



Can *Triticum urartu* (Poaceae) be identified by pollen analysis? Implications for detecting the ancestor of the extinct two-grained einkorn-like wheat

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The domestication of the one-grained einkorn (*Triticum monococcum*) in the Near East is relatively well known. However, an independent two-grained einkorn-like domestication has been archaeobotanically detected and scarce information is available. *Triticum urartu*, a wild wheat, was not fully described until the 1970s because the phenology does not allow it to be distinguished easily from wild einkorn (*Triticum boeoticum* subsp. *thaoudar*), although a genetic separation exists. Both species are mostly two grained and could potentially be the relatives of the extinct two-grained form. Pollen grains of several genetically well-identified wheat species, including *T. urartu* and *T. boeoticum* subsp. *thaoudar*, were studied by measuring the grain diameter and examining the exine sculpturing with phase-contrast microscopy and scanning electron microscopy to gain an insight into differences enabling taxonomic identification. This work showed that, although *T. urartu* pollen is smaller on average, grain diameter is not sufficient because of the size overlap between the species, but *T. urartu* presents a different exine sculpturing (scabrate) from other *Triticum* spp. (aerolate). This outcome is useful for taxonomists and archaeobotanists. First, it will allow a simple re-classification of herbarium materials. Second, further research could establish whether *T. urartu* was cultivated. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, 177, 278–289.

ADDITIONAL KEYWORDS: cultivation – exine pattern – Fertile Crescent – palynology – phase-contrast microscopy – scanning electron microscopy (SEM).

INTRODUCTION

The early Neolithic is one of the most fascinating milestones of human history, as it marks the transition from an economy based on predation to one based on farming. Botanical evidence has shown that the wild ancestors of most Neolithic crops, including the wheat species einkorn (*Triticum monococcum* L.) and

emmer (*T. dicoccum* Schrank), barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), lentils (*Lens culinaris* Medik.) and bitter vetch (*Vicia ervilia* Willd.), were constrained to a ‘core area’ in the Fertile Crescent in present-day south-eastern Turkey/northern Syria (Harlan & Zohary, 1966; Lev-Yadun, Gopher & Abbo, 2000; Nesbitt, 2002) (Fig. 1).

Genetic studies have provided valuable information on which wild cereal forms are the relatives of the modern domesticated ones and which geographical

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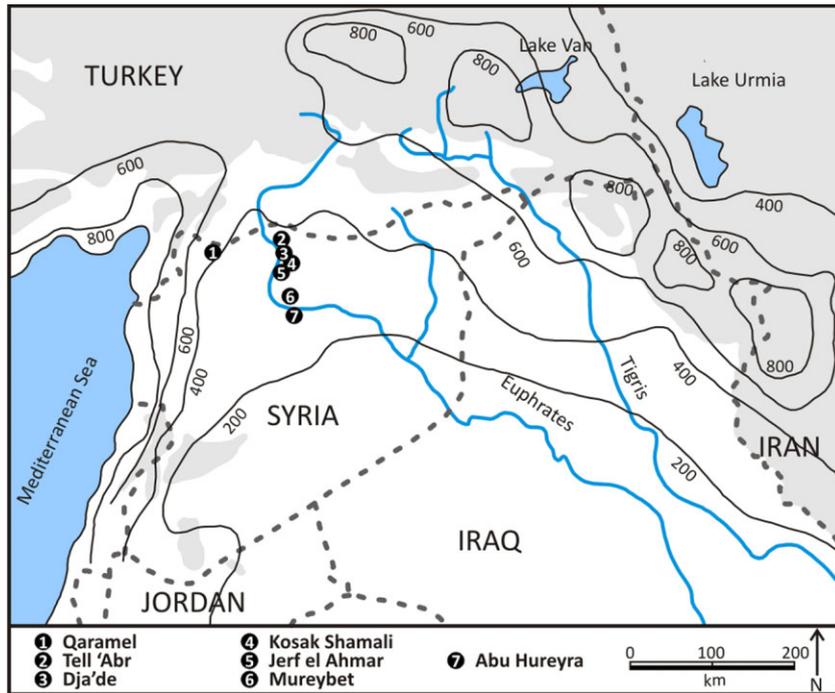


Figure 1. Map showing the location of the archaeological sites cited in the text. Lines present annual precipitation in millimetres (after Evans, Smith & Oglesby, 2004) and grey areas indicate altitude > 1000 m a.s.l.

wild plant stands produced the domesticated forms that are still used in food production [e.g. Heun *et al.*, 1997 for einkorn; Özkan *et al.*, 2002 for emmer and hard wheat (*Triticum durum* Desf.)]. From genetic studies, the Fertile Crescent seems to be the likely focal point of the dawn of agriculture with a rapid transition from cultivation to domestication (e.g. Salamini *et al.*, 2002; Honne & Heun, 2009; Abbo, Lev-Yadun & Gopher, 2010a; Haldorsen *et al.*, 2011; Heun *et al.*, 2012). However, this has been questioned by several archaeobotanical studies, which support a multi-regional protracted model (e.g. Willcox, 2005, 2013; Tanno & Willcox, 2006; Weiss, Kislev & Hartmann, 2006; Fuller, Willcox & Allaby, 2012).

A discussion on whether or not the Younger Dryas cold/dry period had an influence on the start of agriculture is also taking place (e.g. Hillman *et al.*, 2001; Willcox, Buxo & Herveux, 2009; Abbo, Lev-Yadun & Gopher, 2010b; Haldorsen *et al.*, 2011). Consequently, as a result of the difficulties in outlining a general history of cultivation and domestication, it has been recommended to focus in detail on single crops before thinking about general patterns (Haldorsen *et al.*, 2011; Heun *et al.*, 2012).

One case of domestication is that of einkorn (*Triticum monococcum* L. subsp. *monococcum*), a one-grained diploid wheat species that may have been the first cereal to be domesticated at the beginning of the

Holocene (Heun *et al.*, 1997; Salamini *et al.*, 2002; Haldorsen *et al.*, 2011). Although einkorn was important in the Neolithic culture in the Fertile Crescent during the early Holocene, its agricultural importance was limited during the second half of the Holocene, and today it is a relic crop planted in scattered locations (Oliveira *et al.*, 2011). Genetic research has demonstrated that *T. monococcum* subsp. *monococcum* is closely related to the two-grained wild einkorn [*T. boeoticum* Boiss. subsp. *thaoudar* (Reut. ex Hausskn.) E. Schiem.] from the Karacadağ Mountains in south-eastern Turkey (Heun *et al.*, 1997). In some papers, this wild relative is incorrectly attributed to the one-grained *T. boeoticum* subsp. *aegilopoides* (Link) Grossh. (e.g. Willcox, 2005; Fuller, 2007), which is a feral form of domesticated one-grained einkorn occurring in disturbed habitats in the southern Balkans and eastern Turkey (Schiemann, 1948; Heun *et al.*, 1997; Heun, Haldorsen & Vollan, 2008).

Domesticated einkorn is mostly one grained, but a domesticated two-grained einkorn-like form has also been found since the Neolithic in Syria, Turkey and Europe (see Kreuz & Boenke, 2002), although it seems to have become extinct. Whether this was the result of the same domestication process for einkorn or whether another species could have been involved is unresolved. One factor that complicates the discussion about where, how and when einkorn was first

cultivated is the occurrence of another wild einkorn-like wheat, *T. urartu* Thumanjan ex Gandilyan, which may have played a role in early Neolithic cultures (Heun *et al.*, 2008). Wild einkorn (*T. boeoticum* subsp. *thaoudar*) and urartu wheat (*T. urartu*) are phenotypically almost identical, although not interfertile (Johnson & Dhaliwal, 1976). *Triticum urartu* was first properly described by Gandilyan (1972) and was thought to be endemic to Armenia. The much wider distribution of *T. urartu* in areas covered by wild einkorn became later known through the publication of Johnson (1975), who collected and identified 1450 accessions of the two species and performed protein marker comparisons, which showed that the two wild wheat species are different in terms of the analysed seed proteins; this was later confirmed by DNA analyses (e.g. Heun *et al.*, 2008; plant material used here, see below). Because of the relatively recent description of *T. urartu* and the comparatively small differences between the two plants, pre-1970s species determination of herbarium material cannot be trusted. Moreover, because the small differences are not maintained in archaeobotanical remains of kernels and rachis fragments, *T. urartu* is not mentioned in pre-1970s archaeobotanical documents. Consequently, as it has been impossible to distinguish between these two wheat species collected from archaeological sites (Hillman *et al.*, 1993; Nesbitt, 2001), the two species are commonly merged into one group (Fuller, 2007; Willcox, Fornite & Herveux, 2008; Willcox *et al.*, 2009), although the discussions in those publications still mainly concern einkorn alone. Hence, it is difficult to know whether conclusions about early einkorn cultivation are correct when the studied material may consist of wild einkorn, a mixture of wild einkorn and *T. urartu* or *T. urartu* alone (Heun *et al.*, 2008). The confusion also makes it difficult to judge the importance of *T. urartu* in Neolithic cultures.

The issues discussed above need to be resolved. The distinction between einkorn and *T. urartu* has not been achieved through archaeobotanical methods as the kernels from the two species have not been distinguished so far. However, ancient DNA studies, although with problems as a result of DNA degradation in charred seeds, have the potential to provide the most reliable information to resolve this issue, but have not been made so far. Fortunately, another part of the plant is commonly preserved, namely its pollen. Although einkorn pollen was described long ago (Andersen & Bertelsen, 1972; Rajendra *et al.*, 1978), the pollen of *T. urartu* has not been described so far. Therefore, a palynological study of well-characterized material (verified by Heun *et al.*, 2008) of several wheat species (*T. monococcum* subsp. *monococcum*, *T. monococcum* subsp. *sinskajae* Filat. & Kurk.,

T. boeoticum subsp. *aegilopoides*, *T. boeoticum* subsp. *thaoudar* and *T. urartu*) was carried out to determine whether palynological features could be a reliable tool to distinguish between them. The results could be applied in the future: (1) to clarify pre-1970s herbarium material identifications; and (2) to establish whether *T. urartu* was cultivated by studying archaeological contexts.

MATERIAL AND METHODS

Eighteen wheat accessions with well-established genotypes [analysed with amplified fragment length polymorphisms (AFLPs), see Heun *et al.*, 2008] of urartu, wild einkorn and domesticated einkorn subspecies were grown in glasshouses at the Norwegian University of Life Sciences (NMBU) (Table 1). Five to ten ears per individual plant were isolated in cellophane bags before flowering and the bags were harvested when the kernels were fully ripe. Anthers and admixed pollen were transferred into tubes, labelled with the laboratory code and sent to Brunel University in 2008, 2010 and 2011 to perform pollen analysis (carried out in 2009, 2011 and 2012, respectively) to identify palynological criteria to differentiate species.

At Brunel, in the first years, only the sample codes were known, not the species names, to maintain objectivity. In each sample, the anthers were isolated from the ears and acetolysed, as acetolysis destroys most anther material except the sporopollenin of the pollen exine (Erdtman, 1960), enabling future comparison of the modern pollen results with sub-fossil pollen grains. Then, the samples were split into two fractions. The first fraction was mounted in glycerol and used for light and phase-contrast microscopy, and the second fraction was stored in distilled water for subsequent analysis by scanning electron microscopy (SEM). The 2009 batch was not acetolysed for SEM analyses, as they served as a preliminary test to check the viability of the project.

The palynological study was divided into three parts, in which three different microscopic techniques were involved (see Table 1 for the analyses performed on each sample). The reason for combining different microscopic techniques is to find criteria to clearly distinguish *T. urartu* from the other species, and to establish whether phase-contrast microscopy, a tool frequently available in archaeobotanical laboratories for investigating the pollen type of cereals, could be useful in distinguishing *T. urartu* from other wheat species.

Pollen grain diameter measurements were performed under light microscopy at 1000× magnification using immersion oil. The largest diameters of 100

Table 1. Samples studied palynologically in this work. Features of the species, origin of the different accessions, details of the analyses carried out in the different batches. Austral., Australian Winter Cereals Collection, Tamworth, Australia; MPI-Cologne, Max-Plank-Institute für Züchtungsforschung, Köln, Germany; SEM, scanning electron microscopy; USDA, National Small Grain Collection, Aberdeen, ID, USA

Species	Features		Sample code	Source code	Source	Origin	Batch	Grain diameter		Phase contrast	SEM	
<i>T. boeoticum</i> subsp. <i>thaoudar</i>	Wild	Two-grained	INA-58	PI 554488	USDA	Turkey	2009	x	x	x	x	
							2011	x	x	x	x	
							2012	x		x	x	
			INA-54	PI 427741	USDA	Iraq	2009	x	x	x	x	x
							2011	x	x	x	x	
							2012	x		x	x	
			INA-53	PI 427710	USDA	Iraq	2009	x	x	x	x	x
							2011	x	x	x	x	
							2012	x		x	x	
			INA-47	PI 427622	USDA	Turkey	2009	x	x	x	x	x
							2011	x	x	x	x	
							2012	x		x	x	
			INA-45	PI 427620	USDA	Turkey	2009	x	x	x	x	x
							2011	x	x	x	x	
							2012	x		x	x	
<i>T. monococcum</i> subsp. <i>monococcum</i>	Domesticated	One-grained	INA-44	PI 119435	USDA	Turkey	2009	x	x	x	x	
							2011	x	x	x	x	
							2012	x		x	x	
<i>T. monococcum</i> subsp. <i>sinskajae</i>			INA-43	19852	Austral.	Turkey	2009	x	x	x	x	
							2011	x	x	x	x	
							2012	x		x	x	
<i>T. boeoticum</i> subsp. <i>aegilopoides</i>	Feral		INA-40	BGRC 36548	MPI-Cologne	Balkans	2009	x	x	x	x	
							2011	x		x	x	
							2012	x	x	x	x	
<i>T. urartu</i>	Wild	Two-grained	INA-35	PI 538735	USDA	Lebanon	2009	x	x	x	x	
							2011	x	x	x	x	
							2012	x		x	x	
			INA-33	PI 428336	USDA	Lebanon	2009					
							2011					
							2012	x	x	x	x	
			INA-32	PI 428335	USDA	Lebanon	2009	x	x	x	x	x
							2011	x	x	x	x	
							2012	x		x	x	
			INA-27	PI 428320	USDA	Lebanon	2009	x	x	x	x	x
							2011	x		x	x	
							2012	x	x	x	x	
			INA-25	PI 428266	USDA	Lebanon	2009					
							2011					
							2012	x	x	x	x	
			INA-16	PI 428249	USDA	Turkey	2009					
							2011					
							2012	x	x	x	x	
			INA-15	PI 428234	USDA	Turkey	2009	x	x	x	x	x
							2011	x	x	x	x	
							2012	x		x	x	
INA-11	PI 428226	USDA	Turkey	2009	x	x	x	x	x			
				2011	x	x	x	x				
				2012	x		x	x				
INA-7	PI 428203	USDA	Turkey	2009	x	x	x	x	x			
				2011	x	x	x	x				
				2012								
INA-6	PI 428196	USDA	Turkey	2009								
				2011								
				2012	x	x	x	x				

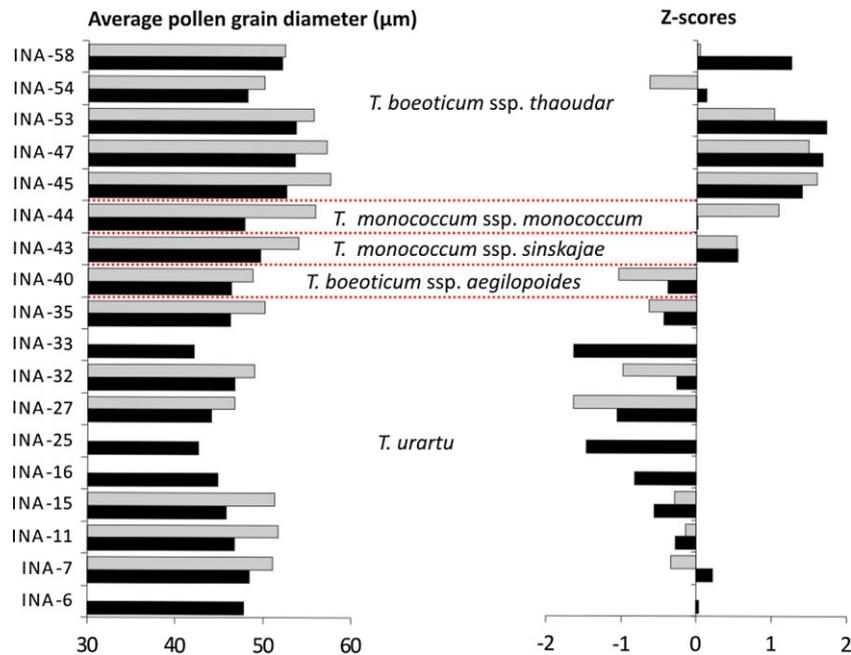


Figure 2. Left: average pollen grain diameters of the Department of Ecology and Natural Resource Management (Institut for naturforvaltning, INA) samples. Grey, measurements of the 2009 batch (several months after acetolysis); black, measurements of the 2011 or 2012 batch (see Table 1) (less than 1 week after acetolysis). Right: Z-scores for each set of measurements.

randomly selected, non-collapsed, unfolded pollen grains were measured per sample. The grain diameter was measured twice. The first measurements were performed on the 2009 batch, but several months after acetolysis. The second measurements were made on the 2011 or 2012 batch (Table 1), less than 1 week after acetolysis in the laboratory. The first set of measurements showed larger diameters, as they remained longer in glycerol. To make the two sets of measurements comparable, the two datasets were independently standardized (Z-scores calculated as $[X_i - X_{avg}]/SD$, where X_i is the average pollen grain diameter in a given sample, X_{avg} is the average of all the samples in the dataset and SD is the standard deviation of the dataset) (Figs 2, 3).

Examination of the exine patterns was performed by means of phase-contrast microscopy at 1000× magnification using immersion oil. Micrographs of pollen exine patterns from several unfolded and non-collapsed randomly selected pollen grains were taken per sample. Representative micrographs for each taxon are presented in Figures 4–6. In addition, to complement the study, pollen grains of several cereal species from pollen reference collection samples or fresh plant material were also studied (data not shown, as many figures are already available in the literature; see Discussion).

The determination of the fine details of the exine ornamentation was completed under scanning elec-

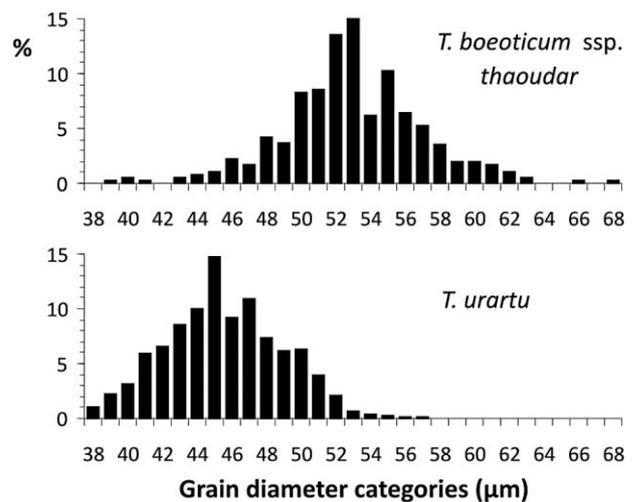


Figure 3. Pollen grain diameter categories in micrometres for the five *Triticum boeoticum* subsp. *thaouдар* samples and the ten *T. urartu* samples studied in this work. The measurements considered are those from the 2011 or 2012 batch, which were performed less than 1 week after acetolysis.

tron microscopy at 5000×, 20 000× and 30 000× magnification. The investigations were made with a Zeiss Supra 35 Field Emission Scanning Electron Microscope. A first attempt to check whether or not it was possible to find differences was performed on

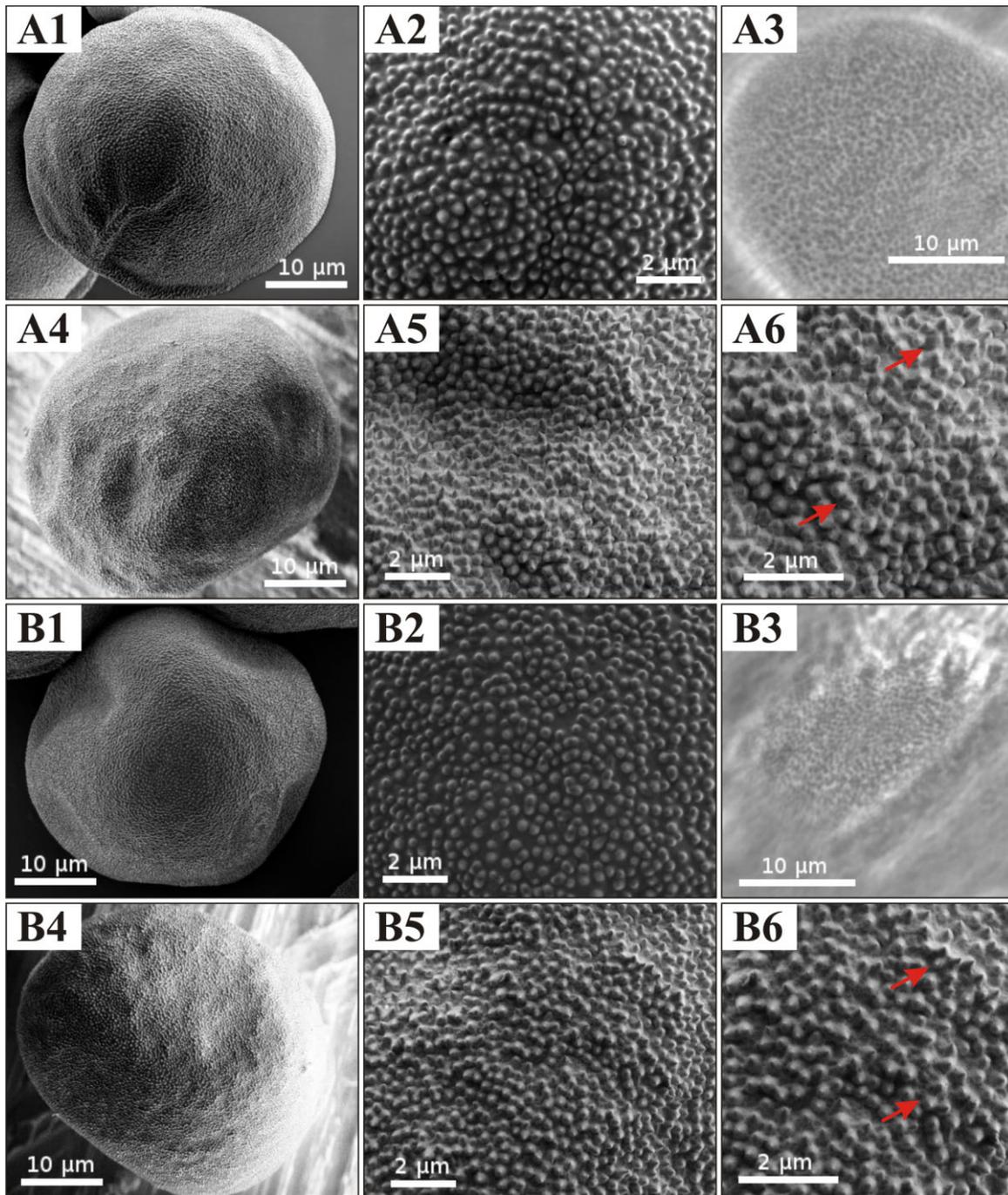


Figure 4. Pollen of *Triticum monococcum* subsp. *monococcum* (sample INA-44): A1, complete non-acetolysed pollen grain at scanning electron microscopy (SEM) at 5000 \times magnification (2009 batch); A2, detail of the non-acetolysed exine at SEM at 20 000 \times magnification (2009 batch); A3, detail of the acetolysed exine at phase-contrast microscopy (2011 batch); A4, complete acetolysed pollen grain at SEM at 5000 \times magnification (2012 batch); A5, detail of the acetolysed exine at SEM at 20 000 \times magnification (2012 batch); A6, detail of the acetolysed exine at SEM at 30 000 \times magnification (2012 batch). Pollen of *Triticum monococcum* subsp. *sinskajae* (sample INA-43): B1, complete non-acetolysed pollen grain at SEM at 5000 \times magnification (2009 batch); B2, detail of the non-acetolysed exine at SEM at 20 000 \times magnification (2009 batch); B3, detail of the acetolysed exine at phase-contrast microscopy (2011 batch); B4, complete acetolysed pollen grain at SEM at 5000 \times magnification (2012 batch); B5, detail of the acetolysed exine at SEM at 20 000 \times magnification (2012 batch); B6, detail of the acetolysed exine at SEM at 30 000 \times magnification (2012 batch). Red arrows highlight some examples of branched spinules.

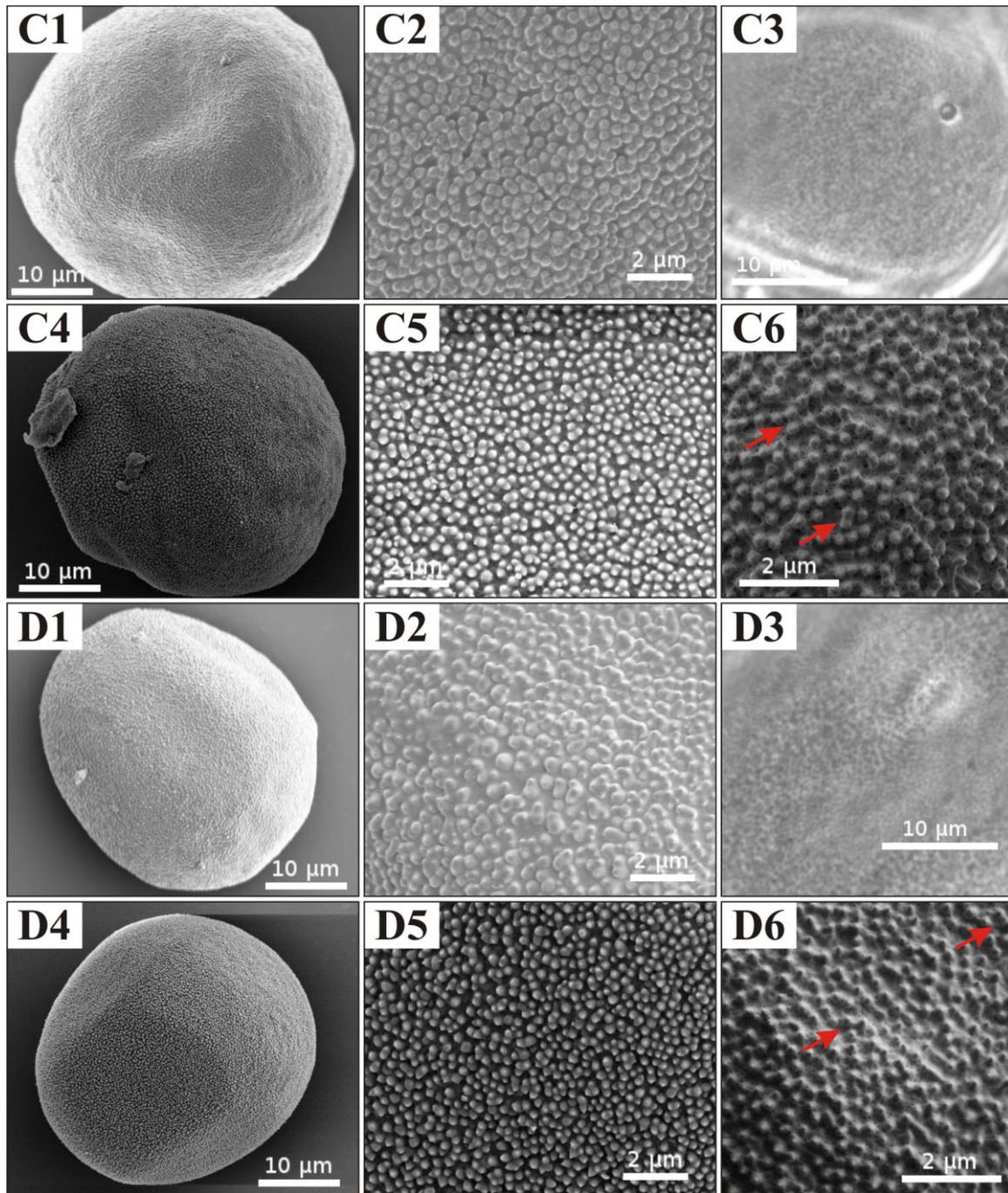


Figure 5. Pollen of *Triticum boeoticum* subsp. *thaoudar*: C1, complete non-acetolysed pollen grain at scanning electron microscopy (SEM) at 5000 \times magnification (2009 batch) (sample INA-53); C2, detail of the non-acetolysed exine at SEM at 20 000 \times magnification (2009 batch) (sample INA-58); C3, detail of the acetolysed exine at phase-contrast microscopy (2011 batch) (sample INA-45); C4, complete acetolysed pollen grain at SEM at 5000 \times magnification (2011 batch) (sample INA-47); C5, detail of the acetolysed exine at SEM at 20 000 \times magnification (2011 batch) (sample INA-54); C6, detail of the acetolysed exine at SEM at 30 000 \times magnification (2012 batch) (sample INA-45). Pollen of *Triticum boeoticum* subsp. *aegilopoides* (sample INA-40): D1, complete non-acetolysed pollen grain at SEM at 5000 \times magnification (2009 batch); D2, detail of the non-acetolysed exine at SEM at 20 000 \times magnification (2009 batch); D3, detail of the acetolysed exine at phase-contrast microscopy (2011 batch); D4, complete acetolysed pollen grain at SEM at 5000 \times magnification (2011 batch); D5, detail of the acetolysed exine at SEM at 20 000 \times magnification (2011 batch); D6, detail of the acetolysed exine at SEM at 30 000 \times magnification (2012 batch). Red arrows highlight some examples of branched spinules.

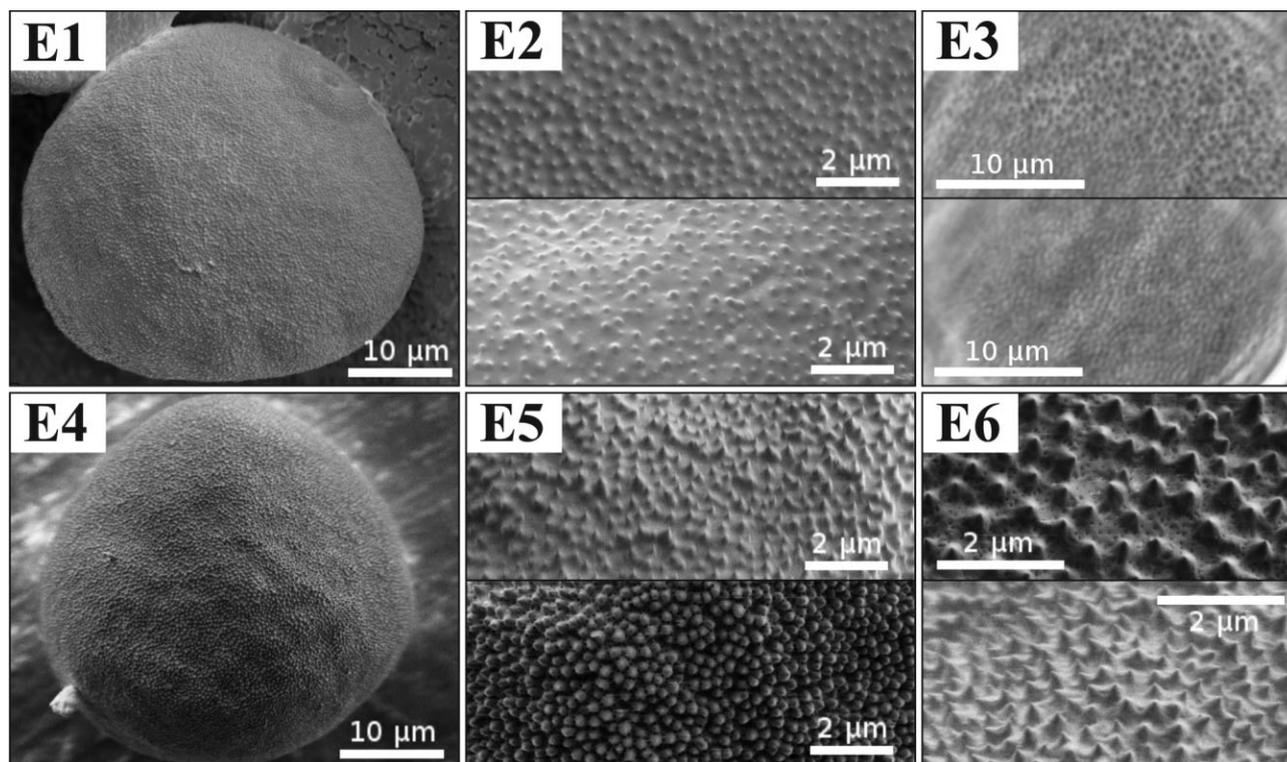


Figure 6. Pollen of *Triticum urartu*: E1, complete non-acetolysed pollen grain at scanning electron microscopy (SEM) at 5000 \times magnification (2009 batch) (sample INA-11); E2, detail of the non-acetolysed exine at SEM at 20 000 \times magnification (2009 batch) [samples INA-11 (top) and INA-32 (bottom)]; E3, detail of the acetolysed exine at phase-contrast microscopy (2012 batch) [samples INA-15 (top) and INA-25 (bottom)]; E4, complete acetolysed pollen grain at SEM at 5000 \times magnification (2012 batch) (sample INA-35); E5, detail of the acetolysed exine at SEM at 20 000 \times magnification (2012 batch) [samples INA-32 (top) and INA-33 (bottom)]; E6, detail of the acetolysed exine at SEM at 30 000 \times magnification (2012 batch) [samples INA-15 (top) and INA-25 (bottom)].

untreated, dry, non-acetolysed pollen grains from the 2009 batch. However, for the 2011 and 2012 batches, acetolysis was performed. As collapse is more pronounced after acetolysis (Hanks & Fairbrothers, 1970), it was difficult, in some samples, to find non-collapsed pollen grains. Additional factors responsible for collapse could be the sample preparation, including fixation, dehydration and gold coating, and the examination in a high vacuum. The use of variable pressure microscopy, where the samples are examined at a lower vacuum in the specimen chamber, did not provide any advantage (2011 batch). Thus, as vacuum is inevitable, samples were finally examined by SEM using the cold-stage (2012 batch). With the cold-stage, freezing the sample at -20°C is possible to achieve dehydration without collapse, although a lower quality of the images is inevitable. Representative micrographs for each taxon are presented in Figures 4–6, with an indication of the sample and batch from which they came. Phase-contrast and scanning electron micrographs were taken in areas away from the pore, as exine sculpturing could be

finer and denser around it (Andersen & Bertelsen, 1972).

RESULTS

GRAIN DIAMETER

The diameters of the two domesticated wheats studied are similar: *T. monococcum* subsp. *monococcum* has an average of $47.76 \pm 0.62 \mu\text{m}$ and *T. monococcum* subsp. *sinskajae* has an average of $49.59 \pm 0.58 \mu\text{m}$ (Fig. 2). These averages and the values presented below come from the second set of measurements. Even though they are not species indicative, the grain diameters of the two wild species are slightly different: in *T. urartu*, the grain diameter (average for ten samples is $45.55 \pm 0.21 \mu\text{m}$) tends to be smaller than that in *T. boeoticum* subsp. *thaoudar* (average for five samples is $51.99 \pm 0.38 \mu\text{m}$) (Figs 2, 3). However, the grain diameter is not a good diagnostic feature because of the size overlap between the species (Fig. 3). For this reason, phase-contrast microscopy

and SEM analyses were undertaken and generated promising results.

EXAMINATION OF THE EXINE PATTERN

In this study, *T. monococcum* (both subsp. *monococcum* and subsp. *sinskajae*) presents a fine aerolate sculpturing, because of the presence of branched spinules. This is clear from SEM observation and could also be inferred by phase-contrast microscopy, suggesting that *T. monococcum* belongs to the *Triticum*-type pollen group (Fig. 4). The same is also true for the wild *T. boeoticum* subsp. *thaouadar* and the feral *T. boeoticum* subsp. *aegilopoides* (Fig. 5), both related to domesticated einkorn, and also showing an aerolate exine sculpturing, as some spinules are branched in groups of two or three. Other *Triticum* spp. present an even more evident aerolate sculpturing, which seems to be more pronounced in polyploids (Rajendra *et al.*, 1978). However, although belonging to *Triticum*, *T. urartu* does not have an evident aerolate exine pattern, and it should be assigned to the *Hordeum*-type pollen group, as this species has a scabrate exine sculpturing with single detached spinules, with an echinulate or granulate appearance, with both SEM and phase-contrast microscopy (Fig. 6). This pollen sculpturing is therefore comparable with that found in the genera *Hordeum* L. and *Secale* L.

DISCUSSION

The pollen size of different *Triticum* spp. tends to increase with increasing number of chromosomes (Cetl, 1960). In our study, only diploid wheat species were included, and so no large variation was expected. In the study by Cetl (1960), *T. monococcum* was the only diploid species measured and the result was $49.0 \pm 0.57 \mu\text{m}$, which is comparable with the data obtained for both domesticated and wild wheats in our study (Fig. 2). In general, *T. urartu* pollen is smaller on average than that of *T. boeoticum* subsp. *thaouadar*. Although grain diameter is not species indicative because of the size overlap between the species (Fig. 3), it could be a parameter worth examining in the first instance. This step could be useful as a first approach, although not sufficient to ascertain the presence or not of the species.

Since the study of Rowley (1960), which demonstrated that cereal exine patterns present isolated or grouped spinules using phase-contrast microscopy, several studies have shown differences in the exine patterns among a number of cereal species. Beug (1961) named three pollen types based on the spinule pattern found by phase-contrast microscopy: the *Hordeum*-type, which included those species with isolated spinules, and the 'Punktgruppen' (*Triticum*-type)

and 'Punktklumped' (*Avena*-type) categories with grouped spinules. Andersen & Bertelsen (1972) studied untreated (non-acetolysed) pollen grains of several cereal species under SEM and distinguished two groups. The first was equivalent to the previous *Hordeum*-type group, with scabrate sculpturing of varying coarseness; the second included those species with aerolate sculpturing, although without distinction between *Triticum*-type and *Avena*-type. In that study, *Hordeum vulgare* and *Secale cereale* L. were placed into the scabrate group with *T. monococcum*, whereas the remaining *Triticum* spp. (*T. dicoccum*, *T. compactum* Host, *T. aestivum* L. and *T. spelta* L.) and *Avena sativa* L. were included in the aerolate group.

Köhler & Lange (1979) investigated, using SEM, the pollen grains of several species, in this case performing acetolysis to reveal the finer details of the exine sculpturing. They were able to distinguish up to four groups: (1) *Hordeum*-type, with single detached spinules and including *H. vulgare* and *S. cereale*; (2) *Triticum*-type, with small insulae bearing from one to three spinules and including *T. dicoccum*, *T. aestivum* and *Panicum miliaceum* L.; (3) *Avena*-type, with larger and irregularly polygonal or elongate insulae with several spinules (mostly from four to six) and including *A. sativa* and *Oryza sativa* L.; and (4) *Setaria*-type, with extensive field-like insulae (with five to eight spinules) of irregularly polygonal shapes and including *Setaria italica* (L.) P.Beauvois. Although they also included *T. monococcum* in the *Hordeum*-type group looking at the images from Andersen & Bertelsen (1972), they did not study this species directly, which would have been interesting because of the incorporation of acetolysis to clean off the pollen grains. Köhler & Lange (1979) were probably not aware of the SEM study performed by Rajendra *et al.* (1978) published one year earlier, also on acetolysed pollen grains and using higher magnification. The latter authors confirmed the scabrate exine sculpturing of *H. vulgare* (spherical pollen) and *S. cereale* (ellipsoidal pollen). The analysed accessions of *T. monococcum* and the other *Triticum* spp. [*T. turgidum* L., *T. aestivum*, *T. speltoides* L. and *T. timopheevii* (Zhuk.) Zhuk.] showed spherical grains with aerolate exine sculpturing with branched spinules isolated in islands. Therefore, not only polyploid wheat species, but also the diploid domesticated einkorn, have aerolate exine sculpturing, although higher magnification was required for the diploid accessions in order to observe the details of the sculpturing. In this study, the findings of Rajendra *et al.* (1978) have been confirmed, as *T. monococcum* (subsp. *monococcum* and *sinskajae*) shows a fine aerolate sculpturing.

Triticum urartu can be distinguished from other *Triticum* taxa, e.g. *T. boeoticum* subsp. *thaouadar*, on

the basis of its scabrate exine sculpturing, in contrast with the aerolate pattern of those found in the *Triticum*-type group. Thus, urartu wheat belongs to the *Hordeum*-type group, and it should therefore be possible to re-study herbarium material using pollen analysis to check whether specimens classified as *T. boeoticum* subsp. *thaoudar* are actually wild einkorn or *T. urartu*. This will also help to outline the natural distribution ranges of both species more accurately, as their habitats are vanishing and the plants are disappearing quickly.

As *T. urartu* shows an exine pattern similar to *Secale* and *Hordeum*, this presents another challenge to overcome when trying to detect it using pollen analysis. Separating *T. urartu* from *Secale* is not a problem, as rye has ellipsoidal pollen grains in contrast with the spherical pollen of *T. urartu*. However, distinguishing *Hordeum* from *T. urartu* could be more problematic. Using the aforementioned difference between *T. urartu* and *T. boeoticum* subsp. *thaoudar* pollen exine sculpturing to detect a possible culture of *T. urartu* could open the debate with regard to the ancestor of the extinct domesticated two-grained einkorn-like form, although only if appropriate archaeological contexts are studied. Examples would be those in which the two-grained einkorn-like form is found abundantly, with *Hordeum* missing, such as the Abu Hureyra 1 site in Syria (Hillman, 2000; Willcox *et al.*, 2009) (Fig. 1). Nevertheless, owing to the difficulties of pollen analysis on archaeological sediments because of different factors, such as contamination, bioturbation, infiltration, selective corrosion, etc. (Bottema, 1975), only profiles with a clear stratigraphy would be satisfactory. In our opinion, the best samples to study would be those coming from seed storage units. High cereal pollen concentration is found in storage structures because of the limited pollen dispersion in cereals, which allows pollen to be trapped in the spikelets or glumes of ripe ears (Bottema, 1992). The Kosak Shamali site in the Upper Euphrates (Fig. 1) includes two storage structures with domesticated emmer and two-grained einkorn-like wheat spikelet forks, awns and glume fragments (Willcox, 2003). As emmer and einkorn have pollen with aerolate sculpturing, if only aerolate pollen grains are found, this two-grained einkorn-like wheat would be related to *T. boeoticum* subsp. *thaoudar*. However, if scabrate pollen grains appear, this could be an unambiguous indication of a link with *T. urartu*.

The two wild wheat species require different growing conditions: *T. urartu* can withstand drought quite well, whereas wild einkorn needs moister conditions (Johnson, 1975; Heun *et al.*, 2008). Therefore, it is likely that *T. urartu* played an important role as a cereal supply in much drier areas, such as northern

Syria, as the natural occurrence of wild einkorn is questionable in such desert areas (Heun *et al.*, 2008). Indeed, the two-grained einkorn-like form is most abundant during the late Pleistocene and early Holocene in northern Syrian archaeological sites, such as Abu Hureyra 1, Tell 'Abr, Mureybet 3, Jerf el Ahmar 1, Jerf el Ahmar 2, Dja'de and Qaramel (Willcox *et al.*, 2008, 2009) (Fig. 1).

CONCLUSIONS

The morphology of the pollen grain of *T. urartu* has been studied microscopically. In comparison with other wheat species that have an aerolate exine sculpturing, usually ascribed to the *Triticum* type, *T. urartu* presents a distinctive scabrate exine sculpturing, which should be assigned to the *Hordeum* type.

This research has applicability not only in taxonomy, but also in archaeobotanical studies. Herbarium materials could be checked when the identification of the species was prior to the discovery of *T. urartu*, with the possibility of it being mistakenly attributed to *T. boeoticum* subsp. *thaoudar*. Palynological studies are cheaper than genetic studies, and therefore more convenient for the re-classification of plant material.

In archaeobotanical studies, this finding, applied in suitable contexts, would help to establish: (1) whether *T. urartu* was cultivated, probably in northern Syria, meaning that it would have to be included as a founder crop in the Neolithic package, and opening up the debate about the ancestor of the domesticated two-grained einkorn-like form; and (2) whether *T. boeoticum* subsp. *thaoudar* was the ancestor, not only of the domesticated one-grained einkorn, but also of the two-grained form.

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REFERENCES

- Abbo S, Lev-Yadun S, Gopher A. 2010a.** Agricultural origins: centers and noncenters; a Near Eastern reappraisal. *Critical Reviews in Plant Sciences* **29**: 317–328.
- Abbo S, Lev-Yadun S, Gopher A. 2010b.** Yield stability: an agronomic perspective on the origin of Near Eastern agriculture. *Vegetation History and Archaeobotany* **19**: 143–150.
- Andersen ST, Bertelsen F. 1972.** Scanning electron microscope studies of pollen of cereals and other grasses. *Grana* **12**: 79–86.
- Beug HJ. 1961.** *Leitfaden der Pollenbestimmung für Mitteleuropa und angrenzende Gebiete. Part 1.* Stuttgart: Gustav Fischer Verlag.
- Bottema S. 1975.** The interpretation of pollen spectra from prehistoric settlements (with special attention to Liguliflorae). *Palaehistoria* **17**: 17–35.
- Bottema S. 1992.** Prehistoric cereal gathering and farming in the Near East: the pollen evidence. *Review of Palaeobotany and Palynology* **73**: 21–33.
- Cetl I. 1960.** The size of pollen grain of the genus *Triticum* L. *Biologia Plantarum (Praha)* **2**: 287–291.
- Erdtman G. 1960.** The acetolysis method. A revised description. *Svensk Botanisk Tidskrift* **54**: 561–564.
- Evans JP, Smith RB, Oglesby RJ. 2004.** Middle East climate simulation and dominant precipitation processes. *International Journal of Climatology* **24**: 1671–1694.
- Fuller DQ. 2007.** Contrasting patterns in crop domestication and domestication rates: recent archaeobotanical insights from the Old World. *Annals of Botany* **100**: 903–924.
- Fuller DQ, Willcox G, Allaby RG. 2012.** Early agricultural pathways: moving outside the ‘core area’ hypothesis in Southwest Asia. *Journal of Experimental Botany* **63**: 617–633.
- Gandilyan PA. 1972.** On the wild-growing *Triticum* species of the Armenian SSR. *Botanicheskii Zhurnal* **57**: 173–181 (in Russian).
- Haldorsen S, Akan H, Çelik B, Heun M. 2011.** The climate of the Younger Dryas as a boundary for einkorn domestication. *Vegetation History and Archaeobotany* **20**: 305–318.
- Hanks S, Fairbrothers DE. 1970.** Effects of preparation technique on pollen prepared for SEM observations. *Taxon* **19**: 879–886.
- Harlan JR, Zohary D. 1966.** Distribution of wild wheats and barley. *Science* **153**: 1074–1080.
- Heun M, Abbo S, Lev-Yadun S, Gopher A. 2012.** A critical review of the protracted domestication model for Near-Eastern founder crops: linear regression, long distance gene flow, archaeological, and archaeobotanical evidence. *Journal of Experimental Botany* **63**: 4333–4341.
- Heun M, Haldorsen S, Vollan K. 2008.** Reassessing domestication events in the Near East: einkorn and *Triticum urartu*. *Genome* **51**: 444–451.
- Heun M, Schäfer-Pregl R, Klawan D, Castagna R, Accerbi M, Borghi B, Salamini F. 1997.** Site of einkorn wheat domestication identified by DNA fingerprinting. *Science* **278**: 1312–1314.
- Hillman G. 2000.** Plant food economy of Abu Hureyra. In: Moore A, Hillman G, Legge T, eds. *Village on the Euphrates, from foraging to farming at Abu Hureyra*. New York: Oxford University Press, 372–392.
- Hillman G, Hedges R, Moore A, Colledge S, Pettitt P. 2001.** New evidence of late glacial cereal cultivation at Abu Hureyra on the Euphrates. *The Holocene* **14**: 383–393.
- Hillman G, Wallis S, McLaren F, Evans J, Butler A. 1993.** Identifying problematic remains of ancient plant foods: a comparison of the role of chemical, histological and morphological criteria. *World Archaeology* **25**: 84–121.
- Honne BI, Heun M. 2009.** On the domestication genetics of self-fertilizing plants. *Vegetation History and Archaeobotany* **18**: 269–272.
- Johnson BL. 1975.** Identification of the apparent B-genome donor of wheat. *Canadian Journal of Genetics and Cytology* **17**: 21–39.
- Johnson BL, Dhaliwal HS. 1976.** Reproductive isolation of *Triticum boeoticum* and *Triticum urartu* and the origin of the tetraploid wheats. *American Journal of Botany* **63**: 1088–1094.
- Köhler E, Lange E. 1979.** A contribution to distinguishing cereal from wild grass pollen grains by LM and SEM. *Grana* **18**: 133–140.
- Kreuz A, Boenke N. 2002.** The presence of two-grained einkorn at the time of the Bandkeramik culture. *Vegetation History and Archaeobotany* **11**: 233–240.
- Lev-Yadun S, Gopher A, Abbo S. 2000.** The cradle of agriculture. *Science* **288**: 1602–1603.
- Nesbitt M. 2001.** Wheat evolution: integrating archaeological and biological evidence. In: Caligari PDS, Brandham PE, eds. *Wheat taxonomy: the legacy of John Percival (Linnean Special Issue 3)*. London: Linnean Society, 37–59.
- Nesbitt M. 2002.** When and where did domesticated cereals first occur in southwest Asia? In: Cappers RTJ, Bottema S, eds. *The dawn of farming in the Near East, studies in early Near Eastern production, subsistence and environment* 6. Berlin: Ex Oriente, 113–132.
- Oliveira HR, Jones H, Leigh F, Lister DL, Jones MK, Peña-Chocarro L. 2011.** Phylogeography of einkorn landraces in the Mediterranean basin and Central Europe: population structure and cultivation history. *Archaeological and Anthropological Sciences* **3**: 327–341.
- Özkan H, Bradolini A, Schäfer-Pregl R, Salamini F. 2002.** AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. *Molecular Biology and Evolution* **19**: 1797–1801.
- Rajendra BR, Tomb AS, Mujeeb KA, Bates LS. 1978.** Pollen morphology of selected Triticeae and two intergeneric hybrids. *Pollen et Spores* **20**: 145–156.
- Rowley JR. 1960.** The exine structure of ‘cereal’ and ‘wild’ type grass pollen. *Grana Palynologica* **2**: 9–15.
- Salamini F, Özkan H, Brandolini A, Schäfer-Pregl R, Martin W. 2002.** Genetics and geography of wild cereal domestication in the Near East. *Nature Reviews Genetics* **3**: 429–441.

- Schiemann E. 1948.** *Weizen, Roggen, Gerste Systematik Geschichte und Verwendung*. Jena: Verlag Gustav Fischer.
- Tanno K, Willcox G. 2006.** How fast was wild wheat domesticated? *Science* **311**: 1886.
- Weiss E, Kislev ME, Hartmann A. 2006.** Autonomous cultivation before domestication. *Science* **312**: 1608–1610.
- Willcox G. 2003.** Chalcolithic carbonised cereals from Ubaid burnt storage structures at Kosak Shamali. In: Nishiaki Y, Matsutani T, eds. *Tell Kosak Shamali vol. II*. University Museum the University of Tokyo, Monograph. Tokyo: University of Tokyo, 267–270.
- Willcox G. 2005.** The distribution, natural habitats and availability of wild cereals in relation to their domestication in the Near East: multiple events, multiple centres. *Vegetation History and Archaeobotany* **14**: 534–541.
- Willcox G. 2013.** The roots of cultivation in southwestern Asia. *Science* **341**: 39–40.
- Willcox G, Buxo R, Herveux L. 2009.** Late Pleistocene and Early Holocene climate and the beginnings of cultivation in northern Syria. *The Holocene* **19**: 151–158.
- Willcox G, Fornite S, Herveux L. 2008.** Early Holocene cultivation before domestication in northern Syria. *Vegetation History and Archaeobotany* **17**: 313–325.