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Grapevine Variety Identification—Methodological Aspects: Sample Preparation, Three-Dimensional Positioning and Morphological Comparison

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The grapevine (*Vitis vinifera*) is one of the most significant fruits in global economy, today as in the past. The international modern wine industry, traditional agriculture and wild populations encompass thousands of grapevine varieties, the differences between them often imperceptible to the naked eye and therefore, necessitating the application of digital technologies. This study aimed at establishing a methodology for analyzing grapevine pips using a 3D scanner, including an optimal protocol for removing the pips' tissue to improve the botanical identification of recent and ancient specimens. The study presents mechanical, chemical and biochemical methods used to prepare the pips for scanning, the sample including 1400 modern grapevine pips belonging to 91 varieties and 300 grape pips from archaeological sites. In addition, several methodological tests were conducted to assess the tool's sensitivity to positioning errors and possible morphological changes during natural ancient desiccation. Our study demonstrates the potential of this methodology for archaeological research and its implications for the wine industry.

Keywords: lab procedure, archaeobotany, 3D model, geometric morphometric methods (GMM), position, drying, scan, grapevine variety

Introduction

In the past two decades, there have been significant advancements in Geometric Morphometric Methods (GMM) and three-dimensional (3D) scanning technologies, with

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practical applications in industry, defense and research. Scanning technology and advanced analysis share an interface; however, scanning is generally perceived as confined to the realm of digital documentation. In archaeology, 3D analyses form an integral part of advanced research, applied to several fields, such as in the study of ceramics (Harush and Grosman 2021; Karasik, Harush and Smilansky 2020), lithics (Caricola et al. 2018), hand axes (Herzlinger, Goren-Inbar and Grosman 2017), coins (Hess, MacDonald and Valach 2018), burial places (Mickleburgh, Stutz and Fokkens 2021) and small objects and artifacts (Cerasoni et al. 2022; Grosman et al. 2022). These studies allow for a precise measurement of minor differences that are often imperceptible to the naked eye. By numerically measuring these differences, researchers can diagnose significant variations and estimate unknown parameters, making GMM studies an indispensable tool. For example, when analyzing flint tools in archaeology, the center of mass is a crucial parameter that cannot be measured accurately without the aid of 3D models. By combining highly precise 3D measurement techniques with statistical methods and innovative algorithms from the field of computer science, researchers of various disciplines have achieved good results (Grosman et al. 2014; Karasik and Smilansky 2008).

In the field of archaeobotany, 3D studies are still in their infancy (see, e.g., Barron 2023; Fermé and Bagur 2023). GMM studies were mainly employed using two-dimensional (2D) analyses on seeds and fruits, wood and charcoal, phytoliths, pollen and starch grains (Portillo et al. 2020). These studies are particularly important in the examination of crop species, including cultivated barley (*Hordeum vulgare*) (Wallace et al. 2019), various wheat species (*Triticum* ssp.) (Goriewa-Duba et al. 2018), pea (*Pisum sativum*) (Braadbaart and Bergen 2005), lentil (*Lens culinaris*), fava bean (*Vicia faba*) and grass pea (*Lathyrus sativus*) (Tarongi et al., 2021). A wide range of fruit species was also studied using these methods, such as olive (*Olea europaea*) (Terral and Arnold-Simard 1996; Terral 2000; Terral et al. 2004; 2012; Newton, Terral and Ivorra 2006), date palm (*Phoenix dactylifera*) (Caspi 2018; Terral et al. 2012), melon (*Cucumis melo*) (Sabato et al. 2017), sweet cherry (*Prunus avium*) (Burger et al. 2011) and grapevine (*Vitis vinifera*) (Terral 2002; Terral et al. 2010; Pagnoux et al. 2014; 2021; Cohen et al. 2023).

Grapevine is known to have been a native plant in the Southern Levant since ancient times, with its domestication dating back to the fourth millennium BCE (Zohary and Spiegel-Roy 1975; Weiss 2015). Recently, it has been shown through a comprehensive genetic study that grapevine domestication occurred about 11,000 years ago concurrently in Western Asia and the Caucasus (Dong et al. 2023) and comprised thousands of domesticated grapevine varieties. During the seventh century CE, with the Muslim conquest of the Levant, viticulture for winemaking was reduced; however, the local population continued to grow grapevines for self-consumption, as small groups of Jews and Christians obtained special permission to produce wine (Amar 2000). This suppression reached its peak in the thirteenth century CE under the Mamluk regime, which put a ban on the cultivation of grapevine and on the production of industrial wine, resulting in the loss of some of the local grapevine varieties, mainly those used for winemaking, as well as the abandonment of wine customs.

Like other cultivated crops, the intensive cultivation of grapevine over millennia has resulted in the emergence of numerous varieties, both in wild and domesticated populations, giving rise to a wide diversity of grapevine morphology. Consequently, for many years, it was widely believed that the morphology of the grapevine pip was not reliable in distinguishing between wild and cultivated varieties, or in identifying different varieties (Zohary et al. 2012).

Traditionally, the distinction between grapevine varieties was primarily based on ampelography, a method of classifying grapes based on the shape and color of their leaves, branches and fruits (Drori et al. 2015). In recent decades, advancements in genetic analysis have become the primary means of differentiation, with various methods employed, such as Amplified Fragment Length Polymorphism (ALEP) (Cervera et al. 1998), Simple Sequence Repeat (SSR) (Emanuelli et al. 2013) and Single Nucleotide Polymorphism (SNPs) (Cabezas et al. 2011). When faced with challenges, such as ancient DNA (aDNA) samples, alternative genomic techniques were utilized (Bacilieri et al. 2017). Presently, the common method is Whole Genome Sequencing (WGS; herein: Full Genome Sequencing), which provides further insights.

The Grapevine in the GMM Studies

Over the past two decades, several researchers have sought to develop digital keys for grapevine variety identification by using image processing and multi-dimensional analysis technologies. GMM Studies have been employed to investigate the morphology of the grapevine varieties, both wild and cultivated, to establish precise criteria for phenotypic diversity. The integration of genetic and morphometric approaches in research has the potential of yielding more robust results and aiding in unraveling the intricate history of grape domestication (Bacilieri et al. 2017; Cohen et al. 2023).

Numerous studies utilized 2D analyses, some focusing on grapevine leaves (Chitwood et al. 2014), but mostly on the pips as the main identification tool. Two-dimensional GMM-based classifications of recent grapevine varieties were conducted in France (Terral et al. 2010), Sardinia (Orrù et al. 2015) and Spain (Cervantes et al. 2021), while 3D analyses were conducted in Israel (Karasik et al. 2018; David 2021; Landa et al. 2021). These works have shown a significant correlation between traditional taxonomy, ampelography and the ability to differentiate between varieties based on pip morphology.

The studies conducted by Terral and others (Terral 2002; Terral et al. 2010; Pagnoux et al. 2014; 2021) focused on archaeological grape pips, applying an analysis based on the Elliptic Fourier Transform (EFT) of the 2D outline of the pips. These ancient grape pips, retrieved from various sites in the Mediterranean basin, were compared to a local modern reference collection to differentiate between wild and cultivated varieties, thereby providing insights into the domestication of grapevine and the history of its varieties (Pagnoux et al. 2014; 2021; Ucchesu et al. 2015; Cohen et al. 2023).

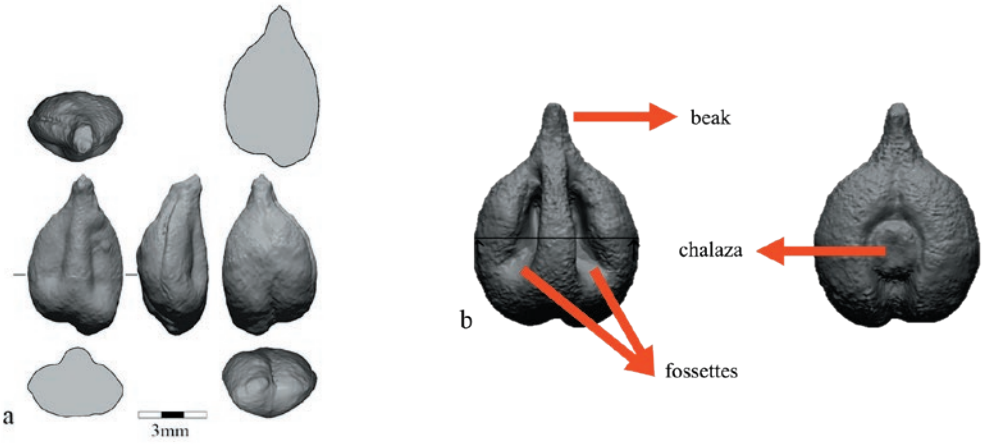

Fig. 1. Different views of a pip scanned into a 3D model: (a) ancient pip, including the tissue covering the fossettes, which interferes with scanning and prevents defining it to variety level; (b) ventral view (left), showing the pair of fossettes and the beak, and dorsal view (right), displaying the chalaza.

The scanning of grape pips is challenging due to their unique biological structure, which consists of a soft tissue that covers the pip and conceals its detailed structure (Fig. 1:a); once the pip dries, the tissue becomes tight and hard to remove. The pip's morphological features include a beak, a pair of fossettes (ventral view), and a chalaza (dorsal view) (Fig. 1:b). The pips of each grape variety have a distinct structure, making the morphology of the fossettes a key characteristic for identification. Hence, it is essential to remove the tissue before scanning while ensuring that the pip is unharmed. Throughout the research, it became evident that a separate set of experiments was necessary to identify an effective method to remove the tissue, which will preserve the integrity of the pip. Various mechanical, chemical and biochemical methods were employed, leading to the development and introduction of protocols for cleaning modern and desiccated ancient grape pips.

The Ancient Grapevine Varieties Project

In 2012, a research project was initiated to locate and classify the local and lost varieties of grapevines that survived the oppression and the medieval Muslim ban on wine consumption and production (Drori et al. 2017). This project involved a comprehensive survey of grapes throughout Israel, including wild (*V. vinifera* ssp. *sylvestris*) and cultivated (*V. vinifera* ssp. *sativa*) ones. Samples of 91 varieties grown in Israel were collected, including European, traditional, cultivars and wild. Over 1400 pips were scanned to a 3D model and processed using various statistical methods. In addition, a 3D tool was developed for the classification of ancient grape pips to the variety level. A total of 300 ancient pips were collected from various archaeological sites, spanning 2000 years of grape cultivation, from the Late Bronze Age to the Byzantine period. The recent samples were subjected to genetic, ampelographic

and analogical tests (Drori et al. 2015; 2017; Sivan et al. 2021), which confirmed that some of them were local varieties.

As part of this research project, we documented the physical characteristics of pips using 3D scanning techniques. Our objective was to create a digital identification tool capable of distinguishing between pips of recent grapevine varieties, and then to identify ancient pips. The ancient grape pips were predominantly preserved in a desiccated condition and underwent a cleaning and preparation process before scanning. Subsequently, they were scanned from all directions to obtain a 3D model that can be used for classification, using various statistical tools.

Here, we consider the methodological challenges in applying a morphological comparison by means of a 3D model. First, the sample must be prepared for scanning, including the removal of the tissue covering the grapevine pips, while ensuring that the pips are undamaged. For this, we have developed protocols for cleaning both modern and ancient pips preserved through desiccation. In the second stage, which was described in our previous study of GMM analysis (Karasik et al. 2018), selected Fourier coefficients and an optimal set of weights were used and create clear distinctions between most varieties with very few overlaps. Below is a comprehensive description of the methodological aspects of scanning and analysis, including an outline of the adaptations made to the 3D scanner, both in software and hardware, to fit grapevine pips. In the last stage, methodological testing of the tool were performed, primarily focusing on its sensitivity to changes in pip positioning. The tool was further tested to determine whether ancient pips underwent morphological changes due to their preservation by desiccation in archaeological sites.

Material and Methods

The body of data in this research includes some 1400 grapevine pips belonging to 91 varieties, including European, traditional, cultivars and wild. Additionally, 300 ancient pips were collected from several archaeological sites, dating from the Late Bronze Age (1300 BCE) to the Byzantine period (fourth–fifth centuries CE). In one of the methodological tests—the drying test—we used three recent grapevine varieties: Cabernet Sauvignon (international grapevine variety, N = 20), Black Tzuriman (local grapevine variety, N = 20) and Variety 62 (wild grapevine variety, N = 20) (see *Discussion*, below).

Tissue Removal Techniques

A 3D scanner based on structured-light technology has specific requirements, including the color and shine of the scanned object. The scanner cannot effectively scan black items as they absorb light, as well as shiny items, regardless of their color, due to light reflection and glare. Recent grape pips, and even ancient ones that have been preserved through desiccation, are typically dark brown in color and thus, allow for a successful scanning (Fig. 2). This technology, however, cannot be applied to charred seeds of black color and shine,

which would require pre-treatment of a gentle spray-painting in shades of light brown. Recently, another method was developed for charred pips, which is based on photographs taken by a binocular microscope, resulting in a very high accuracy in the classification tests (Landa et al. 2021).

The covering tissue was observed in all 1400 scanned recent grape pips, as well as in most of the ancient pips. The tissue was removed by using special tools, such as a laboratory needle, tweezers, fine and coarse brushes, a medical syringe and a surgical scalpel, as well

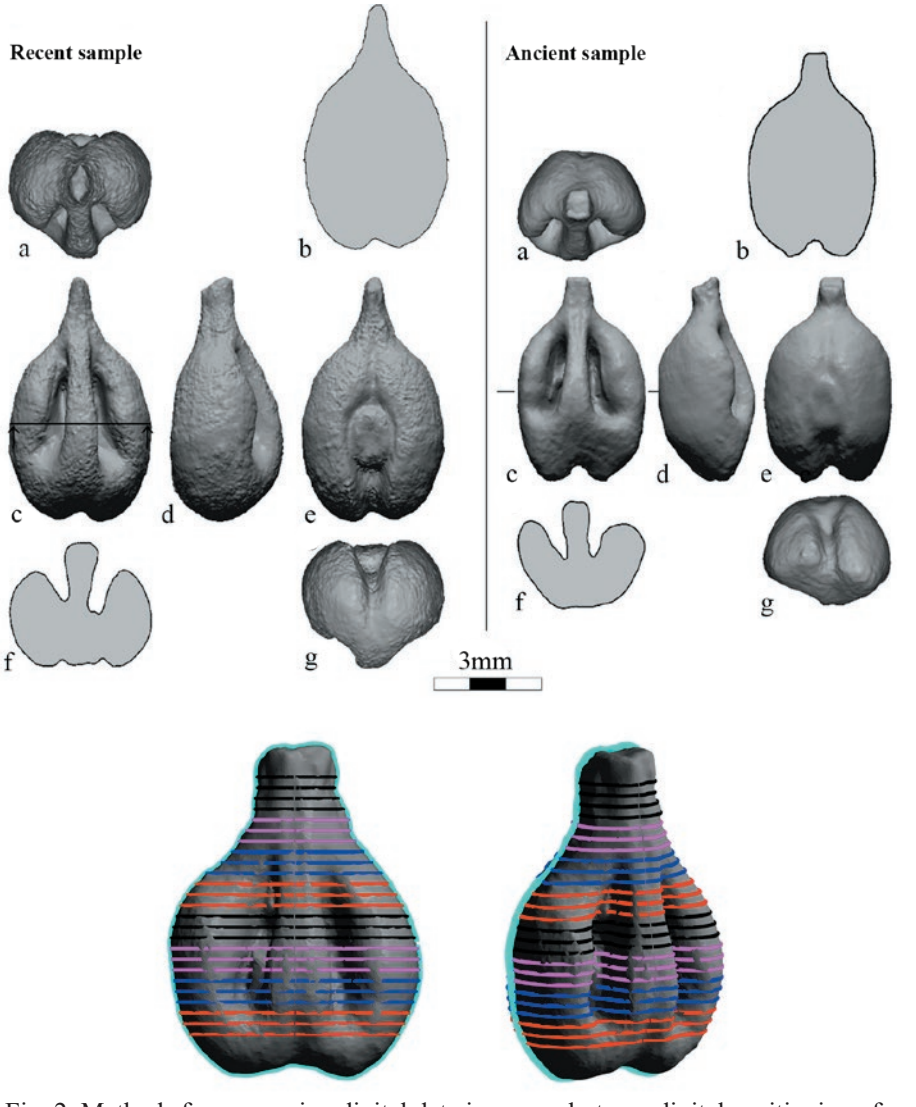

Fig. 2. Methods for processing digital data in research: top—digital positioning of two pips for morphological parameter calculation; bottom—imaging of 50 sections processed in each pip.

as by softening in various materials, such as HCl and NaCl, pectolytic enzyme and distilled water. First, the tissue was softened and then, the fossettes were meticulously cleaned using brushes and needles. The thorough cleaning and drying of the fossettes are crucial to prevent fungal growth (see Fig. 3:e). To determine the most efficient and non-destructive method of tissue removal from both recent and ancient pips, we conducted a series of experiments:
Mechanical: Dry removal of the tissue, sometimes aided by wetting the pip with water.
Biochemical: Overnight soaking of the pips in a pectolytic enzyme (here we used endozyme éclair, from AEB technologies, Italy), in a dosage of 2.5 milliliters per liter, within an incubator maintained at a temperature of 37°C. This enzyme aided in breaking down the pectin, a polysaccharide present in plant cell walls which facilitates tissue removal.
Chemical: Soaking in solutions of hydrochloric acid (HCl) in different concentrations (200 mM; 100 mM; 50 mM) and durations (15; 30; 60; 120; 180; 240 seconds) to enable chemical decomposition.
Boiling (for recent pips only): Boiling the pips in water for 120 seconds to soften the tissue.

After applying each of these methods, we removed the tissue using brushes and tweezers, while paying special attention to cleaning the fossettes. Then, we left the pips to dry for several hours and stored them in silica gel to maintain low humidity. These comprehensive experiments allowed us to identify the most effective approach for successful removal of the tissue, while preserving the structural integrity of the pips for subsequent scanning.

Three-Dimensional (3D) Scanning

The 3D scanner is a projector flanked by two cameras, positioned to cover the imaging area—a rotating table. The projector illuminates the object placed on the table with a pattern of light stripes, while the cameras capture the reflected light. This process enables the precise measurement of the coordinates of dense points on the object. Before scanning, the number of photographs required to complete a full 360-degree rotation ('cluster') is determined. The scan is initiated by rotating the table, fully controlled by computer. Finally, the software merges all captured views, resulting in a 3D digital model of the scanned object (Grosman et al. 2014).

A new FlexScan3D Duo scanner, manufactured by GoMeasure3D, USA, was purchased and customized to meet the specific requirements of the project. This scanner was chosen due to its cost efficiency, user-friendly software, scanning speed and high-quality output. The scanner's hardware and software were modified to adapt to the narrow and vertical structure of the pips' fossettes. To achieve the desired horizontal pattern of light reflections while scanning, we designed a new part using a 3D printer. Additionally, adjustments were made to the software structure, specifically to address the issue of overlapping information between captured images. This enabled the creation of clean and detailed models, ensuring accurate identification of the crucial characteristics of fossettes.

Three-dimentional scanning has secondary advantages, which are particularly important regarding archaeological contexts: it allows for the documentation of a digital representation

of the object and does not damage it. Consequently, the scanned object remains intact and can be utilized for additional tests, such as aDNA analysis or tissue culture (Bacilieri et al. 2017).

Three-Dimensional (3D) Analysis

Software Aspects

The scanned models (WRL or PLY files) were converted into processing files compatible with MATLAB software. Before conducting any morphological comparisons, it was crucial to establish the correct positioning of the scanned models to ensure accurate and repeatable measurements. To achieve this, we utilized a tool based on the eigenvectors of the Inertia Tensor, which determines the way that asymmetric shapes are expressed in the Torquet (Grosman et al. 2014). However, the computerized tool sometimes failed to work automatically, necessitating a manual, uniform positioning of the models. Figure 2 illustrates the methods employed to perform the positioning with the partition parallel to the Y-axis. Following our previous study (Karasik et al. 2018), we opted for a mathematical representation and computerized analysis of the pips by extracting consecutive horizontal sections from the 3D model (Fig. 2) to reveal significant morphological features. Thus, it was essential to clean these sections of the models by removing unnecessary data that were captured during the scanning process. In this study, 50 sections per pip were analyzed, each section serving as a meaningful piece of information.

By addressing the software aspects of model preparation, including positioning and cleaning of sections, we established a solid foundation for conducting precise morphological comparisons. These steps were essential to ensure accurate measurements and enable a meaningful analysis of the scans.

Mathematical Processing

Mathematical processing plays a crucial role in analyzing the information obtained from the scanned pip models. Each section of the model was treated as a closed curve on a plane, allowing for the characterization of the 3D pips' morphology through a dense sequence of plane curves. The Fourier Transform (FT) technique enabled the representation of a close curve as a sum of trigonometric functions with varying levels of detail.

The obtained Fourier coefficients capture different orders of magnitude of morphological characteristics in the curve. For example, a simple closed curve, such as a circle, may be represented by a single coefficient, namely the radius, while more complex curves, like ellipses, require two coefficients to describe the ratio between their axes. Higher-order coefficients reflect characteristics with frequencies of 5, 4, 3, and so on.

Simplifying the comparison process necessitates transitioning from a curve, represented by numerous X and Y coordinates, to a smaller set of parameters, namely the Fourier coefficients. This step enables the isolation of different morphological components and helps eliminate irrelevant data, often referred to as 'noise.' For example, certain deviations

may be visible in specific samples but not consistent across the entire set, thus considered 'noise.'

The Fourier coefficients represent formal characteristics in various areas of the samples, with their strength diminishing as the coefficient index increases. To optimize the differentiation between different groups of varieties, we created an optimal set of weights for the coefficients (Karasik et al. 2018). This weight distribution allows for an enhanced distinction, which can further ignore coefficients representing 'noise' while assigning greater importance to coefficients that are more indicative of variety identification.

After numerous attempts, we selected seven coefficients for the analysis: the average radius (r_0) and the first six non-trivial parameters (r_2–r_7). The lower coefficients, which dominate and capture the majority of the shape, received higher weights. It is noteworthy that altering the weight distribution does not introduce a bias to the sorting process, but rather emphasizes specific aspects of the scanned samples. This mathematical processing, utilizing Fourier coefficients and optimized weight distribution, proved valuable for automatically classifying grapevine varieties based on pip's morphology.

Results and Discussion

Sample Preparation

Recent Pips

The recent grape samples were obtained from the research winery in Ariel University, after undergoing initial fermentation. They were collected in two different states: moist, following freezing (Fig. 3:a, b), and dry (Fig. 3:c, d). The results revealed that removing the tissue from the dry pips was extremely more challenging when applying the mechanical, biochemical and chemical methods. Therefore, it is recommended to avoid preserving grape pips in this state for future research. In the case of the moist pip samples, the mechanical method did not uniformly remove the tissue, often causing damage to the pips' structure; the biochemical, chemical and boiling methods yielded similar results, taking approximately seven minutes to clean each pip. Attempts to remove the tissue without thoroughly cleaning the fossettes proved unsuccessful, as fungi grew inside the fossettes within a short time (Fig. 3:e).

Ancient Pips

In the context of ancient pips, where the external tissue was present, many of them were tightly wrapped by the tissue due to their preservation in a desiccated state (Fig. 1:a). Two main methods were employed: mechanical and chemical. The mechanical method failed to completely remove the tissue, often causing damage to the pips' structure. The chemical removal using HCl acid solution at different concentrations: 200 mM, 100 mM, and 50 mM. These tested concentration solutions resulted in a successfully removal of the tissue, but unfortunately caused immediate or subsequent fissuring of the pips. This issue was attended

Fig 3. Different views of grape pips in various conditions: (a, b) moist pip from grape waste, covered in tissue, ventral (a) and dorsal (b) views, Pinotage, Ramat Negev, Vintage 2017; (c, d) dried pip wrapped in tissue, ventral (c) and dorsal (d) views, Cabernet Sauvignon; (e) moist pip after semi-cleaning, ventral view, fossettes were covered by fungi shortly after cleaning, Pinotage, Ramat Negev, Vintage 2017.

by using a higher osmotic modified solution—by adding 100 mM NaCl to a concentration of 50 mM HCl—to selectively remove only the outer layer. This solution proved successful in entirely removing the tissue without damaging the pips' structure.

Protocol Development

The experimental results revealed that the mechanical method was inadequate for the complete removal of the tissue from recent and ancient pips, sometimes even causing damage to their structure. Conversely, the biochemical and chemical methods proved

successful in achieving thorough cleaning. Considering the variation in tissue composition between recent and ancient pips, it is recommended to employ different methods for cleaning each type. The biochemical method, utilizing a pectolytic enzyme to break down fresh pectin, is recommended for recent pips, while the chemical method is suitable for cleaning ancient pips preserved in a desiccated state, as the old pectin is less responsive to enzymatic breakdown, in addition to other components that may be present. Two laboratory protocols were developed based on these recommendations:
(1) For recent material, use the biochemical method of overnight soaking in a pectolytic enzyme in a dosage of 2.5 milliliters per liter, within an incubator maintained at a temperature of 37°C. Alternatively, employ the boiling method for 120 seconds to soften the tissue.
(2) For ancient remains, use the chemical method. Apply the solution (HCl 50 mM and NaCl 100 mM in a 1:1 ratio) to intact pips, and rinse with distilled water after 15 seconds.

After applying each of these methods, remove the tissue using brushes and tweezers, paying special attention to the fossettes. Then, make sure that the pips are dried for several hours and stored with silica gel to maintain low humidity.

Methodological-Computational Testing of the Tool

Methodological tests were conducted to evaluate the capabilities, accuracy, repeatability and robustness of the developed digital identification tool. These tests aimed at assessing the tool's performance, its sensitivity to small errors and accuracy in classifying the samples. Additionally, tests were carried out to examine potential changes of ancient pips preserved by desiccation.

Position Test

The first test focused on examining the tool's sensitivity to the precise positioning of the samples to determine whether the tool can successfully classify the samples despite slight variations in their positioning due to computer errors or human mistakes. Proper positioning of the scanned object (e.g., Fig. 2) is crucial, as it directly affects its corresponding horizontal sections (e.g., Fig. 2:b, f) and the resulting classification.

The experiment involved a single scanned pip, whose position was changed in the software, yielding seven variations (Fig. 4:a). The results demonstrated successful classification outcomes. In Fig. 4:b, the green markers correspond to the seven different positions of the pip shown in Fig. 4:a. They are close to one another and are grouped within a small area. Our understanding is that slight inaccuracies in the positioning of the 3D model will not affect their classification. Importantly, they were consistently located within their original area, effectively distinguishing them from other tested varieties (Fig. 4:b). These findings highlight the tool's ability to accurately classify pips, even when minor positioning deviations occur. Thus, the tool demonstrates robustness and reliability in identifying distinct varieties.

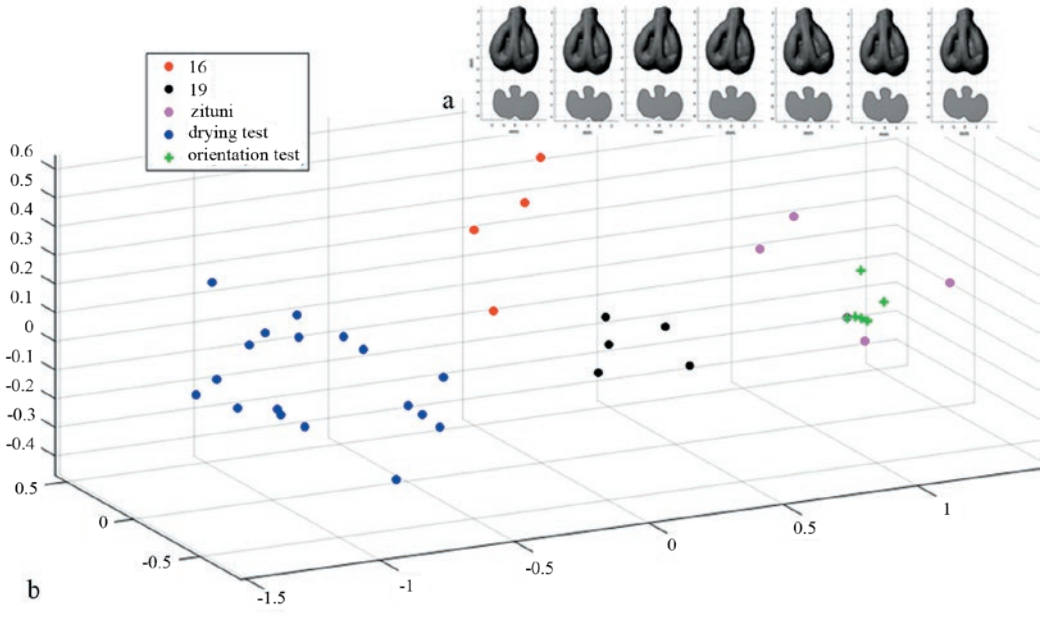

Fig. 4. Position test: (a) seven different positions of the same pip; (b) results for four varieties. The seven positions are marked in green. The dispersion of the different positions is small and contained within the volume of its variety's group, which is marked in pink.

Drying Test

Given that most of the archaeobotanical pips in this research project were preserved under desiccated conditions, the second test aimed at exploring potential morphological changes that may occur during long-term archaeological preservation. Understanding these changes is crucial as the developed identification tool will compare recent and ancient remains based on their morphology.

For this, three recent grapevine varieties were selected: Cabernet Sauvignon (international grapevine variety, N = 20), Black Tzuriman (local grapevine variety, N = 20) and Variety 62 (wild grapevine variety, N = 20). After removing the tissue from the pips, they were scanned into a 3D model and subsequently subjected to drying in an oven for 60 days, simulating soil conditions in a desert climate (50°C, sand cover). Weight measurements were conducted every three days to monitor the rate of water loss from the pips. The drying test results indicated that a significant weight loss occurred in the first two weeks, after which the pips reached a stable weight (Fig. 5). The extent of weight loss varied among the grapevine varieties, ranging between 6% and 13%, corresponding to 30–140 mg each.

Then, the pips were scanned again to 3D models to compare them to their corresponding scans and identify possible changes. The results showed that on average, there was a decrease of 3–4% in each dimension (height, depth and width) (Fig. 6); however, these changes were minor and did not result in substantial differences between the dried and fresh pips. Visual

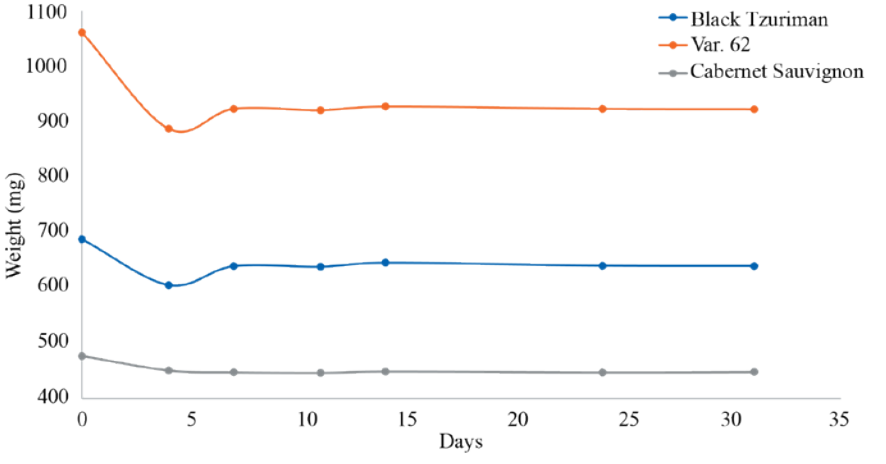

Fig 5. Experimental phase of drying test, three recent grapevine varieties: Cabernet Sauvignon (N = 20), Black Tzuriman (N = 20) and Variety 62 (N = 20).

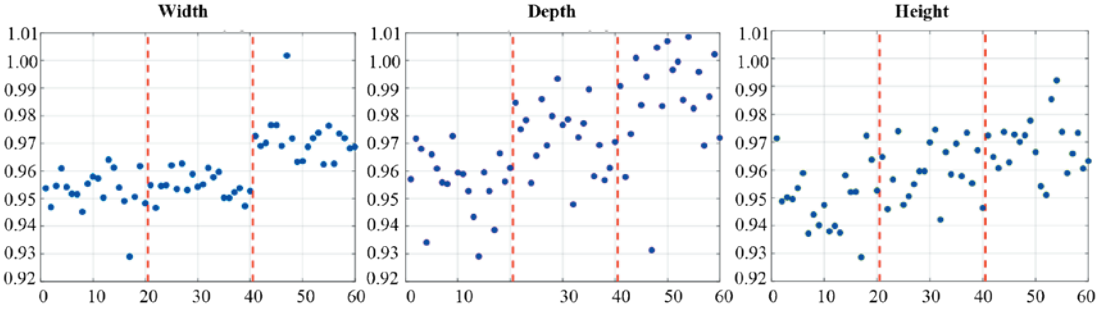

Fig 6. Change in dimensions after drying, each blue dot corresponding to a single pip: pips 1–20 belong to Variety 62; pips 21–40 belong to Black Tzuriman; pips 41–60 belong to Cabernet Sauvignon; the dashed red line separates the different varieties.

comparison of the pips confirmed their high similarity, with no deformation caused by their drying (Fig. 7:a–c).

An additional crucial test applied to the dried pips involved assessing the impact of these slight changes on the distinctiveness of group separation. Figure 7:d demonstrates that despite the changes, the samples continued to be successfully classified into their original respective groups. In other words, dried pips of the same variety remained closer to their fresh counterparts than to pips of other varieties. Therefore, the drying tests confirmed that

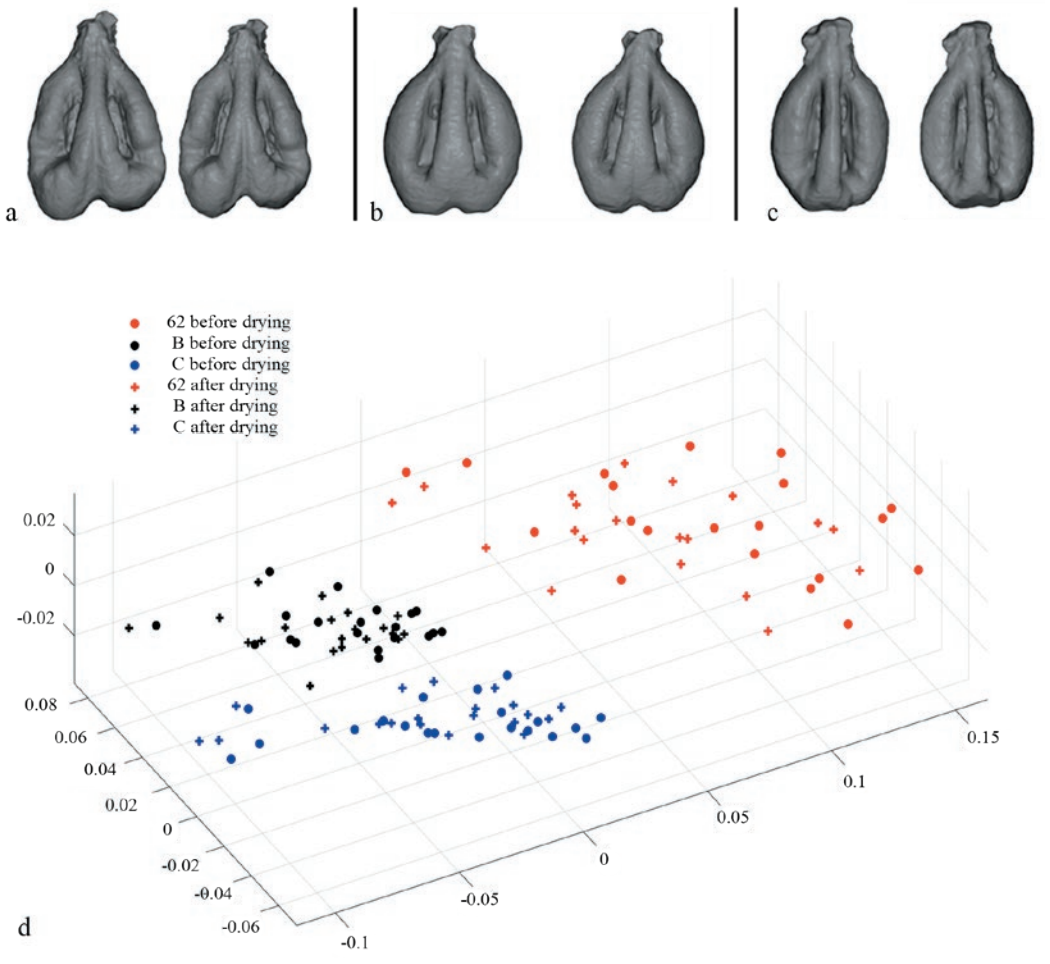

Fig. 7. Results of the drying test: (a) Variety 62, before (left) and after (right) drying; (b) Black Tzuriman variety, before (left) and after (right) drying; (c) Cabernet Sauvignon variety, before (left) and after (right) drying; (d) Classification of fresh and dried pips: blue—Cabernet Sauvignon, black—Black Tzuriman, red—Variety 62, dot—fresh pips, plus—dry pips.

comparing ancient pips to a recent reference collection is feasible, as the drying process does not induce significant morphological changes or introduce biases. This implies that the identification tool successfully used in classifying recent grapevine varieties can also be effective in identifying ancient pips of an unknown variety by morphological comparison.

Conclusions

This paper focused on the methodological aspects of developing a digital-analytical tool for classifying and identifying grapevine varieties based on their pips' morphology. For this, over 1400 recent pips and 300 ancient pips were scanned to a 3D model and processed using various statistical methods.

While developing the tool, different preparation methods for scanning were examined, tested and refined. It was found that in order to allow identification, different methods of tissue removal should be applied to recent and ancient pips: the biochemical method, involving overnight soaking in a pectolytic enzyme, was recommended for recent pips, while the chemical method, with a solution of sodium chloride (NaCl) and hydrochloric acid (HCl), was suggested for ancient specimens.

Further tests were conducted to assess the tool's performance, such as the evaluation of the impact of positioning changes on the accuracy of classification and the study of possible morphological changes following archaeological preservation by natural desiccation. In both tests, the tool successfully classified the pips.

In conclusion, the novel approaches presented in this paper to address methodological challenges in biological materials through 3D scanning and analysis may prove beneficial for other studies encountering difficulties in sample preparation and morphological comparisons. The developed tool was found to effectively classify recent grapevine varieties with a potential to identify ancient grape pips. It provides insights into ancient grapevine varieties in the Southern Levant and opens avenues for further exploration and understanding of the grapevine cultivation history.

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