

High definition 3D models of small legume seed: a close up application to support ancient plant genomics

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Abstract

Legume seeds are essential for nutrition and play crucial roles in food security, climate change mitigation, biodiversity conservation, and sustainability. Among them, the common bean (*Phaseolus vulgaris* L.) is the most widely cultivated and vital to the food value chain. Originating in Mesoamerica and independently domesticated there and in the Andes about 8,000 years ago, common beans have adapted to diverse environments globally following the Columbian exchange. Ancient DNA (aDNA) sequencing offers insights into the bean's adaptation and evolution, but traditional methods struggle with detailed 3D phenotypic analysis due to the beans' small size and the destructive nature of aDNA extraction. Expensive high-detail optical instruments are not commonly available in genetic labs. This research proposes macro-photogrammetry as a low-cost technique for creating detailed 3D digital replicas of beans. By using samples from archaeological sites in northern Peru, this method preserves phenotypic information despite the destruction of physical samples during DNA extraction. The study details the process of capturing high-detail images with specialized equipment to build a digital phenotype library, preserving the morphological features of ancient beans for further study and future comparison with modern varieties.

1. Introduction

Legume seeds are an important source of proteins, minerals, and vitamins for human and animal diets and represent a key-stone for food security and have a fundamental role in several domains; fighting climate changes, biodiversity conservation, sustainability. Among legumes, common bean (*Phaseolus vulgaris* L.) is the most grown food legume crop worldwide and it is crucial for the food value chain (Nartea et al., 2023, Perez-Hernandez et al., 2021). Originated in Mesoamerica, the wild progenitor of common bean colonized South America along the Andes, and its domestication occurred independently more or less simultaneously in both Mesoamerica and the Andes about 8,000 years ago, followed by divergence into distinct landraces due to drift, local selection and adaptation. Following the travels of Columbus the common bean was rapidly and successfully disseminated in Europe with both the Andean and Mesoamerican gene pools. Among the centuries its diffusion reached a global scale, undergoing a genetic evolution given by migration phenomena, highlighting selection of different genomic groups for the adaptation in different areas at worldwide scale. Common bean evolutionary history and its dissemination in new environments make it a model for the study of plant adaptation in a changing environment. Ancient DNA (aDNA) sequencing offers a unique opportunity to retrieve genetic information from past individuals and it is a great tool for shedding light the complex process of local adaptation, domestication and evolution. In particular, the screening of the ancient genome of common bean, one of the three major crops grown by Native Americans, is critical to reconstructing the origin and diffusion of agriculture in the sub-continent. A sample of 26 seeds remains from Peruvian archaeological sites, which dating by radiocarbon with the Accelerator Mass Spectrometry (AMS) technique has confirmed to be all pre-Columbian, are used for DNA analyses. Nucleic acids extraction from seeds

remains a disruptive procedure and ancient seeds are no longer available for further phenotypic analysis and for possible correlations with the genotype and the genetic variants, as well as comparison with modern samples.

Digital technologies can facilitate and improve this process, providing experts and insiders with essential information which could not be achieved with standard laboratory practices (Clini et al., 2016); images, for instance, are useful for archiving, while the sole RGB information might lack of maintaining the characterization features necessary for phenotypic analysis. Indeed, while considerable progress has been made in the last two decades in the theory and instrumentation for the indirect measurement of phenotypes, 3D models reconstructions has posed a challenge because standard procedures of photogrammetry have generally been incapable of measuring small objects with the required level of detail. Detail that, for this characterization, shall be in terms of both colour and dimension. Moreover, a 3-D reconstruction technique makes it possible to compare ancient seeds with well described genetic resources collections (Bellucci et al., 2021). Conversely, optical instrumentations like laser scanner are able to achieve the desired results, but they are expensive and not available in genetic labs (Pisek et al., 2023). All these obstacles translated into a relatively low number of studies reporting actual measurements of beans 3D reconstruction. Anyway, the study of genetic ends up with the destructive action for the aDNA sequencing, making the generation of 3D replicas essential. Thus, detailed 3D representations of beans bring many opportunities for further improvements of measurement methods in the up scaling of optical and dimensional knowledge of legumes. In this work, we introduce macro-photogrammetry as a highly efficient and low-cost technique for creating 3D Digital Replicas of small seeds. This paper argues over a selection of several samples of beans, discovered during different archaeological site located in the north

of Peru. A sample of seeds remains from Peruvian archaeological sites will be used for DNA analyses, but nucleic acids extraction is a destructive procedure and seeds are no longer available for further phenotypic analysis and for possible correlations with the genotype, as well as for comparison with modern samples. Such ancient beans date back more than 600 years, though well preserved; these samples will undergo genetic studies that will shed the light over the species evolution. Such studies will bring to the destruction of these invaluable samples, losing the morphological and radiometric features and making any correlation of genetic variants with seed phenotype impossible. Thanks to the digitisation practices, which is the core of this manuscript, digital replicas will allow to build up a library of phenotypes.

2. Related works

In this paragraph we offer a comprehensive overview of current methodologies and applications in macro-photogrammetry and 3D modeling, particularly for small objects and phenotypic analysis. They provide valuable insights into the technological advancements and practical implementations in this field, supporting this research on preserving phenotypic information of ancient seeds and other small artifacts.

In the ambit of macro-photogrammetry for small objects, a review of various techniques and methodologies for photogrammetric 3D reconstruction of small and micro-sized objects is presented (Galantucci et al., 2018). It discusses the challenges and solutions in capturing high-detail features using close-range photogrammetry, emphasizing the importance of camera calibration and software algorithms in improving measurement accuracy. This is particularly useful for applications requiring precise 3D models of objects with complex surfaces and sub-millimeter features. A less recent work (Gajski et al., 2016) explores the use of macro photogrammetry for the detailed documentation and analysis of small archaeological artifacts. The primary aim is to address the limitations of traditional methods and the inadequacies of available laser scanners in capturing fine details of artifacts that are only a few centimeters in size. In the same field, another study highlights the application of photogrammetry for creating 3D models of small objects. It compares various photogrammetric setups and their effectiveness in capturing detailed features of small artifacts, providing insights into optimizing equipment and methods for high-resolution imaging (De Paolis et al., 2020). Focus stacking combined with photogrammetry is used for 3D reconstruction of small objects in the paper of (Marziali and Marziali, 2017). They detail the workflow from image acquisition to post-processing, addressing common issues such as depth of field limitations and the integration of multiple focus-stacked images to create detailed 3D models. Focus stacking approach is also employed in the paper of (Ravanelli et al., 2022). They present a photogrammetric workflow that utilizes focus-stacked macro images to achieve high-resolution 3D models of small objects, such as Aegean inscriptions. It demonstrates the capability of close-range digital photogrammetry to capture fine details with a 3D density up to 30 microns, making it suitable for detailed phenotypic analysis. The use of Structure from Motion (SfM) photogrammetry to model the 3D structure of lichens and monitor changes over time in the work of (Peterson, 2019). The author puts in evidence the technical challenges and solutions in achieving sub-millimeter accuracy for small biological specimens, providing a detailed methodology for setting up spatial control and capturing high-quality images. Moreover, the study

conducted by (Li and Nguyen, 2019) presents a method for capturing multiple partially focused images of small objects using a fixed-lens setup. It addresses challenges in 3D reconstruction due to the limited depth of field in macro photography and proposes solutions for achieving high-detail 3D models. In a recent study (Fawzy et al., 2023), the authors focus on a specific methodology for close-range photogrammetry, using rotational scanning to create precise 3D models of tiny objects. The research validates the approach by comparing the 3D photogrammetry results with high-resolution transmission electron microscopy, ensuring high accuracy in the models produced.

In the field of bean phenotyping, the paper proposed by (Walter et al., 2015) discusses the advancements in image-based plant phenotyping, highlighting the use of digital photogrammetry to capture detailed phenotypic traits of plants, including beans. The study emphasizes the importance of non-destructive techniques for high-throughput phenotyping, which allows for the detailed characterization of plant traits such as size, shape, and color. Another study examines the adequacy of two low-cost systems for plant reconstruction. The SfM technique was used to create 3D models using a low-cost process. Additionally, an algorithm using an RGB-Depth Kinect v2 sensor was tested for acquiring and reconstructing plant images. The SfM method is slightly better in reconstructing final details and in the accuracy of plant height estimation (Martinez-Guanter et al., 2019). Moreover, a recent review provides a comprehensive overview of 3D imaging methods used in plant phenotyping, including photogrammetry. The authors discuss various techniques and their applications in capturing detailed 3D models of plant structures, which are crucial for understanding phenotypic variations in beans and other crops (Harandi et al., 2023).

3. Materials and methods

The methodology proposed for the 3D reconstruction of the seeds mainly relies on two different techniques which can be applied in image acquisition: cross polarization (CP) and macrophotography. The SfM algorithm reconstructs a sparse point cloud of a surface after recognizing corresponding features visible on different pictures depicting the same areas. Therefore, its success mainly depends on image quality and on the characteristics of the portrayed surface, which is the reason why not all objects are suitable for being reconstructed using this method. Particularly, featureless, or high-reflective surfaces may result in image matching errors and in extremely noisy point clouds. Focusing on our case study, the use of polarizing filters was mandatory to avoid specular reflections on the surface of the seeds and thus make their details visible. Moreover, the dimensions of these details required the use of a macro lens to be perceived, ensuring recognizable features for the image alignment and the subsequent geometric reconstruction.

3.1 Data capturing

To capture the features on the seeds surface, a Sony $\alpha 7RIV$ mirrorless full-frame camera (61 MPx) mounting a macro-lens (90 mm) was used at an approximately object-to-camera distance of 50 cm, ensuring thereby a magnification ratio of 1:4. This setting was based on two main aspects: features detection and depth of field. Indeed, the 1:4 magnification ratio ensured a GSD of 0,03 mm, an adequate value to ensure the identification of the details on the surface of the seed (Table 1). Moreover, it allowed the entire seed to be framed and in focus, avoiding the need for focus stacking, which would have required a greater

number of images, thus excessively long times. Seeds were positioned on a support equipped with metric references and fixed to a turntable. The images were acquired by rotating the seeds by 10° between each shot and according three different positions, to warrant their full coverage. The camera and lighting system were fixed in a permanent position, to avoid any variation of the acquisition settings to not affect image capturing (Figure 1). Since the shiny surface of the seeds generated specular reflections, polarizing filters were mounted on the camera lens and the flash-ring. Highlights were thus eliminated by adjusting the rotation angle of the filter mounted on the camera lens. Due to the object geometry and acquisition settings, 108 images were needed to represent the whole object, providing an adequate overlap (Figure 2).



Figure 1. Set Pictures of the controlled environment for the images acquisition.

Camera details	
Width (mm)	37.5 mm
Height (mm)	23.8 mm
Resolution	
Width (pixel)	9504
Height (pixel)	6336
Dim. Pixel (mm/pixel)	0.0039 mm/pixel
Objectives	
FOV	27°
Focal Length (mm)	90 mm
Distance camera/subject (m)	0.57 m
Gsd (Ground Sample Distance, mm/pixel)	0.025 mm/pixel

Table 1. Main features of the acquisition system.

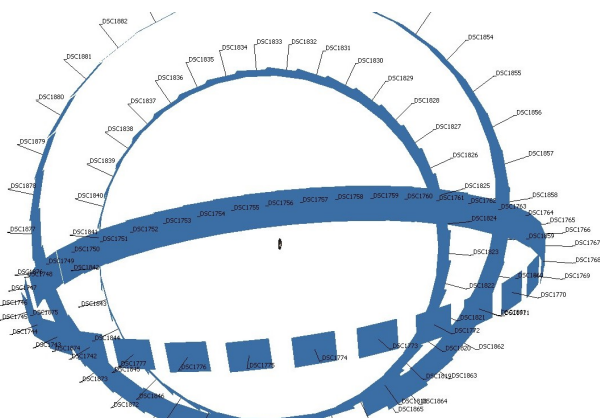


Figure 2. Acquisition scheme with 3D model and camera positions in Metashape.

3.2 Data processing

The raw images were pre-processed in Adobe Camera Raw, paying particular attention to colour representation, that was

managed using a color-checker. Data sets were then imported in Agisoft Metashape where they were separately processed according to the same settings for image alignment (Highest) and point cloud building (Ultra high). The use of CP combined to the macrophotography proved to be particularly relevant. By strongly reducing reflections, it thus increased the number of matchable details, rising the number of matched points, as can be observed in Figure 3. This was clearly shown by SfM-DMVR results, as represented in Figure 4. To assess the accuracy of the SfM-DMVR carried out by integrating macrophotography and CP, measurements on the obtained point cloud were compared with measurements carried out on the real objects using a sub-millimetric caliber.

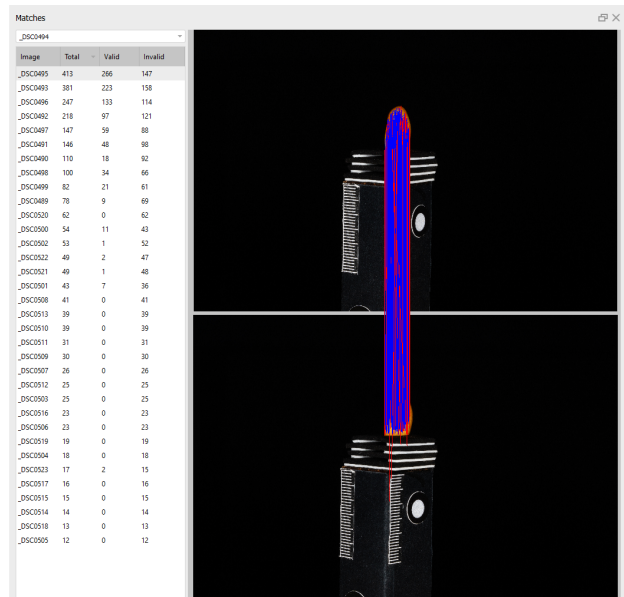


Figure 3. Example of point matches in metashape, the blue lines are the valid ones and the red are the invalid.

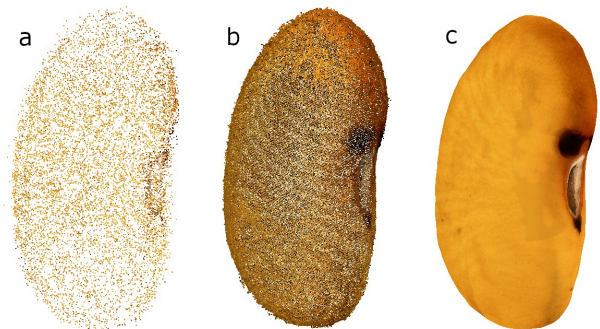


Figure 4. An example of the SfM-DMVR results: a) tie points b) dense cloud c) final 3D model.

4. Experimental results

Each seed length and height of every ancient bean were measured with a precision caliper in the laboratory (Table 2). Once the 3D models were obtained and scaled using the metric references placed on the support used for photogrammetric acquisition, the following information was retrieved for each digitally reconstructed bean: length (cm), height (cm), width (cm), hilum width (cm), hilum length (cm), seed shape, and seed coat

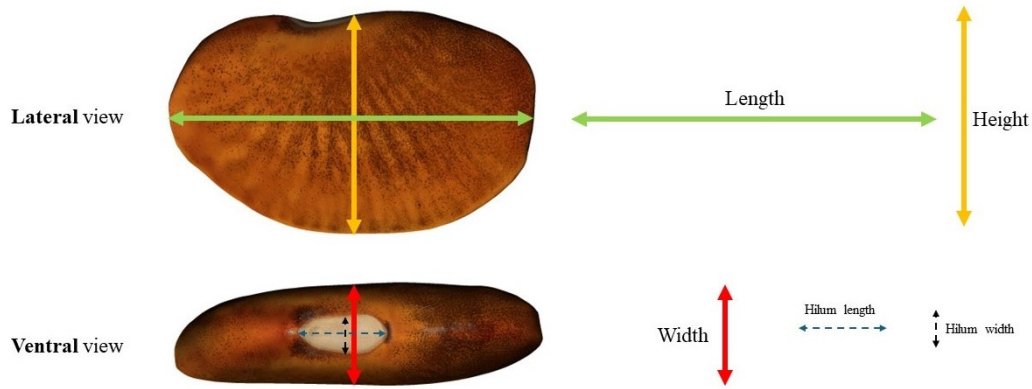


Figure 5. Lateral and ventral view of the seed length, height, and width measurements. Hilum length and width.

pattern (Figure 5). The results of these measurements on the 3D model of each ancient bean are shown in Figure 6 and Figure 7. The seed shape and seed coat pattern were defined according to the protocol developed for characterizing bean seeds in (Cortinovis et al., 2021). For the seed shape*, the numbers indicate: 1: round, 2: oval, 3: cuboid, 4: kidney shaped, 5: truncate fastigiate. For the seed coat pattern*, the numbers indicate: 0: absent, 1: constant mottled, spotted, 2: striped, 3: mottled, 4: constant mottled, marmorated, 5: spot near the hilum (swallow, geometric type), 6: spot near the hilum (soldier, irregular spot), 7: spot near the hilum (large, diffuse), 8: bipartite/tripartite, 9: covered, 10: coated, 11: other (specify in the notes). A total of 26 ancient bean seeds were measured and, thanks to the extracted metric data, the Bias and RMSE (Root Mean Square Error) values were calculated, both in absolute values and in percentage. For the length measurement, the RMSE is 0.09 cm and the RMSE% is 4.49. The bias is 0.03 cm and the bias% is 1.38. For the height measurement, the RMSE is 0.07 cm and the RMSE% is 5.91. The bias is 0.01 cm and the bias% is 1.06.

4.1 Exploitation in VR

Digitisation allows the creation of accurate and detailed 3D models of biological specimens. In the context of this study, digitisation of bean seeds offers several key advantages. One of the most important benefits is the preservation of the physical integrity of the seeds. As stated, traditional DNA extraction methods require the destruction of the seed, which poses a significant challenge for subsequent studies that require the original sample. Digitised 3D models ensure that a virtual representation of the seed remains available for current and future research, overcoming this problem. In addition, the creation of a digitised database improves accessibility, allowing researchers worldwide to access and study these models without the need for physical transport. This will encourage collaborative research and data sharing on an unprecedented scale. In addition, the detailed phenotypic analysis facilitated by 3D models is crucial for understanding the evolutionary dynamics of beans. The ability to examine fine morphological details through digitisation leads to more accurate and comprehensive phenotypic characterisations. This is particularly important for identifying subtle phenotypic variations that may be related to genetic differences or environmental factors.

To support this practice, the integration of Virtual Reality with 3D models further enhances the research experience and offers unique and significant benefits. VR provides an immersive environment where users can interact with the 3D models

Bean name	Caliber measurement	
	Length (cm)	Height (cm)
5A	2.4	1.3
5B	1.6	0.9
5C	1.5	0.9
6A	2.3	1.3
6B	2.1	1.2
6C	1.4	1
257_N-16	1.84	1.4
258_N-37_1	1.57	1.11
258_N-42_3	2.25	1.39
258_N-49_1	2.49	1.54
258_N-49_2	2.07	1.31
258_N-49_3	2.19	1.48
EL_BRUJO_7A	1.5	0.77
EL_BRUJO_8A	2.74	1.28
EL_BRUJO_8C	2.59	1.13
EL_BRUJO_9A	2.45	1.4
HUACA_TICMAR_CAPA_4_3A	2.34	1.22
HUACA_TICMAR_CAPA_4_3B	2.12	1.23
HUACA_TICMAR_CAPA_4_4A	2.15	1.14
MANCHAN_2_1-104_A	1.43	0.93
MANCHAN_2_9-540_A	1.93	1.1
MANCHAN_2_9-540_B	2.75	1.34
MANCHAN_2_7-560	1.41	0.8
MANCHAN_2-389_a	1.8	1.12
MANCHAN_2-389_b	1.41	0.93
MANCHAN_2-389_c	1.02	0.85

Table 2. Caliber measurements of the length (cm) and height (cm) of each ancient bean.

of legume seeds, enhancing the understanding and analysis of seed phenotype. Users can manipulate and explore the models in a detailed way, which helps to better understand complex biological structures. The inclusion of annotations within the VR environment allows users to access additional information and insights directly while examining the models. This feature facilitates the correlation of specific phenotypic traits with genetic and environmental factors, thereby deepening the understanding of evolutionary biology.

We performed a test by including the 3D model in a VR online platform, to determine whether the 3D models are suitable for and enhanced visualization in laboratory settings. The results of this test, made within Sketchfab platform are visible in Figure 8.














Bean code	Length (cm)	Height (cm)	Width (cm)	Hilum Width (cm)	Hilum Length (cm)	Seed Shape*	Seed Coat Pattern*	Bean picture
5A	2.51	1.3	0.66	0.21	0.55	3	0	
5B	1.51	0.97	0.73	0.2	0.39	1	7	
5C	1.49	0.96	0.64	0.15	0.41	2	0	
6A	2.28	1.18	0.59	0.17	0.56	5	7	
6B	2.16	1.27	0.61	0.18	0.5	3	0	
6C	1.43	1.03	0.71	0.17	0.36	2	0	
257_N-16	1.91	1.42	0.78	0.19	1.02	1	7	
258_n-37_1	1.62	1.18	0.66	0.23	0.41	3	5	
258_N-42_3	2.24	1.41	1.09	0.23	1	2	3	
258_N-49_1	2.53	1.54	0.7	0.25	0.61	2	1	
258_N-49_2	2.07	1.24	0.83	0.22	0.51	2	3	
258_N-49_3	2.12	1.38	0.89	0.23	1.02	2	2	
EL_BRUJO_7A	1.59	0.77	0.71	0.16	0.4	3	2	

Figure 6. 3D ancient bean model measurements and bean pictures. Seed shape and seed coat pattern were defined according to the protocol developed for characterizing bean seeds in (Cortinovis et al., 2021).











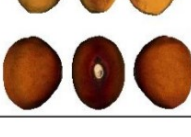
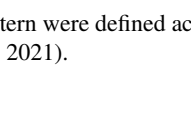
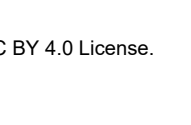
Bean code	Length (cm)	Height (cm)	Width (cm)	Hilum Width (cm)	Hilum Length (cm)	Seed Shape*	Seed Coat Pattern*	Bean picture
EL_BRUJO_8A	2.79	1.4	0.8	0.2	0.53	4	5	
EL_BRUJO_8C	2.58	1.09	0.65	0.19	0.72	3	5	
EL_BRUJO_9A	2.57	1.33	0.71	0.2	0.52	4	6	
HUACA_TICMAR CAPA_4_3A	2.43	1.26	0.67	0.2	0.49	4	6	
HUACA_TICMAR CAPA_4_3B	2.16	1.27	0.65	0.16	0.44	5	5	
HUACA_TICMAR CAPA_4_4A	2.16	1.22	0.64	0.2	0.54	4	0	
MANCHAN 2_1-104_A	1.29	0.82	0.62	0.16	0.41	2	0	
MANCHAN 2_9-540_A	1.94	1.08	0.7	0.15	0.39	3	5	
MANCHAN 2_9-540_B	2.71	1.36	0.59	0.22	0.66	4	7	
MANCHAN 2-7-560	1.4	0.94	0.75	0.15	0.24	1	0	
MANCHAN 2-389_a	2.13	1.21	0.8	0.28	0.51	3	0	
MANCHAN 2-389_b	1.42	0.94	0.75	0.2	0.47	2	3	
MANCHAN 2-389_c	1.02	0.82	0.74	0.13	0.2	1	5	

Figure 7. 3D ancient bean model measurements and bean pictures. Seed shape and seed coat pattern were defined according to the protocol developed for characterizing bean seeds in (Cortinovis et al., 2021).

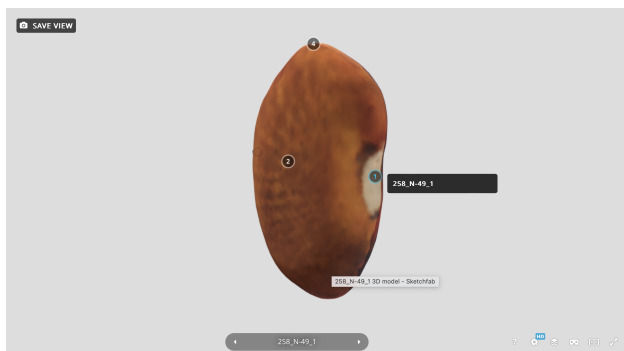


Figure 8. Example of VR visualization in Sketchfab, depicting one of the phenotypes with additional tabs and information.
<https://shorturl.at/YZVT5>

5. Conclusions and future directions

The integration of digitisation techniques and virtual reality into laboratory practices has revolutionised biological research, offering transformative benefits that significantly enhance the study of 3D legume seeds, particularly beans. This paper highlights the importance of these technologies by creating a comprehensive database of 3D bean models, which is an invaluable resource for researchers, especially in the context of DNA extraction and phenotype analysis. Moreover, the methods and tools developed can be extended to other plant species and biological specimens, significantly enhancing the study of plant phenotypes, genetics and evolutionary biology. Using the SfM technique, it was possible to generate 3D models that represent in detail the shape, size, structure and color of ancient seeds (Figure 4c), characteristics destined to be lost following DNA extraction for genomic sequencing in the laboratory. Moreover, such 3D models can also be recreated for modern beans and used as a control to compare their phenotypic characteristics with those of ancient beans, facilitating phenotype-genetic variant correlation. The three-dimensional reconstruction technique outlined in this article, applied for the first time to ancient seeds, will allow us to preserve valuable phenotypic information that would otherwise have been lost. This information will enrich the library built by the INCREASE project (Bellucci et al., 2021) by helping to elucidate the causal relationships between genetic variation at the DNA level and corresponding variation in the plant phenotype. The use of photogrammetric techniques for the digitization and 3D modeling of cultural heritage is now widespread (Bekele et al., 2018, Aicardi et al., 2018). Advances in photogrammetry have significantly enhanced the creation of high-resolution 3D models of historical buildings, archaeological sites and cultural heritages (Balloni et al., 2023). The digital form of these culturally relevant objects enables detailed analysis without physical interference (Pierdicca et al., 2016). This technology facilitates the creation of interactive and immersive virtual tours, broadening public access to cultural heritage and aiding in education and tourism. In addition to its applications in cultural heritage and archeological sites preservation, photogrammetry is also increasingly used in forestry and agricultural fields for the conservation and digitalization of trees of particular historical or cultural interest, such as the case of monumental or ancient trees (Balestra et al., 2023, Uslu, 2022, Maravelakis et al., 2012). As technology advances, photogrammetric techniques will further refine the accuracy and efficiency of 3D modeling. This will facilitate the reconstruction and the analyses of high-detail features using close-range pho-

togrammetry. Additionally, improvements in sensors will enhance the capability to create high resolution models that will ensure preservation of unique specimens with unprecedented detail, contributing significantly to the fields of conservation, research and knowledge transfer. The ability to digitise and virtually preserve biological specimens can contribute to better conservation strategies. By maintaining digital archives of endangered species, researchers can ensure that valuable genetic and phenotypic data are preserved for posterity, aiding conservation efforts and biodiversity studies.

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