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PAPER

Functionally graded structures in the involucre of Job's tears

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**Keywords:** functionally graded materials, Job's tears, organic-inorganic hybrid materialsSupplementary material for this article is available [online](#)

Abstract

Nature is filled with materials that are both strong and light, such as bones, teeth, bamboo, seashells, arthropod exoskeletons, and nut shells. The insights gained from analyzing the changing chemical compositions and structural characteristics, as well as the mechanical properties of these materials, have been applied in developing innovative, durable, and lightweight materials like those used for impact absorption. This research concentrates on the involucre of Job's tears (*Coix lacryma-jobi* var. *lacryma-jobi*), which are rich in silica, hard, and serve to encase the seeds. The chemical composition and structural characteristics of involucre were observed using scanning electron microscopy and energy-dispersive x-ray spectroscopy and optical microscopy with safranin staining. The hardness of the outer and inner surfaces of the involucre was measured using the micro-Vickers hardness test, and the Young's modulus of the involucre's cross-section was measured using nanoindentation. Additionally, the breaking behavior of involucre was measured through compression test and three-point bending tests. The results revealed a smooth transition in chemical composition, as well as in the orientation and dimensions of the tissues from the outer to the inner layers of involucre. Furthermore, it was estimated that the spatial gradient of the Young's modulus is due to the gradient of silica deposition. By distributing the hard, brittle silica in the outer layer and elastoplastic organic components in the middle and inner layers, the involucre effectively respond to compressive and tensile stresses that occur when loads are applied to the outside of the involucre. Furthermore, the involucre are reinforced in both meridional and equatorial directions by robust fibrovascular bundles, fibrous bundles, and the inner layer's sclerenchyma fibers. From these factors, it was found that involucre exhibit high toughness against loads from outside, making it less prone to cracking.

1. Introduction

Strong materials in nature such as bones, teeth, bamboo, seashells, exoskeletons of arthropods, and nutshells are examples of biological functionally graded materials (FGMs) [1–14]. These biological FGMs feature gradual transitions in chemical compositions/constituents, as well as in structural characteristics, including the arrangement, distribution, dimensions, and orientations of the building units [1]. These transitions, observed either across the entire material or specifically near the interfaces of dissimilar components, lead to continuous changes in mechanical properties such as hardness, strength, elasticity, and toughness. The resulting continuous changes not only suppress fractures at the interfaces

of dissimilar components but also enable the localization of specific properties within the material. By appropriately positioning the right chemical composition and structure in targeted areas, it is possible to tailor local properties to meet specific requirements, thereby creating multiple functionalities within a single material and enhancing its overall mechanical properties. Biological FGMs exhibit a rich diversity as they express the aforementioned gradual transitions, either singularly or in combination, within the constraints imposed by their environment and resources. In recent years, inspired by these biological FGMs, numerous studies have been conducted to improve the lightness and mechanical properties of materials [1, 9, 15–24].

In this study, we focused on Job's tears (*Coix lacryma-jobi* var. *lacryma-jobi*), a member of the Poaceae family [25]. These plants have hard and glossy involucre that enclose their seeds (figures 1(a) and (b)). Ge *et al* reported that in the epidermis of Job's tears, phytoliths with a blocky ameboid shape are interconnected and form a unified structure [26]. Phytoliths are hard, fine structures formed by the accumulation of amorphous silica [27, 28].

In the Poaceae family plants, silicic acid absorbed by the roots moves with the transpiration stream to reach the shoots, where it is unloaded from the xylem [29–31]. The silicic acid then polymerizes as solid silica particle at several locations within the plant body. The most significant silica deposition in grasses occurs in the cell walls of leaf epidermal cells, the abaxial epidermal cells of inflorescence bracts (glumes and lemma), and the root endodermal cells [29]. Kumar *et al* reported that the mechanism of silica deposition in the Poaceae family plants varies by cell type, involving either a passive process, an active process, or both, depending on the cell type [32]. The passive process is particularly observed in cells of mature organs or those with intense transpiration. Dehydration or transpiration continuously supplies silicic acid to the cell walls, leading to the concentration and polymerization of silicic acid. This deposition occurs without metabolic control by the cell. In the active process, before organ dehydration, silicic acid polymerization is promoted by proteins that feature unique sequences of acidic and basic amino acids, occurring within a scaffold made up of cellulose nanofibers and additional polysaccharides located in the cell wall [33–35].

It is known that silicon present on the surface of plants is beneficial because it helps in defense against insects and fungi [26, 36–41]. Furthermore, producing silica as a compression material is considered to be 20 times more energy-efficient on a volume basis than producing lignin [42]. Therefore, the formation of phytoliths in the involucre is thought to be a relatively economical and effective method for protecting seeds. Furthermore, the dispersal methods of Job's tears seeds are thought to involve being carried away by floodwaters or transported by birds and rodents [43]. The robust involucre is considered to be useful in protecting the seeds from abrasion while being transported by river currents and from physical and chemical digestion by birds and rodents. Previous studies measuring the elasticity modulus of the involucre's cross-section have reported that, beyond 100 μm from the outer surface, the outer part of the involucre exhibits a slightly higher elasticity modulus than the inside [43].

In this study, we observed the microstructure and chemical composition of the involucre, measured the hardness of both its outer and inner surface, and quantified the elastic modulus across its

cross-section. Additionally, we conducted compression tests and three-point bending tests on the involucre. These experiments are intended to elucidate the gradual changes in material properties within the involucre and to clarify the factors contributing to its resistance to cracking. Understanding the gradient functional structure of the involucre of Job's tears in detail can add one more piece to our knowledge of diverse and robust biological structures. This could potentially serve as inspiration for designing robust and lightweight materials (e.g. for impact resistance in advanced applications) in industries facing a variety of constraints, as is the case in the natural world.

2. Materials and methods

2.1. Material

We primarily used mature Job's tears collected from Gifu and Aichi prefectures in Japan during the winter seasons of December 2020 to March 2021 and December 2021. The fruits used for the energy-dispersive x-ray spectroscopy (EDX) analysis of the involucre from immature fruit were collected in July 2021. These fruits were stored at room temperature and ambient humidity. These fruits are moist, soft, and green in the summer (figure 1(c)). But in winter, they dry out and change color from green to gray or brown. Concurrently, they develop a glossy appearance and become harder and more robust. We shattered 15 mature fruits with a hammer, extracted only the involucre, and measured their moisture content using a moisture meter (MOC63u, SHIMADZU, Kyoto, Japan). Similar to the report by Torres *et al* the involucre contained 10.5% moisture (figure S1). The fruits have two small holes along the y -axis: one at the apex of the fruit and the other at the point of attachment to the plant. The fruits displayed a symmetrical drop shape when viewed on the xy plane. However, when viewed on the yz plane, a clear asymmetry is observable, with one side of the fruit exhibiting less curvature, while the other side is significantly more curved. The length along x axis was 7.7 mm (with a standard deviation (SD) of 0.4), the length along y axis measured 10.3 mm (SD 0.6), and the length along z axis was 6.6 mm (SD 0.4). The thickness of involucre is 742 μm (SD 68).

2.2. Observation by scanning electron microscopy

We used a knife and hammer to carefully split the involucre along the xz and yz planes. We then coated the observation surfaces of the samples with gold using a sputter coater and attached them to a stage using carbon tape. Next, the samples were examined using scanning electron microscopy (SEM) (JCM-5000, JEOL Ltd, Tokyo, Japan). To obtain more detailed images, we cut slices of the involucre parallel to the yz plane into 500 μm sections using a wire saw (DWS3500P, Meiwafoods Co., Ltd, Tokyo,

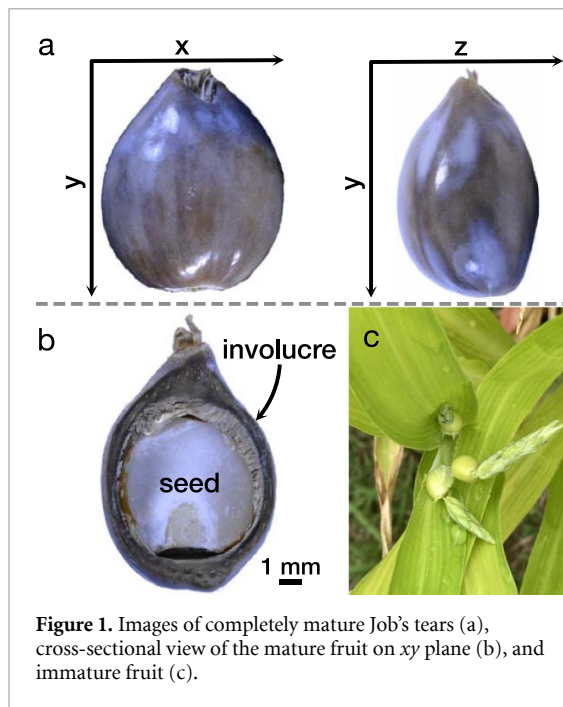


Figure 1. Images of completely mature Job's tears (a), cross-sectional view of the mature fruit on xy plane (b), and immature fruit (c).

Japan) and embedded them in epoxy resin (NER-814M, Nissin EM Co., Ltd, Tokyo). Using an ultramicrotome (Leica EM UC7, FC7; Leica Microsystems, Wetzlar, Germany), we prepared a surface parallel to the xz plane for observation. This surface was then coated with a layer of osmium, and higher magnification observations of the samples were conducted using SEM (JSM-7800F, JEOL Ltd, Tokyo, Japan) and EDX analysis.

2.3. Observation by optical microscopy

Following the procedure described in section 2.2, we prepared a sample embedded in epoxy resin. We then used an ultramicrotome to create slice with a thickness of $15\ \mu\text{m}$. Using safranin (Chroma Gesellschaft Schmid & Co., Munster, Germany), we prepared a solution with $0.014\ \text{mol l}^{-1}$ safranin in 45 vol% ethanol water and used it to stain the lignin in the samples. The observations were conducted using an optical microscopy (VHX-2000, Keyence, Osaka, Japan).

2.4. Micro-Vickers hardness test

To measure the hardness of the involucre, we shattered ten mature fruits using a hammer, resulting in ten sample pieces, each approximately 2 mm square, with minimal surface curvature. The measurements were conducted on both the inner and outer surface of each piece using a micro-Vickers hardness tester (HMV-1, SHIMADZU, Kyoto, Japan). Hardness was measured by pressing a diamond indenter at HV0.05 (490.3 mN) for 30 s. We applied Welch's t-test to compare the hardness of the inner and outer surfaces of the involucre [44].

2.5. Nanoindentation

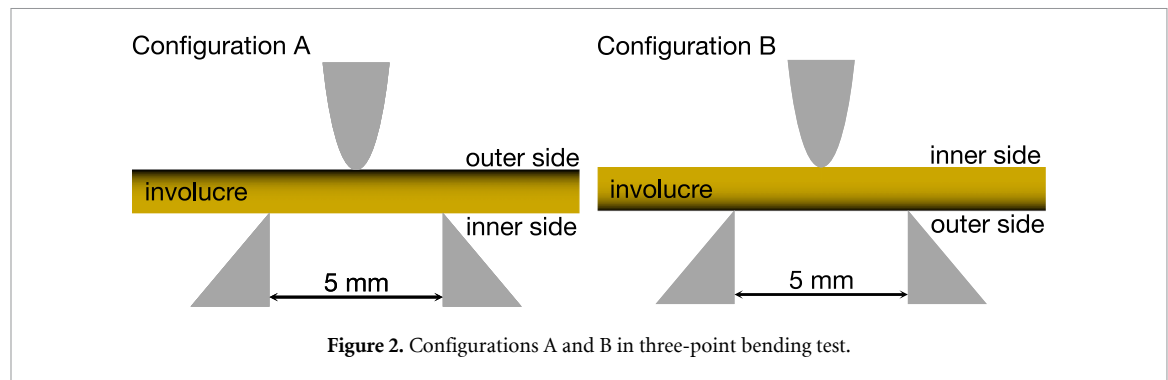
Following the procedure described in section 2.2, we prepared a sample embedded in epoxy resin and used an ultramicrotome to expose its surface parallel to the xz plane. Next, we utilized a cryo-cross-section polisher (CCP) to conduct beam irradiation at an accelerating voltage of 5.0 kV for approximately six hours, followed by a 30 min finishing process to achieve a smooth surface. We then measured the local hardness of the involucre cross-section transversally from the outer to the inner surface using a nanoindentation tester (ENT-2100, ELIONIX Inc., Tokyo, Japan). Starting $10\ \mu\text{m}$ inward from the outer surface of the involucre, we performed measurements every $20\ \mu\text{m}$ until a depth of $230\ \mu\text{m}$ was reached. Beyond this point, measurements were performed at intervals of $50\ \mu\text{m}$. To avoid excessive indentation, which could distort the measurements, we adjusted the test force between 3 and 30 mN. Three separate measurements were conducted at each defined distance from the outer surface of the involucre. All measurement points were carefully selected to avoid vascular bundles. We ensured a minimum distance of $20\ \mu\text{m}$ between each measurement point to guarantee adequate spacing.

2.6. Compression test

Compression tests were carried out on fruits utilizing a universal testing machine (Model SL-6002; IMADA Co., Ltd, Aichi, Japan) to evaluate their mechanical properties under different loading conditions. The fruits were subjected to loads in both y and z directions, with a set of ten fruits tested for each orientation. To ensure stability during testing, the fruits were affixed to the machine's platform using double-sided and masking tape. The strain of the fruit was defined as $\epsilon = \frac{\Delta l}{l_0}$, where l_0 represents the initial dimension of the involucre in the direction of the applied load before testing, and Δl denotes the deformation of the involucre in the direction of the load during the test. We measured the load and strain at the point of fracture. After the measurement, the cracks in the involucre were observed using an optical microscopy (VHX-2000, Keyence, Osaka, Japan).

2.7. Three-point bending test

Using a wire saw (DWS3500P, Meiwafoods Co., Ltd, Tokyo), We sliced the involucre along the yz plane to produce sections approximately 3.5 mm in width from its flatter parts, yielding a total of ten samples. The width and thickness of each sample were measured using digital calipers. Three-point bending tests were conducted on the samples using a universal testing machine (SV-55C, IMADA CO., Ltd, Aichi, Japan) with a support span of 5 mm and a test speed of $10\ \text{mm min}^{-1}$. By utilizing the less curved parts of the involucre and measuring with a narrow support span, we minimized the influence of the involucre's



shape on the measurement values. The tests were conducted five times each in two setups: configuration A tensile force acted on the inner side of the involucre, and configuration B, where the tensile force acted on the outer side of the involucre (figure 2). We used the following equation to determine the stress and strain, and the toughness was determined by integrating the stress–strain curves[45].

$$\sigma = 3FL/2bh^2$$

$$\varepsilon = 6\delta h/L^2$$

where:

σ : stress (MPa).

L : length between supports (mm).

b : sample width (mm).

F : applied force (N).

ε : deformation in the loading direction.

δ : deflection in the middle of the specimen (mm).

h : thickness of specimen (mm).

Furthermore, we observed the cracks in the samples that had reached fracture stress using SEM (JCM-5000, JEOL Ltd, Tokyo, Japan).

3. Results

3.1. Microstructures

Cross-sections of the involucre, cut along the yz plane (figure 3(a)) and xz plane (figure 3(b)), were examined using SEM. The study revealed that the involucre are primarily composed of sclerenchyma cells, which undergo a process known as lignification, leading to thickening and hardening of the secondary cell wall. The inner layer of the involucre consisted of tubular sclerenchyma fibers oriented in the equatorial direction (figures 3(a)–(c)). In the middle layers, sclerenchyma cells are irregular in shape, and as they approach the outer layer, the cells interconnect and the boundaries between them become indistinct (figure 3(d)). A continuous phytolith layer without gaps was formed in the outer layer (figure 3(e)). Transitional structures were observed at points transitioning from the inner layer to the middle layer and

from the middle layer to the outer layer (figures 3(a), (b) and (d)).

Additionally, upon high-magnification observation of the outer layer of the involucre, structures consisting of aggregated nanoparticles ranging from a few nanometers to approximately 100 nm were frequently identified (figures 3(f) and (g)). It was also confirmed that fine fibrous components were interspersed within these nanoparticles.

Fibrovascular bundles were located in the middle of the involucre (figures 3(a) and (b)). The fibrous bundles were scattered throughout the middle layer (figure 3(b)). Both bundles extended from the point of attachment of the fruit towards its apex. Fibrovascular bundles are composed of vascular tissue reinforced by a bundle cap made of sclerenchyma fibers, whereas fibrous bundles consist solely of sclerenchyma fibers.

SEM observations were also conducted on cross-sections of immature fruit involucre, cut along the xz and yz planes (figures S3(a) and S4(a)). Similar to the mature fruits, the involucre of immature fruits consist of an inner layer of tubular sclerenchyma fibers, a middle layer of irregularly shaped interconnected sclerenchyma cells, and an outer layer densely filled with phytoliths without gaps. However, compared to the involucre of mature fruits, the outer layer was significantly thinner, about 15 μm , and there were more gaps observed in the middle and inner layers.

3.2. Chemical compositions

EDX analysis measures the energy and intensity of the characteristic x-rays emitted when a specific area is irradiated with electron beams, allowing for elemental and qualitative analyses. Intensity refers to the number of detected characteristic x-rays at a particular energy level and is expressed as counts per second. This intensity can be used to estimate the presence and approximate concentrations of elements within the analyzed area. EDX analysis was conducted on a cross section of the involucre cut along the xz plane (figures 4(a)–(d)). Line analysis was performed along the path indicated by the yellow arrow (figure 4(e)). In the line analysis, carbon peaks at the

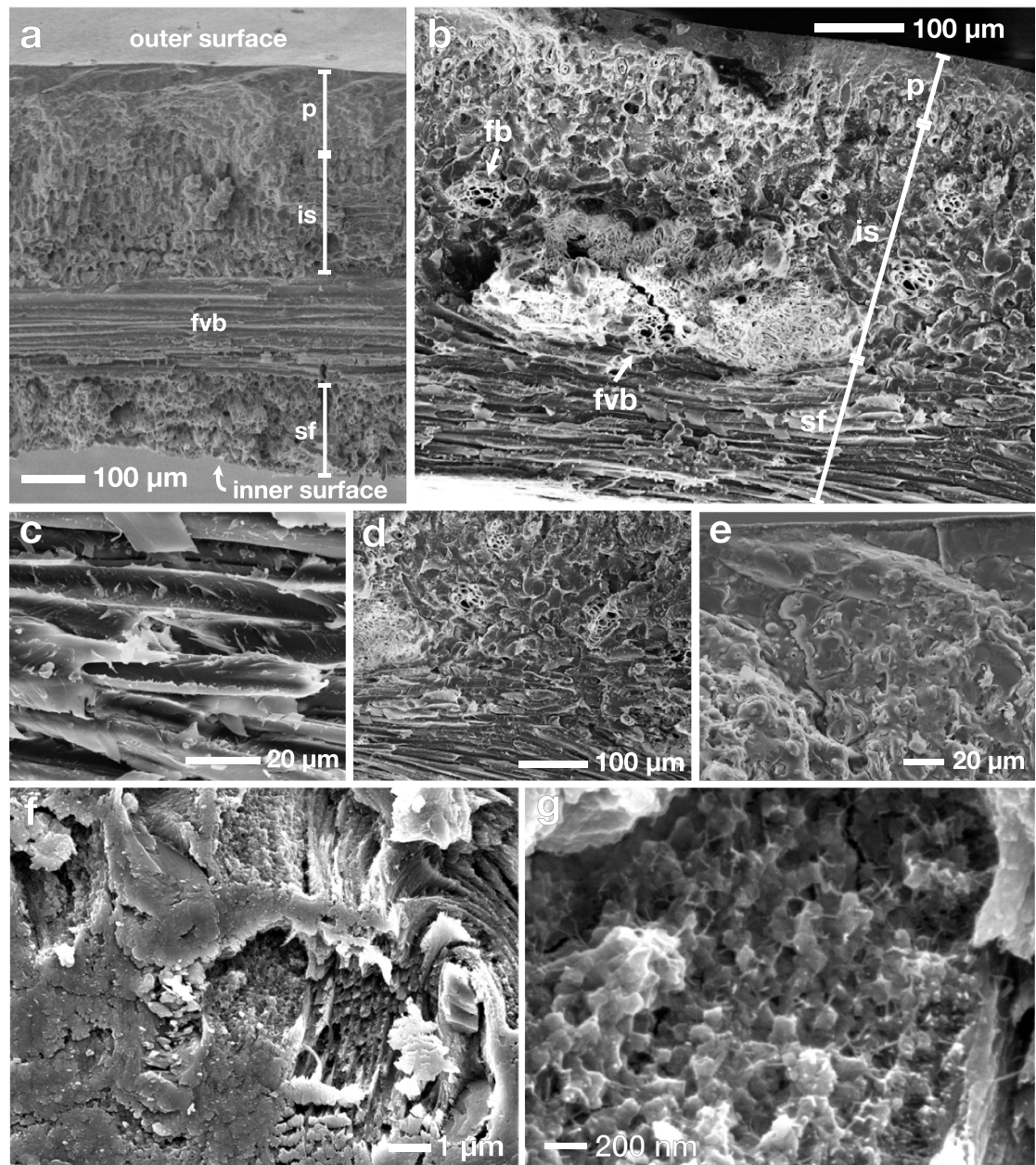


Figure 3. SEM images of cross-sections of the involucre of Job's tears, cut along the *yz* plane (a) and *xz* plane (b). The involucre consists of an outer layer of phytoliths (p), a middle layer of interconnected sclerenchyma cells (is), and an inner layer of sclerenchyma fibers (sf). The fibrovascular bundles (fvb) and fibrous bundles (fb) are located in the middle layer. The sclerenchyma fibers are tubular (c). The intermediate layer is composed of irregularly shaped interconnected sclerenchyma cells (d). The outer layer is characterized by interconnected phytoliths that form a unified structure (e). High-magnification observations of the outer layer revealed that structures are formed by the aggregation of microparticles ranging from several nanometers to approximately 100 nm, with fine fibrous components interspersed among these nanoparticles (f), (g).

beginning (0 μm) and end (570 μm) were observed and attributed to the epoxy resin. The analysis indicated trends within the involucre layers: the outer layer appeared rich in oxygen and silicon but poor in carbon, whereas the inner layers showed a decrease in oxygen and silicon and an increase in carbon. Specifically, oxygen and silicon appeared to reduce inward from the outer surface to 250 μm . The regions

with high silicon and less carbon corresponded to the structures characterized by the aggregated nanoparticles ranging from a few nanometers to approximately 100 nm. These structures were primarily found in the outer layer but were also observed in other parts of the involucre (figure S2). These structures were considered to be composed of amorphous silica.

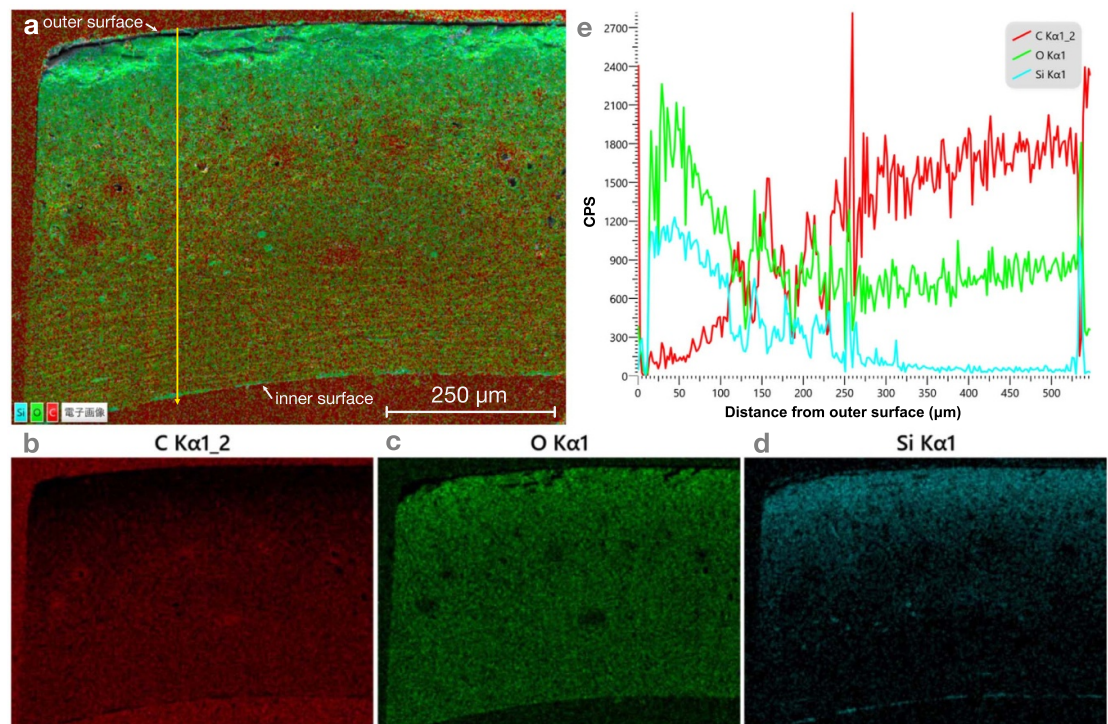


Figure 4. EDX elemental mapping of cross-sections of the involucre of Job's tears, cut along the xz plane (a)–(d), and line analysis was conducted along the yellow arrow (e).

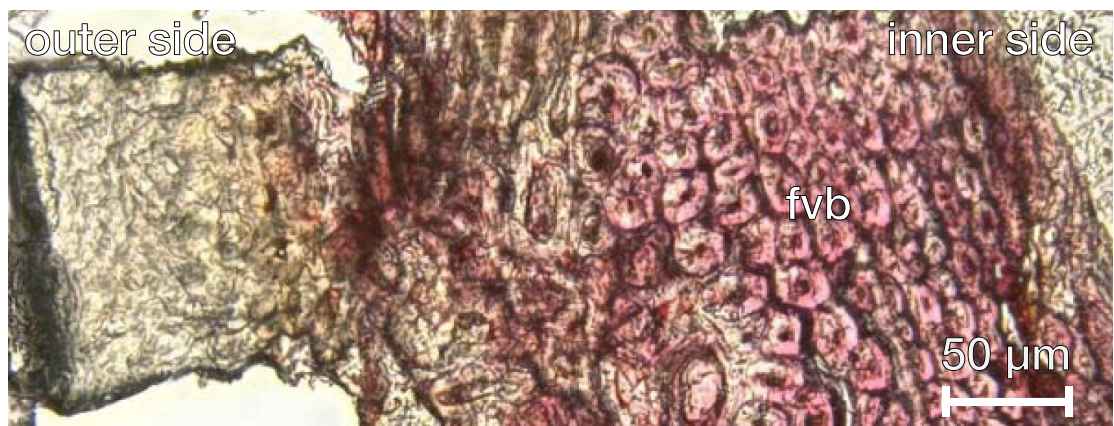


Figure 5. Detection of lignin in the secondary cell wall of involucre by safranin staining.

Compared to the involucre of mature fruits, the amorphous silica layer in the outer layer of the involucre from immature fruits was very thin, at 15 μm, and showed no spatial gradient in the distribution of amorphous silica (figures S3(b)–(e) and S4(b)–(e)).

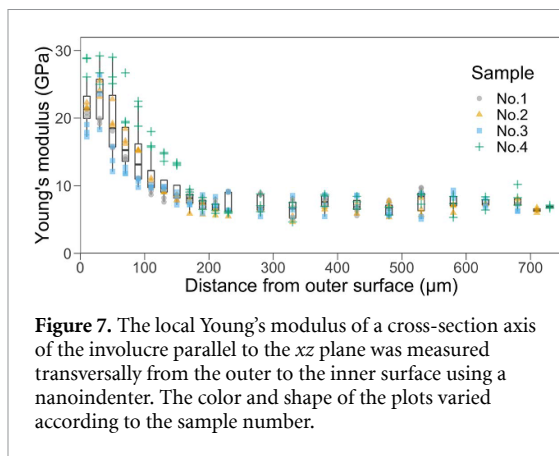
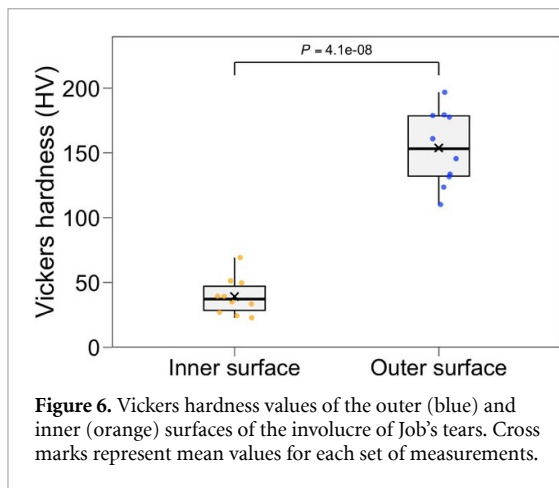
Lignin in the secondary cell walls of the involucre of mature fruits was stained with safranin and observed under an optical microscope (figure 5). Within the phytolith-composed outer layer, no staining was observed from the outer surface up to 60 μm, followed by slight staining for the next 40 μm. Beyond 100 μm from the outer surface, areas of dense and light staining were interspersed. Tissues composed

of sclerenchyma fibers, such as the bundle caps of fibrovascular bundles, were prominently stained.

3.3. Micro-Vickers hardness test

The outer and inner surfaces of the involucre were tested for Vickers hardness (figure 6). The outer surface had a hardness of 153.8 HV (SD 29.0), while the inner surface had a hardness of 39.1 HV (SD 14.3). This suggests that the outer surface was approximately four times harder than the inner surface.

Compared to the involucre thickness of 742 μm (SD 68), the depth of the indentation on the outer surface was only 9.5 μm (SD 0.9), and on the inner



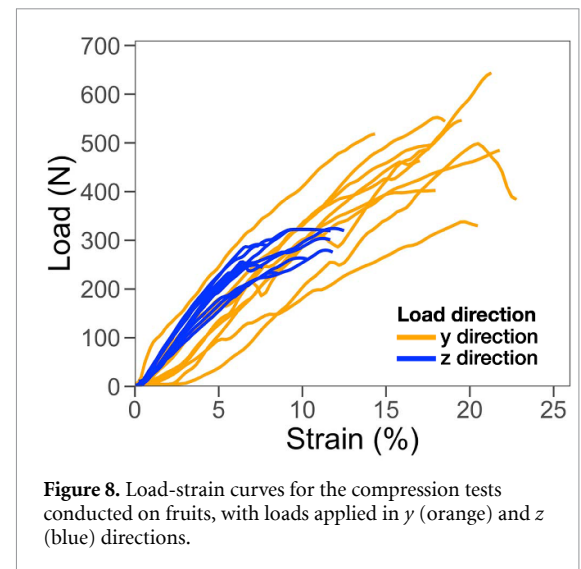
surface, it was $19.4 \mu\text{m}$ (SD 3.3). These indentation depths were sufficiently small, indicating that the measurements satisfied the test conditions.

3.4. Nanoindentation

The Young's modules of a cross-section of the involucre parallel to the xz plane was measured transversally from the outer to the inner surface using a nanoindenter (figure 7). The measurement was performed at $10 \mu\text{m}$ from the outer surface of the involucre, revealing the Young's modules of 22.1 GPa (SD 3.9). The Young's modulus gradually decreased from the outer surface to $200 \mu\text{m}$, after which it converged to 7.1 GPa (SD 1.2).

3.5. Compression test

Figure 8 shows the load-strain curves from compression tests conducted with loads applied in two orientations. When the load was applied in the y direction, the fracture strain was 18.2% (SD 2.5), and the maximum load at fracture was 502 N (SD 82). When the load was applied in the z direction to the fruit, the fracture strain was 8.8% (SD 1.9), and the maximum load at fracture was 288 N (SD 33). Upon removing the cracked involucre after the compression test, intact seeds were found. Since there was a



gap between the involucre and the seeds, the applied load was primarily absorbed by the involucre.

Upon observing the cracks in the involucre following the compression tests, it was found that the cracks formed parallel to the loading direction. Furthermore, loading in the y direction resulted in fractures within the sclerenchyma fibers of the inner layer (figure 9(a)). Conversely, loading in the z direction caused the cracks to interconnect through the fibrovascular bundles (figure 9(b)).

3.6. Three-point bending test

We conducted bending tests of two types, each with a different set-up (figure 1). In configuration A tensile force was applied to the inner side of the involucre, whereas in configuration B, it was applied to the outer side of the involucre. Configuration A resulted in approximately twice the fracture stress, three times the fracture strain, and approximately seven times the toughness compared with configuration B (table 1).

The process of crack propagation in the involucre upon reaching fracture stress was found to vary depending on the configuration, as revealed by SEM observations. In configuration A, cracks were observed to propagate immediately from the tensile side to the compressive side (figures 11(a) and (b)). In contrast, in configuration B, the progression of cracks halted on the tensile side (figures 11(c) and (d)). Furthermore, in configuration A, it was also observed that the cracks interconnected through fibrovascular bundles (figure 11(b)).

4. Discussions

Sclerenchyma cells contain mostly polymers, such as cellulose, hemicellulose, and lignin, which are primarily composed of carbon and oxygen [46, 47]. The amorphous silica is composed of silicon and

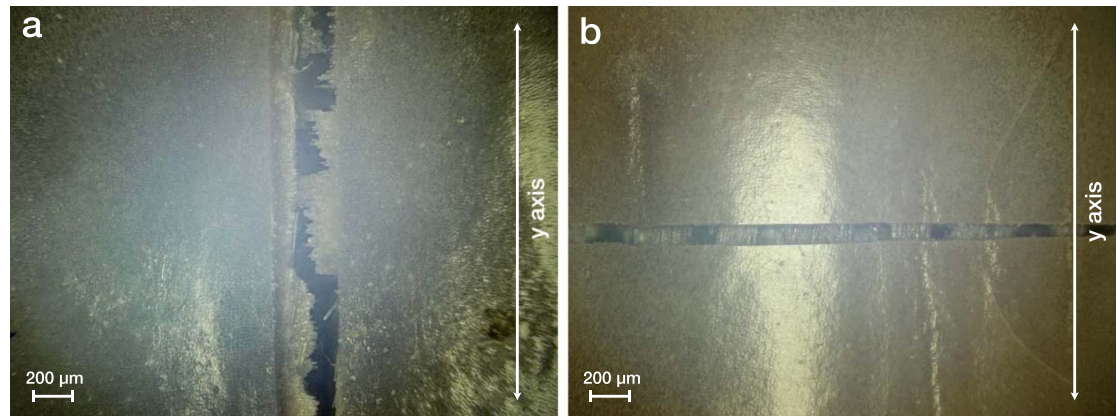


Figure 9. Images of involucre cracks when loads were applied in the y (a) and z (b) directions.

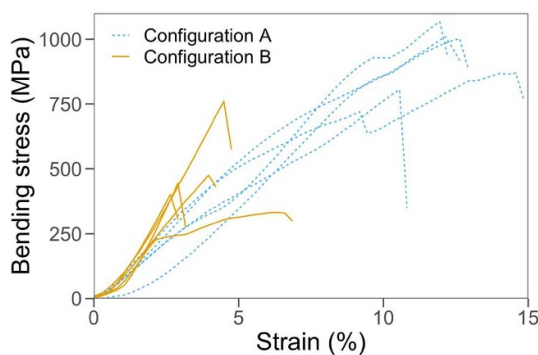


Figure 10. Stress-strain curves from the three-point bending test on the involucre in two setups: configuration A (blue dotted line) and configuration B (orange line).

oxygen. The distribution of these tissues and compounds was clearly identified through SEM observations, EDX elemental mapping, and observations using optical microscopy with safranin staining. The inner layer of the involucre consists of sclerenchyma fibers oriented in the equatorial direction, whereas the middle layer contains interconnected sclerenchyma cells with trace amounts of silica. The outer layer is composed of phytoliths, which are formed by the deposition of amorphous silica particles ranging from several nanometers to 100 nm. Furthermore, each layer is continuously connected through transitional structures. The involucre can be viewed as a structure in which well-lignified and robust fibrovascular and fibrous bundles are embedded within a rigid matrix formed by sclerenchyma cells. The involucre is considered to be a structure reinforced in meridional and equatorial directions by fibrovascular bundles, fibrous bundles, and the inner layer's sclerenchyma fibers (figure 12).

It is evident that throughout the entire involucre, there is a continuous transition in chemical composition, as well as in the orientation and dimensions of the tissues, moving from the inner to the outer

layers. Moreover, it is suggested that there are few points within the involucre where there is a significant change in chemical composition and structural characteristics, which could potentially serve as initiation sites for fractures.

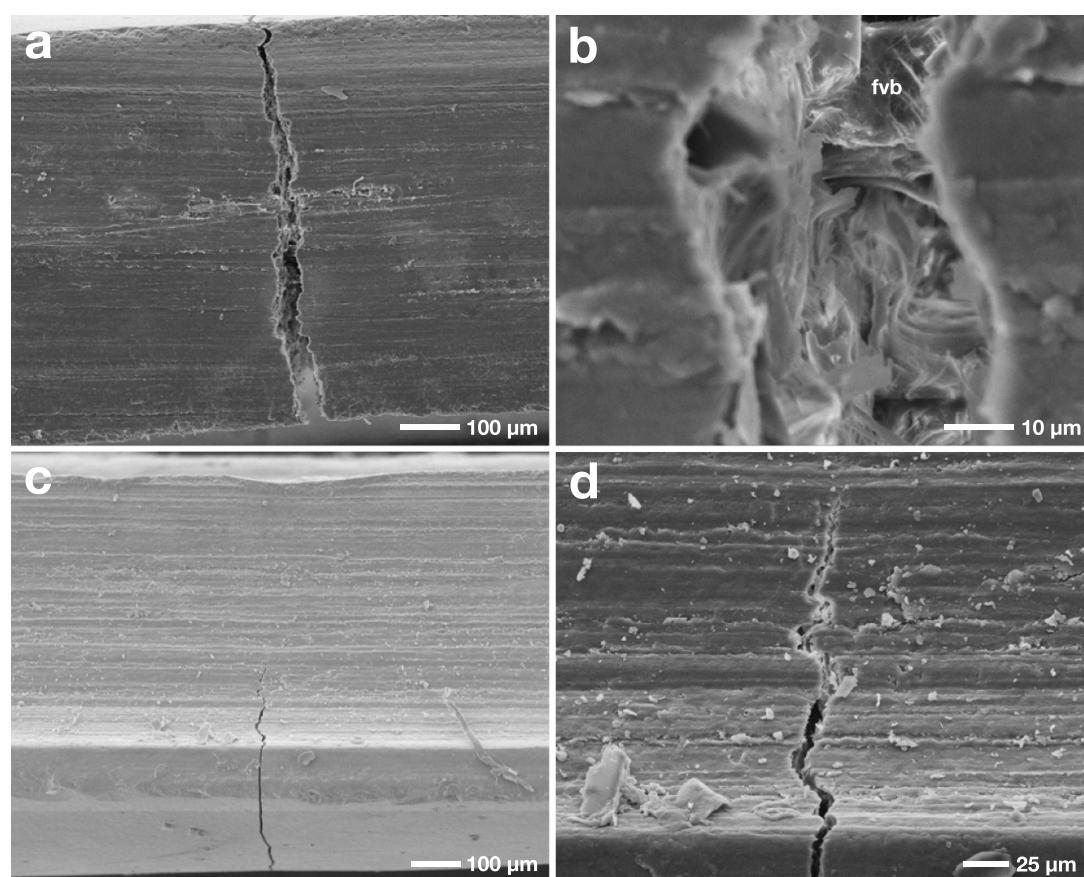
The involucre of immature fruits initially have areas with thin deposits of amorphous silica. As maturation progresses, these areas expand, and silica accumulates in a gradient distribution. This suggests that at the immature stage, silica deposition occurs through an active process controlled by proteins that promote silicification in the outer layer's epidermal cells. Furthermore, it is expected that transpiration and dehydration from the outer surface during maturation lead to the deposition of silica in a gradient distribution through a passive process. Additionally, involucre of immature fruits are thought to have numerous gaps due to less lignification and thinner cell walls.

In compression tests on Job's tears fruits, applying load in the y direction resulted in a higher maximum load at fracture compared to loading in the z direction. The main reason is that the hoop tensile force, which occurs in the circumferential direction of the involucre on the plane orthogonal to the load direction, increases as the curvature of the circumference decreases[48]. Observations of cracks after compression tests reveal that when the load is applied in the y direction, the inner layer's sclerenchyma fibers break, whereas when the load is applied in the z direction, robust fibrovascular bundles are pulled out from the matrix of sclerenchyma cells during crack formation. It is also considered that the difference in type of fracture contributes to the variations in fracture strain and fracture load depending on the direction of the load.

Both the micro-Vickers and nanoindentation tests clearly demonstrated that the outer layer of the involucre is significantly harder than its inner layer. EDX analysis revealed that the silica content increases from the outer surface to a depth of approximately

Table 1. Fracture stress, fracture strain, and toughness for each sample in the three-point bending test, along with their average and standard deviation (SD).

Configuration			Configuration A				
Sample No.	1	2	3	4	5	Average	SD
Fracture stress (MPa)	991.2	1067.7	1002.3	864.7	805.2	946.2	107.8
Fracture strain (-)	0.119	0.119	0.126	0.143	0.106	0.123	0.014
Toughness (MJ m ⁻³)	57.3	64.3	72.9	80.7	43.4	63.7	14.4
Configuration			Configuration B				
Sample No.	6	7	8	9	10	Average	SD
Fracture stress (MPa)	332.3	445.3	759.6	474.3	400.8	482.5	163.9
Fracture Strain (-)	0.063	0.029	0.045	0.040	0.026	0.041	0.015
Toughness (MJ m ⁻³)	14.8	4.6	14.7	8.7	4.3	9.4	5.2

**Figure 11.** SEM images of the cracks in the involucre reached at the fracture stress during the three-point bending tests conducted in configurations A (a) and (b) and B (c) and (d).

200 μm . Considering this finding alongside the nanoindentation test results, it is believed that the increased silica content contributes to the enhanced the Young's modules of the outer layer. Additionally, the standard deviation in hardness values obtained from the micro-Vickers tests was observed to be greater in the outer layer than in the inner layer. This variation is thought to arise from differences in the amount of silica deposition across individual samples. Specifically, samples with higher levels of silica deposition were expected to show higher hardness values.

A similar trend was observed in the nanoindentation test results.

The results of the three-point bending test indicate that the fracture stress, strain, and toughness were higher in configuration A than in configuration B. Through SEM observations and EDX analyses, it was determined that the outer layer of the involucre contained an accumulation of nano sized silica particles, forming a structure like ceramics. Ceramics, in general, have a high hardness and are resistant to compression but demonstrate brittle characteristics

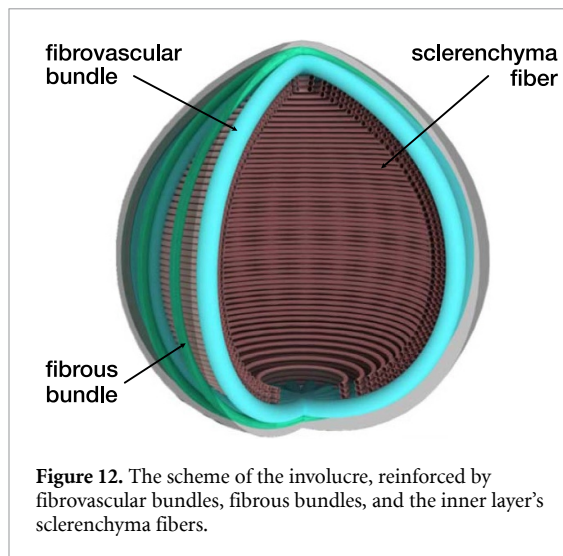


Figure 12. The scheme of the involucre, reinforced by fibrovascular bundles, fibrous bundles, and the inner layer's sclerenchyma fibers.

under tension. On the other hand, the inner and middle layers of the involucre were primarily composed of secondary cell walls of sclerenchyma cells, which were thickened due to lignification. Within the secondary cell wall, cellulose microfibrils are cross-linked by hemicellulose, and the spaces between this cross-linked structure are further filled and cross-linked by lignin, resulting in the formation of an extremely robust structure, similar to fiber reinforced plastics. This presumes that the sclerenchyma cells are strong against tension. Within the involucres, these components with different material properties are distributed layers in a smooth spatial gradient from the outer to the inner. In configuration A, the outer layer, which accumulates silica nanoparticles, is subjected to compression, while the inner layer, composed of thick-walled cells, undergoes tension. It is considered that such an arrangement of tissues means that the right materials are in the right places. Conversely, in configuration B, the outer layer, which is weak against tension, is positioned on the tension side. Furthermore, it is believed that fibrovascular bundles and fibrous bundles, which reinforce the involucre in the meridional direction, also contribute to withstanding tension in configuration A.

The differences in the crack formation process during a three-point bending test are closely related to the changes in material properties along the direction of crack propagation. In configuration A, the crack propagates rapidly due to a transition from elastoplastic to brittle materials along the propagation path. Conversely, in configuration B, the transition from brittle to elastoplastic materials halts crack progression. Moreover, the involucre of configuration A, compared to that of configuration B, has notably higher fracture stress (twice as much), fracture strain (three times as much), and toughness (seven times as much). Therefore, it is presumed that the stress and strain accumulated due to bending are

instantaneously released, triggered by the delamination between sclerenchyma fibers on the tension side.

5. Conclusion

In the involucre of Job's tears, the chemical composition and structural characteristics including orientation and dimensions of tissues, exhibit a smooth transition from the outer to the inner layers. Furthermore, it was presumed that the spatial gradient in the Young's modulus within the involucre is attributed to the gradient in silica deposition. The outer layer is densely deposited with silica, enhancing its compression resistance, while the middle and inner layers are composed of sclerenchyma cells that are resistant to tension. The tissues of the outer, middle, and inner layers are smoothly connected through transitional structures, meaning seamlessly integrating tissues with different material properties. Moreover, the involucre is reinforced in both meridional and equatorial directions by robust fibrovascular bundles, fibrous bundles, and the inner layer's sclerenchyma fibers. The gradient in material properties within the involucre appropriately responds to the compressive and tensile stresses caused by loads from outside. Furthermore, combined with the reinforcement by fibers, the involucre achieves high toughness and becomes less prone to cracking.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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