 11.1091	<u>19.0966 ± 11.4680</u>
Pearl millet	2.4956 ± 5.4887	1.7735 ± 2.4207
Finger millet	0.0476 ± 0.1131	<u>0.1969 ± 0.3141</u>
<b>Elongates crenate</b>	<b>0.1825 ± 0.3015</b>	<b>0.0633 ± 0.1488</b>
Sorghum	0.1789 ± 0.3710	0.0213 ± 0.0453
Pearl millet	0.1757 ± 0.2515	0.0584 ± 0.1396
Finger millet	0.1949 ± 0.2960	0.1218 ± 0.2264
<b>Elongates dentate</b>	<b>0.8560 ± 0.9326</b>	<b>0.3247 ± 0.3838</b>
Sorghum	0.5884 ± 0.5830	0.1010 ± 0.1175
Pearl millet	0.5154 ± 0.6362	0.4189 ± 0.4732
Finger millet	1.5859 ± 1.1987	0.4864 ± 0.3853
<b>Elongates entire</b>	<b>2.8426 ± 3.4397</b>	<b>1.1869 ± 0.9328</b>
Sorghum	0.7267 ± 0.9134	0.4978 ± 0.3438
Pearl millet	2.5596 ± 1.2667	1.5309 ± 0.8375
Finger millet	5.7213 ± 5.0119	1.6184 ± 1.1145
<b>Elongates sinuate</b>	<b>6.6661 ± 5.1782</b>	<b>4.5050 ± 3.9180</b>
Sorghum	7.2338 ± 6.4446	6.8265 ± 4.6219
Pearl millet	5.7275 ± 2.9102	3.6539 ± 3.6949
Finger millet	7.1113 ± 5.9480	2.6670 ± 1.2635
<b>Polylobates</b>	<b>0.0405 ± 0.0705</b>	<b>0.0738 ± 0.1050</b>
Sorghum	0.0663 ± 0.0863	0.1410 ± 0.1462
Pearl millet	0.0484 ± 0.0721	<u>0.0615 ± 0.0463</u>
Finger millet	0	<u>0.0053 ± 0.0151</u>
<b>Rondels</b>	<b>0.2041 ± 0.2855</b>	<b>0.1218 ± 0.1723</b>
Sorghum	0.0367 ± 0.0824	0.0070 ± 0.0153
Pearl millet	0.1932 ± 0.3149	0.0916 ± 0.0980
Finger millet	0.4182 ± 0.2846	0.3032 ± 0.2099
<b>Saddles</b>	<b>0.4174 ± 0.7264</b>	<b>0.2399 ± 0.5277</b>
Sorghum	0.0093 ± 0.0323	0
Pearl millet	0.0936 ± 0.2194	0.0146 ± 0.0340
Finger millet	1.2955 ± 0.8101	0.8215 ± 0.7291
<b>Stomata</b>	<b>0.7478 ± 0.7588</b>	<b>0.3870 ± 0.5224</b>
Sorghum	1.3474 ± 0.9246	0.8473 ± 0.6679
Pearl millet	0.3951 ± 0.3082	0.1408 ± 0.0639
Finger millet	0.4517 ± 0.4560	0.1194 ± 0.0537

**Table 3**

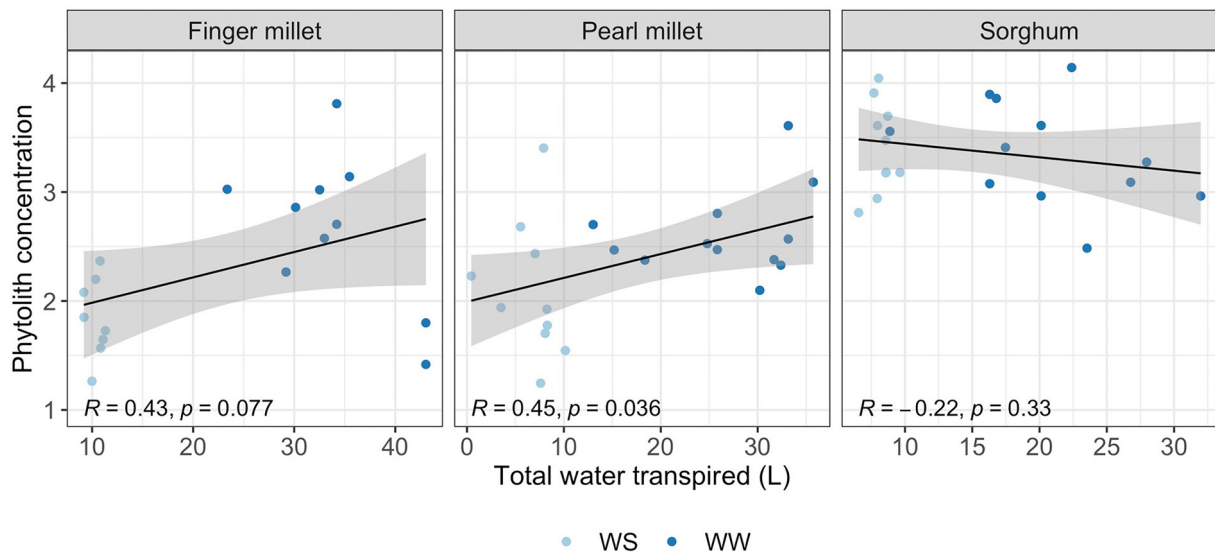
p-value and Akaike information criterion (AIC) of the gaussian generalized linear models tested using treatment (WW-WS), species (sorghum, pearl millet and finger millet) as the independent variables (x = TREATMENT; x = SPECIES). Light gray cells show the almost significant values; dark gray cells highlight statistically significant results (p-value <0.05 and low AIC values) i.e. those morphotypes whose concentration is predicted by the independent variable (or treatment or species). In the case of the species, the p-value <0.05 reports when the PM or S proved to be statistically different from the FM by concentration of the morphotype.

Table 3	Morphotype	x = TREATMENT		x = SPECIES (FM VS PM and S)	
<i>SENSITIVE FORMS</i>	<i>Acute bulbosus</i>	p-value AIC	0.633 151.32	PM p-value S p-value AIC	0.5520 9.47e-07 122.54
	<i>Bulliforms (sum of blockies + flabellate)</i>	p-value AIC	0.0555 -32.75	PM p-value S p-value AIC	0.9962 0.0323 -33.759
	<i>Blockies</i>	p-value AIC	0.0798 -45.441	PM p-value S p-value AIC	0.7292 0.0298 -46.304
	<i>Bulliforms flabellate</i>	p-value AIC	0.156 -209.72	PM p-value S p-value AIC	0.2034 0.4579 -210.36
	<i>Elongates (sum of all elongates)</i>	p-value AIC	0.219 483.58	PM p-value S p-value AIC	0.787 1.08e-06 452.1
	<i>Elongates clavate</i>	p-value AIC	0.769688 473.44	PM p-value S p-value AIC	0.366 1.25e-10 423.77
	<i>Elongates crenate</i>	p-value AIC	0.0613 5.4846	PM p-value S p-value AIC	0.62129 0.49623 10.621
	<i>Elongates dentate</i>	p-value AIC	0.00648 142.25	PM p-value S p-value AIC	0.00868 0.00242 141.03
	<i>Elongates entire</i>	p-value AIC	0.0164 299.66	PM p-value S p-value AIC	0.0231 8.2e-05 291.23
	<i>Elongates sinuate</i>	p-value AIC	0.0738 370.58	PM p-value S p-value AIC	0.815 0.206 372.94
	<i>Stomata</i>	p-value AIC	0.03707 128.96	PM p-value S p-value AIC	0.8913 2.52e-05 109.31
<i>FIXED FORMS</i>	<i>Bilobates</i>	p-value AIC	0.49124 139.63	PM p-value S p-value AIC	0.0117 7.54e-06 120.86
	<i>Crosses</i>	p-value AIC	0.721 240.17	PM p-value S p-value AIC	0.00187 1.51e-07 213.08
	<i>Polylobates</i>	p-value AIC	0.141 -121.8	PM p-value S p-value AIC	0.047157 0.000326 55.292
	<i>Rondels</i>	p-value AIC	0.18656 3.6088	PM p-value S p-value AIC	0.00112 1.49e-06 -17.341
	<i>Saddles</i>	p-value AIC	0.2851 125.48	PM p-value S p-value AIC	5.40e-10 1.23e-10 77.93

species with a key role in prehistoric agriculture in arid areas, can be used as a proxy for plant water availability. To this end, we analyzed archeological phytolith assemblages from distinct Indus Valley sites in order to understand the water practices habits according to

which available C<sub>4</sub> crops grew. The results of the study will therefore be discussed starting from the outcomes on the experimental plants and then analyzing the extent to which they contributed to the archaeology of the Indus Valley.





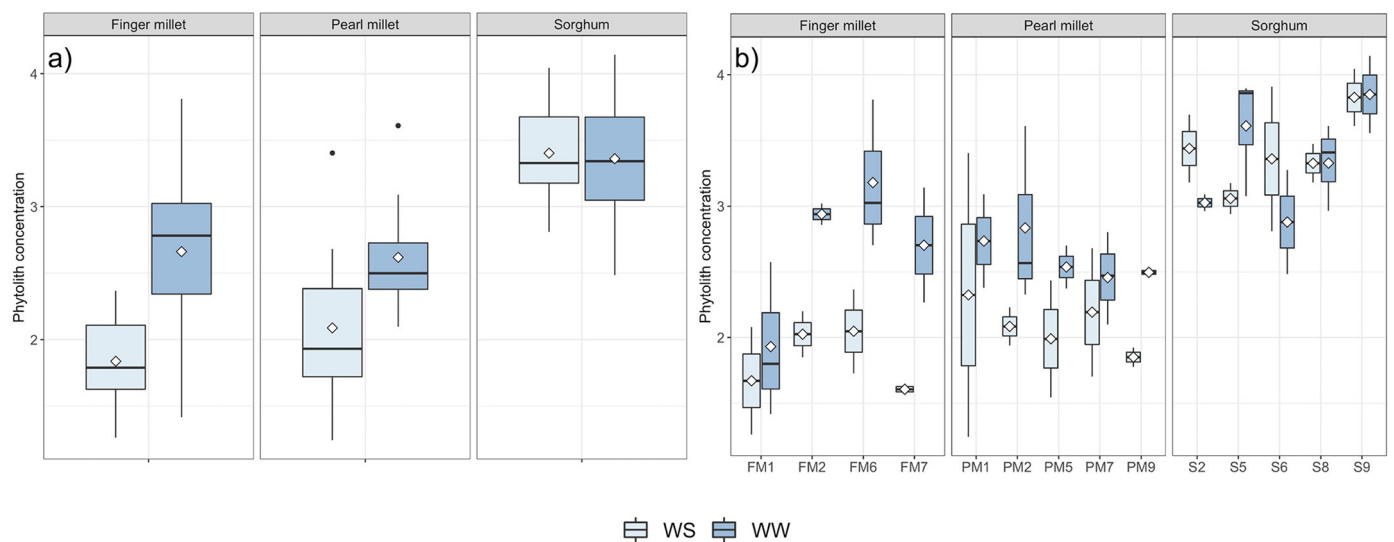
**Fig. 2.** Linear regression for phytolith concentration (n° of cells per gram of dry leaf in millions) tested on finger millets, pearl millet and sorghum. Total water transpired (L) is used as an independent variable, and phytolith concentration extracted from leaves normalized with natural logarithm as dependent variable. Gray bands represent 95% of confidence intervals.

4.1. Modern phytolith assemblage

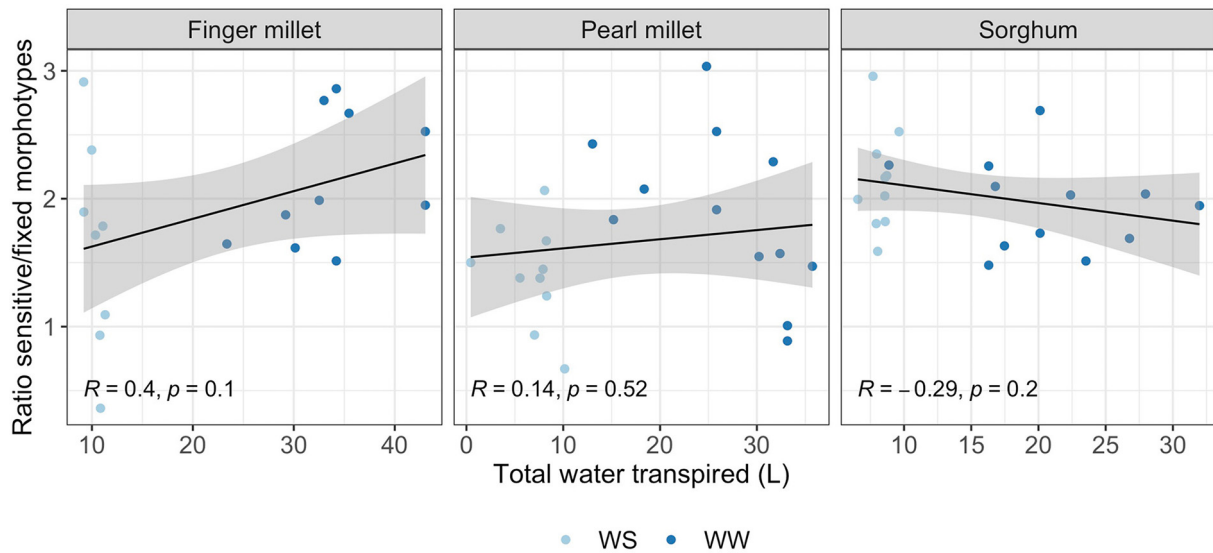
The results from the experimental phytolith assemblage indicate that the concentration values of several morphotypes can be predicted by both the treatments and the species, indicating that phytoliths can be used as proxies for water availability in millet species. The concentration of most morphotypes is higher in well-watered conditions (Table 2), supporting the hypothesis by Schulz-Kornas et al. (2017) from which when there is an increase in silica supply plants produce more phytoliths instead of bigger and/or heavier ones (Schulz-Kornas et al., 2017). In addition, most of the sensitive morphotype concentrations are well predicted by the treatments (Table 3). These outcomes support previous results in different species (Ermish and Boomgarden, 2022; Jenkins et al., 2016, 2020; Madella et al., 2009). Silica skeleton size, however, did not show any variation related to plant water availability and therefore might not be a reliable proxy for water availability in the studied landraces. Only sorghum seems to produce silica skeletons of larger

dimension, suggesting that high silica accumulators, as in the case of sorghum, have a bigger chance to produce conjoined silica cell structures.

Nonetheless, phytolith production exhibits high variability among and within the species which differ both in the morphotypes produced and in Silicon accumulation trends (Table 3, Figs. 2, 3, 4, 5). On the one hand, phytolith composition assemblages are discriminating the three species: sorghum is characterized by high concentrations of polylobates and crosses, especially in WS conditions, and by an almost total absence of saddles and rondels, supporting results in experiments by Novello and Barboni (2015) and Jenkins et al. (2020). Saddles and elongates are produced in high numbers in the leaves of finger millet. Bilobates, polylobates and crosses are almost absent in pearl and finger millets. These results align with previous studies that indicate that short-cell could differentiate Pooideae from Chloridoideae while bulliforms and hairs are more generic and redundant morphotypes (Barboni and Bremond, 2009; Neumann et al., 2017).



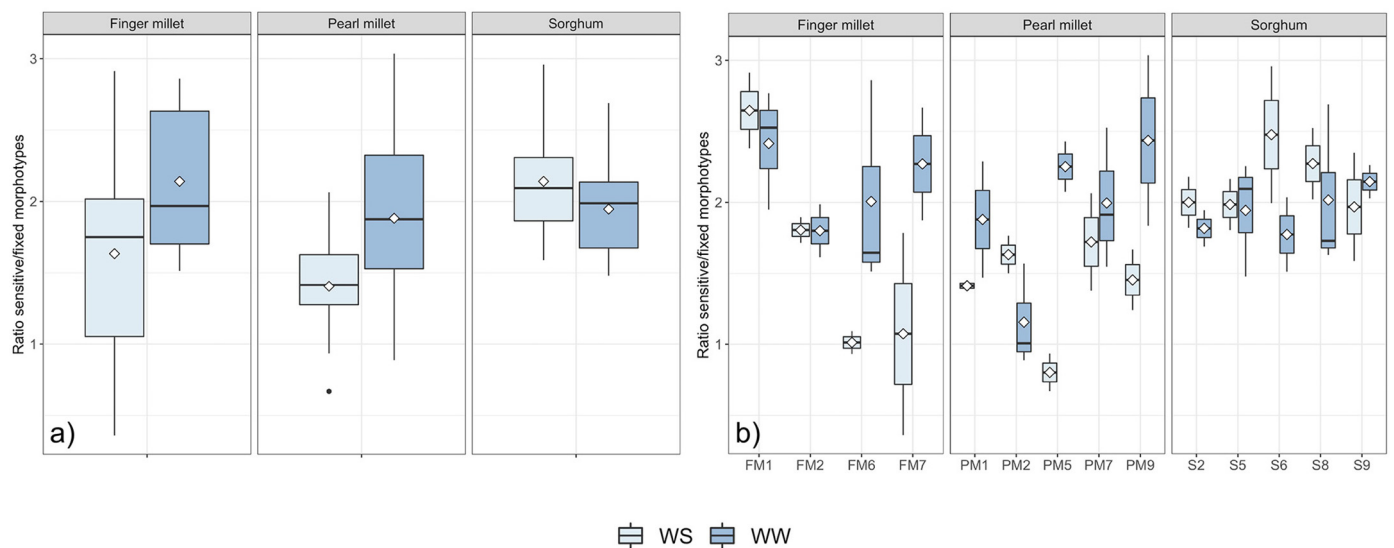
**Fig. 3.** Boxplots of phytolith concentration (n° of cells per gram of dry leaf in millions) by water treatments sorted for a) species and b) landraces. Horizontal bar: median, white diamond: mean, black spot: outliers.



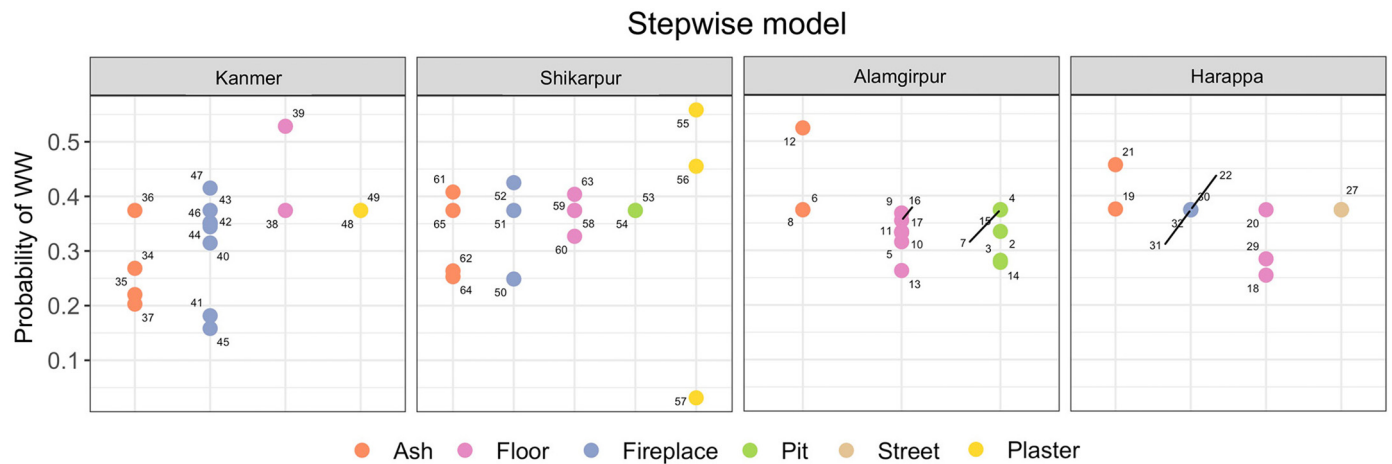
**Fig. 4.** Linear regression for the sensitive to fixed phytoliths ratio tested on finger millet, pearl millet and sorghum. Total water transpired (L) is used as an explanatory variable, and the ratio, normalized using natural logarithm, as dependent variable. Gray bands represent 95% of confidence intervals.

On the other hand, the accumulation of phytoliths seems to follow different trends. Sorghum is the species that not only produced the highest concentration of phytoliths (more than double if compared with the other two species) but also produced more sensitive morphotypes, especially in WS conditions, with low variability among landraces. Neither phytolith concentration nor sensitive morphotype production alone are good predictors of the water availability in sorghum. We can assume that phytoliths in sorghum play a key role in implementing the fitness of the species, being their accumulation constant and independent of treatment, as also suggested by Katz (2019). The beneficial effects of Si in plants are well documented for *C<sub>3</sub>* species such as *Triticum durum* L. (Meunier et al., 2017) or *Triticum aestivum* L. (Daoud et al., 2018). Studies conducted on *Sorghum bicolor* (L.) Moench (Hattori et al., 2005) and *Zea mays* L. Sp. Pl. (Liang et al., 2005) demonstrated that Silicon could enhance drought tolerance even in crops well adapted to survive stress conditions. It is known from literature that epidermal phytoliths can play a role in giving a structural support to the

tissues (e.g. elongates) (Meunier et al., 2017; Rodrigues et al., 2003); they can contribute to the osmotic adjustment and the biosynthetic mechanisms (e.g. stomata) (Cooke and Leishman, 2016; Goto et al., 2003; Hosseini et al., 2017); and/or influence the mineral balance by protecting tissues from toxic elements and insect/fungi attack (Fauteux et al., 2005; Mateos-Naranjo et al., 2013; Oliva et al., 2021). Thus, as phytoliths can play a key role in implementing plant physiology response to biotic and abiotic stresses we can assume that phytolith production can be regulated and stimulated. On this consideration we can presume that in sorghum, silicification of elongates, trichomes, stomas, bulliforms and crosses can be genetically controlled. Nevertheless, we also observed a surprising variability for phytolith concentration among the sorghum genotypes, with some positively and some negatively related to water availability (Fig. 3). Available literature indicates that the wide expression profile of Lsi1 and Lsi2 transporter proteins and their regulation can be related to different absorption rates of monosilicic acid (Coskun et al., 2021). Furthermore, the presence of



**Fig. 5.** Boxplot of the ratio sensitive to fixed morphotypes by water treatments sorted for a) species, and b) landraces. Horizontal bar = median, white diamond = mean, black spot = outliers.



**Fig. 6.** Plot of the probability of each archeological phytolith sample to be derived from a well watered crop-phytolith assemblage. Each dot is labeled with the number corresponding to the archeological sample.

mutants unable to absorb high quantities of silica in sorghum has been suggested by Markovich et al. (2019). We then suggest that the high variability in phytolith production in sorghum is probably in connection to such genetic features more than on the environmental factors. Notwithstanding, sorghum phytolith assemblages contributed to obtaining statistically significant results to discriminate treatments. Specifically, although taken separately sorghum seems to show no correlation between watering and phytolith production, the total database comprising the three millets (including sorghum) shows that phytolith production can be correlated with watering and that robust predictive models can be created with them. Sorghum was not removed from the model both because there was evidence that sorghum was present in the archeological record at the sites of interest (through independent proxies e.g. seeds) and because there was an intention to produce an inclusive model, containing phytoliths of as many species as possible, in order to approximate the archeological assemblage. Indeed, it is often impossible to reach full identification to genus/species level in archeological assemblages and these can be formed by plants that are more responsive (such as finger millet and pearl millet in our study) and plants that are less responsive (such as sorghum in our study). Thus, including sorghum in the model makes it stronger when applied to an unknown archeological dataset. In finger millet and pearl millet, the concentration of phytoliths is actually positively related to watering, as was also discussed in our previous work regarding biosilica accumulation, where we hypothesize a biosilica accumulation driven by mostly transpiration (D'Agostini et al., 2022). The ratio of sensitive/fixed morphotypes does not seem to show positive relationships for millets, underlining a great variability between landraces (Fig. 5) in the relationship between the different morphotype classes. We can therefore conclude that fixed morphotypes are useful to distinguish the three species; phytolith concentration and total composition predicts the treatment while sensitive/fixed morphotype ratio is not a good proxy for water availability. We can deduce that since the biosilica accumulation trends are different for each species and since the production of fixed shapes is not constant between species, a common ratio for the three millet is not efficient in highlighting environmental changes in a mixed dataset. The full assemblage, on the other hand, which includes all morphotypes, allows for a model sensitive to water changes.

#### 4.2. Archeological assemblage and interpretation

Results from the model applied to the archeological phytolith dataset indicate that most of the cereals grew in water scarcity conditions (Fig. 6). All sites gave the same result without any particular differences, despite the fact that the water and climatic conditions are somewhat different

among settlements. Harappa and Alamgirpur were possibly located near watercourses, with extensive *doabs* where it was very easy for agricultural practices to take place, perhaps aided even by irrigation systems (Kenoyer, 1998). In contrast, Kanmer and Shikarpur stood most likely on drier sites with no alluvial plains to exploit for agricultural purposes (Lancelotti, 2010). In spite of these differences, and considering that the presence of domesticated  $C_4$  cereals has been reported at each of these sites (Harappa: *Panicum* L. Sp. Pl.; Kanmer: *Pennisetum galucum* e *Setaria* Sp. P. Beauv.; Shikarpur: *Brachiaria ramosa* L. Stapf., *Coix lacryma-jobi* L., *Digitaria* Sp. Haller, *Eleusine coracana*, *Setaria* Sp. P. Beauv.; Alamgirpur: small millets in general) (Bates, 2019; Bates et al., 2021) the results obtained in this study seem to emphasize that, at these sites, cereals  $C_4$  grew under a low water regime. Several hypotheses can explain this result: in areas with ample water availability (e.g. Harappa and Alamgirpur) there could be a system of double cropping (*rabi* and *khari*) with  $C_3$  cereals (e.g. wheat and barley or rice) and vegetables grown under a water regime while others species more resistant to drought, such as  $C_4$  employed during the hot and less rainy period (Petrie et al., 2016). This is supported by ethnographic evidence from the area, where millet crops are mostly rain-fed (Giosan et al., 2012). On the other hand, it is also possible that more drought-tolerant resistant cereals were cultivated alongside  $C_3$  throughout the year, but were used in drier areas of the settlement, growing in a controlled or uncontrolled manner. The results of the model also do not rule out the hypothesis that the area included between southern Pakistan and northwestern India has experienced some form of drying and that the cultures have therefore adapted to more consistent dry periods over time, as suggested by Wright et al. (2008) (Wright et al., 2008). The authors suggested that after a millennium of riverine agriculture based on the overbank flooding, around 2800–2500 BCE, the rivers failed to deliver the usual balance of water and forced farmers to base their agricultural system on the rainfall. The same study suggests that around 2000 BCE the reduction of rainfall could lead to a minimum mean peak of 240 mm. All this information matches with the results obtained in this study, which hypothesized the possible presence of  $C_4$  cereals grown in water stressed conditions with less than 300 mm of available water. Whether the adoption of millets was due to a question of progressive aridification, or whether there was an opportunistic cultivation of  $C_4$  to exploit drier areas or that  $C_4$  grew wild in arid parcels of the sites, what emerges from these findings is that most likely  $C_4$  grew in water scarcity conditions.

#### 5. Conclusion

Firstly, we constructed a methodological framework using phytolith assemblage to observe changes in water availability. Albeit the

miscellaneous assemblage of landraces highlighted different trends in phytolith production, the complete dataset allows us to make predictions on the water availability with a good fitting. Even if sorghum, in all likelihood, is an active biosilica accumulator which does not respond only to water availability for phytolith production, it contributed to construct a solid database for archeological comparison if considered together with the other millets. Bulliforms and stomata, specific sensitive morphotypes are well predicted by the treatments and gave sensible results when tested as explanatory variables in the archeological dataset. Fixed morphotypes on the contrary are better associated with the species. These findings confirm the validity of leaf phytoliths to identify past water availability for the three species under study when considered together. Nevertheless, further experimental cultivations have been set to assess whether phytolith production are influenced by different growing conditions, where environmental variables as relative humidity, soil composition and silica availability, light intensity and exposition could lead to a variation on sorghum, pearl millet and finger millet biomass production and transpiration rate which in turn could influence phytolith production. Secondly, we aim to answer an important archeological question about water management in the Indus Valley. Through the study of the dataset of phytoliths from the four archeological sites Harappa, Kanmer, Shikarpur and Alamgirpur, we can hypothesize the presence of millets grown under water stress regimes, leaving open the hypothesis of double cropping/opportunistic management, where millets were possibly planted in marginal areas with little access to water. The hypothesis would be best confirmed with the addition of alternative proxies such as isotopes or the study of macro remains. However, these results mark a preliminary finding that may open up new theories on millet consumptions in the Indus Valley.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.revpalbo.2022.104783>.

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